

चने (साइसर एराइटीनम एल.) में न्यून जल-तनाव के  
सुधार में पैक्लोब्यूट्राज़ोल की भूमिका

**ROLE OF PACLOBUTRAZOL IN  
AMELIORATION OF WATER DEFICIT STRESS  
IN CHICKPEA (*Cicer arietinum* L.)**

By

**SOUMYA P. R.**



**DIVISION OF PLANT PHYSIOLOGY  
INDIAN AGRICULTURAL RESEARCH INSTITUTE  
NEW DELHI-110012  
2014**



**Division of Plant Physiology  
Indian Agricultural Research Institute  
New Delhi -110012**

**Dr. Pramod Kumar**  
Senior Scientist

**CERTIFICATE**

This is to certify that the thesis entitled “**Role of Paclobutrazol in Amelioration of Water deficit Stress in Chickpea (*Cicer arietinum* L.)**” submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE in PLANT PHYSIOLOGY**, embodies the results of bona fide research work carried out by **Ms. Soumya P. R.**, under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by her.

**Date:** 30 June, 2014

**Place:** New Delhi

**Dr. PRAMOD KUMAR**  
Chairperson Advisory Committee

# **Role of Paclobutrazol in Amelioration of Water deficit Stress in Chickpea (*Cicer arietinum* L.)**

**A Thesis**

**By**

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**Approved by:**

Chairman : Dr. Pramod Kumar \_\_\_\_\_

Co-chairman : Dr. Madan Pal Singh \_\_\_\_\_

Member : Dr. Rakesh Pandey \_\_\_\_\_

Dr. Aruna Tyagi \_\_\_\_\_

Dr. Supradeep Saha \_\_\_\_\_

*Dedicated to my beloved  
parents & my dear sister*



*for their love, unconditional support &  
endless encouragement*

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*Dated: 30<sup>th</sup> June, 2014*

*(Soumya P. R.)*

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## **CONTENTS**

| <b>Sl. No.</b> | <b>CHAPTERS</b>       | <b>Page No.</b> |
|----------------|-----------------------|-----------------|
| 1              | INTRODUCTION          | 1               |
| 2              | BACKGROUND            | 5               |
| 3              | MATERIALS AND METHODS | 24              |
| 4              | RESEARCH PAPER 1      | 43              |
| 5              | RESEARCH PAPER 2      | 64              |
| 6              | DISCUSSION            | 74              |
| 7              | SUMMARY               | 84              |
| 8              | ABSTRACT (ENGLISH)    | 87              |
| 9              | ABSTRACT (HINDI)      | 88              |
| 10             | BIBLIOGRAPHY          | i-xx            |

## LIST OF TABLES

| Table         | TITLE                                                                                                                                       | After page |
|---------------|---------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 4.3.1.1.a.    | Optimization of the foliar application dose of PBZ in <i>Kabuli</i> (Pusa 1108) and <i>Desi</i> (Pusa 362) under water deficit condition    | 47         |
| 4.3.1.1.b.    | Optimization of the drenching application dose of PBZ in <i>Kabuli</i> (Pusa 1108) and <i>Desi</i> (Pusa 362) under water deficit condition | 47         |
| 4.3.1.2.1.    | Effect of PBZ on relative water content (%) in chickpea genotypes under well watered and imposed water stress conditions.                   | 48         |
| 4.3.1.2.2.    | Effect of PBZ on membrane stability index (%) in chickpea genotypes under well watered and imposed water stress conditions.                 | 48         |
| 4.3.1.2.3.1.  | Effect of PBZ on shoot length in chickpea genotypes under well watered and imposed water stress conditions.                                 | 48         |
| 4.3.1.2.3.2.  | Effect of PBZ on root length in chickpea genotypes under well watered and imposed water stress conditions.                                  | 48         |
| 4.3.1.2.3.3.  | Effect of PBZ on leaf number in chickpea genotypes under well watered and imposed water stress conditions.                                  | 49         |
| 4.3.1.2.3.4.  | Effect of PBZ on total leaf area in chickpea genotypes under well watered and imposed water stress conditions.                              | 49         |
| 4.3.1.2.3.5.  | Effect of PBZ on shoot dry weight in chickpea genotypes under well watered and imposed water stress conditions.                             | 49         |
| 4.3.1.2.3.6.  | Effect of PBZ on root dry weight in chickpea genotypes under well watered and imposed water stress conditions.                              | 49         |
| 4.3.1.2.3.7.  | Effect of PBZ on leaf dry weight in chickpea genotypes under well watered and imposed water stress conditions.                              | 50         |
| 4.3.1.2.3.8.  | Effect of PBZ on specific leaf weight in chickpea genotypes under well watered and imposed water stress conditions.                         | 50         |
| 4.3.1.2.3.9.  | Effect of PBZ on specific leaf area in chickpea genotypes under well watered and imposed water stress conditions.                           | 50         |
| 4.3.1.2.3.10. | Effect of PBZ on leaf area ratio in chickpea genotypes under well watered and imposed water stress conditions.                              | 50         |
| 4.3.1.2.5.1.  | Effect of PBZ on photosynthetic rate in chickpea genotypes under well watered and imposed water stress.                                     | 51         |
| 4.3.1.2.5.2.  | Effect of PBZ on stomatal conductance in chickpea genotypes under well watered and imposed water stress conditions.                         | 51         |

|              |                                                                                                                               |    |
|--------------|-------------------------------------------------------------------------------------------------------------------------------|----|
| 4.3.1.2.5.3. | Effect of PBZ on transpiration rate in chickpea genotypes under well watered and imposed water stress conditions.             | 51 |
| 4.3.1.2.5.4  | Effect of PBZ on water use efficiency in chickpea genotypes under well watered and imposed water stress conditions.           | 52 |
| 4.3.1.2.6.1. | Effect of PBZ on chlorophyll a in chickpea genotypes under well watered and imposed water stress conditions.                  | 52 |
| 4.3.1.2.6.2. | Effect of PBZ on chlorophyll b in chickpea genotypes under well watered and imposed water stress conditions                   | 52 |
| 4.3.1.2.6.3. | Effect of PBZ on total chlorophyll in chickpea genotypes under well watered and imposed water stress conditions.              | 52 |
| 4.3.1.2.6.4. | Effect of PBZ on total carotenoids in chickpea genotypes under well watered and imposed water stress conditions.              | 53 |
| 4.3.1.2.7.a. | Effect of PBZ on post harvest parameters in chickpea genotypes under well watered and imposed water stress conditions.        | 54 |
| 4.3.1.2.7.b. | . Effect of PBZ on post harvest parameters in chickpea genotypes under well watered and imposed water stress conditions       | 55 |
| 4.3.1.2.7.c. | Effect of PBZ on post harvest parameters in chickpea genotypes under well watered and imposed water stress conditions         | 55 |
| 5.3.1.1.     | Effect of PBZ on lipid peroxidation in chickpea genotypes under well watered and imposed water stress conditions.             | 67 |
| 5.3.1.2.     | Effect of PBZ on proline content in chickpea genotypes under well watered and imposed water stress conditions.                | 67 |
| 5.3.1.3.     | Effect of PBZ on total free amino acids content in chickpea genotypes under well watered and imposed water stress conditions. | 67 |
| 5.3.1.4.     | Effect of PBZ on total soluble sugar content in chickpea genotypes under well watered and imposed water stress conditions.    | 68 |
| 5.3.1.5.     | Effect of PBZ on total soluble protein content in chickpea genotypes under well watered and imposed water stress conditions.  | 68 |
| 5.3.1.6.     | Effect of PBZ on catalase activity in chickpea genotypes under well watered and imposed water stress conditions.              | 68 |

|           |                                                                                                                               |    |
|-----------|-------------------------------------------------------------------------------------------------------------------------------|----|
| 5.3.1.7.  | Effect of PBZ on peroxidase activity in chickpea genotypes under well watered and imposed water stress conditions.            | 68 |
| 5.3.1.8.  | Effect of PBZ on superoxide dismutase activity in chickpea genotypes under well watered and imposed water stress conditions.  | 68 |
| 5.3.1.9.  | Effect of PBZ on glutathione reductase activity in chickpea genotypes under well watered and imposed water stress conditions. | 69 |
| 5.3.1.10. | Effect of PBZ on abscisic acid content in chickpea genotypes under well watered and imposed water stress conditions.          | 69 |

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# LIST OF FIGURES

| Figure No.      | TITLE                                                                                                                                       | After page |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Fig. 1.         | Weather records during the course of investigation                                                                                          | 25         |
| Fig. 2.         | Soil moisture content and field capacity recorded during the preliminary experiment                                                         | 26         |
| Fig. 3.         | Soil moisture content recorded during the main experiment                                                                                   | 26         |
| Fig.4.3.1.1.a.  | Relative water content response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition.   | 47         |
| Fig.4.3.1.1. b. | Relative water content response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during recovery.                                 | 47         |
| Fig.4.3.1.1.c.  | Membrane stability index response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition. | 47         |
| Fig.4.3.1.1.d.  | Membrane stability index response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during recovery.                               | 47         |
| Fig.4.3.1.1.e.  | Photosynthesis rate response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition.      | 47         |
| Fig.4.3.1.1.f.  | Photosynthesis rate response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during recovery.                                    | 47         |
| Fig.4.3.1.1.g.  | Chl a response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition.                    | 47         |
| Fig.4.3.1.1.h.  | Chl a response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during recovery.                                                  | 47         |
| Fig.4.3.1.1.i.  | Chl b response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition.                    | 47         |
| Fig.4.3.1.1.j.  | Chl b response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during recovery.                                                  | 47         |
| Fig.4.3.1.1.k.  | Total Chlorophyll response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition.        | 47         |
| Fig.4.3.1.1.l.  | Total Chlorophyll response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during recovery.                                      | 47         |
| Fig.4.3.1.1.m.  | Total carotenoids response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed water deficit stress condition.        | 47         |
| Fig.4.3.1.1.n.  | Total carotenoids response of <i>desi</i> and <i>kabuli</i> chickpea varieties to PBZ during imposed recovery.                              | 47         |
| 4.3.1.2.1.      | Effect of PBZ on relative water content (%) in chickpea genotypes under well watered and imposed water stress conditions.                   | 48         |

|               |                                                                                                                                    |    |
|---------------|------------------------------------------------------------------------------------------------------------------------------------|----|
| 4.3.1.2.2.    | Effect of PBZ on membrane stability index (%) in chickpea genotypes under well watered and imposed water stress conditions.        | 48 |
| 4.3.1.2.3.1.  | Effect of PBZ on shoot length in chickpea genotypes under well watered and imposed water stress conditions.                        | 48 |
| 4.3.1.2.3.2.  | Effect of PBZ on root length in chickpea genotypes under well watered and imposed water stress conditions.                         | 48 |
| 4.3.1.2.3.3.  | Effect of PBZ on leaf number in chickpea genotypes under well watered and imposed water stress conditions.                         | 49 |
| 4.3.1.2.3.4.  | Effect of PBZ on leaf area in chickpea genotypes under well watered and imposed water stress conditions.                           | 49 |
| 4.3.1.2.3.5.  | Effect of PBZ on shoot dry weight in chickpea genotypes under well watered and imposed water stress conditions.                    | 49 |
| 4.3.1.2.3.6.  | Effect of PBZ on root dry weight in chickpea genotypes under well watered and imposed water stress conditions.                     | 49 |
| 4.3.1.2.3.7.  | Effect of PBZ on leaf dry weight in chickpea genotypes under well watered and imposed water stress conditions.                     | 49 |
| 4.3.1.2.3.8   | Effect of PBZ on specific leaf weight in chickpea genotypes under well watered and imposed water stress conditions.                | 50 |
| 4.3.1.2.3.9.  | Effect of PBZ on specific leaf area in chickpea genotypes under well watered and imposed water stress conditions.                  | 50 |
| 4.3.1.2.3.10. | Effect of PBZ on leaf area ratio in chickpea genotypes under well watered and imposed water stress conditions.                     | 50 |
| 4.3.1.2.4.    | Effect of PBZ on chloroplast structure in chickpea genotypes under imposed water deficit stressed conditions.                      | 50 |
| 4.3.1.2.5.1.  | Effect of PBZ on photosynthetic rate in chickpea genotypes under well watered and imposed water stress conditions.                 | 51 |
| 4.3.1.2.5.2.  | Effect of PBZ on stomatal conductance in chickpea genotypes under well watered and imposed water stress conditions.                | 51 |
| 4.3.1.2.5.3.  | Effect of PBZ on transpiration rate in chickpea genotypes under well watered and imposed water stress conditions.                  | 51 |
| 4.3.1.2.5.4.  | Effect of PBZ on water use efficiency in chickpea genotypes under well watered and imposed water stress conditions.                | 52 |
| 4.3.1.2.5.5.  | Effect of PBZ on quantum efficiency of PS II (Fv/Fm) in chickpea genotypes under well watered and imposed water stress conditions. | 52 |
| 4.3.1.2.6.1.  | Effect of PBZ on chlorophyll a in chickpea genotypes under well watered and imposed water stress conditions.                       | 52 |
| 4.3.1.2.6.2.  | Effect of PBZ on chlorophyll b in chickpea genotypes under well watered and imposed water stress conditions                        | 52 |
| 4.3.1.2.6.3.  | Effect of PBZ on total chlorophyll in chickpea genotypes under well                                                                | 52 |

|                   |                                                                                                                                                   |    |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                   | watered and imposed water stress conditions                                                                                                       |    |
| 4.3.1.2.6.4.      | Effect of PBZ on total carotenoids in chickpea genotypes under well watered and imposed water stress conditions                                   | 53 |
| 4.3.1.2.6.5.      | Effect of PBZ on photosynthetic pigments profile of <i>Kabuli</i> and <i>Desi</i> chickpea genotypes under imposed water deficit stress condition | 53 |
| 4.3.1.2.7.a.      | Effect of PBZ applications on podding of chickpea varieties under well watered condition                                                          | 54 |
| 4.3.1.2.7.b.      | Effect of PBZ applications on podding of chickpea varieties under water stressed condition                                                        | 54 |
| 4.3.1.2.7.15.(a). | Effect of PBZ on dry matter partitioning in <i>Kabuli</i> chickpea variety under well watered and imposed water stress conditions                 | 56 |
| 4.3.1.2.7.15.(b). | Effect of PBZ on dry matter partitioning in <i>Desi</i> Chickpea variety under well watered and imposed water stress conditions                   | 56 |
| 4.3.1.2.7.16.     | Effect of PBZ on harvest index in chickpea genotypes under well watered and imposed water stress conditions                                       | 56 |
| 4.3.1.2.7.17.     | Effect of PBZ on drought susceptibility index (DSI) in chickpea genotypes under well watered and imposed water stress conditions                  | 56 |
| 4.3.1.2.7.18.     | Effect of PBZ on drought tolerance efficiency (DTE) in chickpea genotypes under well watered and imposed water stress conditions                  | 56 |
| 5.3.1.1.          | Effect of PBZ on lipid peroxidation in chickpea genotypes under well watered and imposed water stress conditions.                                 | 67 |
| 5.3.1.2.          | Effect of PBZ on proline content in chickpea genotypes under well watered and imposed water stress conditions.                                    | 67 |
| 5.3.1.3.          | Effect of PBZ on total free amino acids content in chickpea genotypes under well watered and imposed water stress conditions.                     | 67 |
| 5.3.1.4.          | Effect of PBZ on total soluble sugar content in chickpea genotypes under well watered and imposed water stress conditions.                        | 68 |
| 5.3.1.5.          | Effect of PBZ on total soluble protein content in chickpea genotypes under well watered and imposed water stress conditions.                      | 68 |
| 5.3.1.6.          | Effect of PBZ on catalase activity in chickpea genotypes under well watered and imposed water stress conditions.                                  | 68 |
| 5.3.1.7.          | Effect of PBZ on peroxidase activity in chickpea genotypes under well watered and imposed water stress conditions.                                | 68 |
| 5.3.1.8.          | Effect of PBZ on glutathione reductase activity in chickpea genotypes under well watered and imposed water stress conditions                      | 69 |
| 5.3.1.9.          | Effect of PBZ on superoxide dismutase activity in chickpea genotypes under well watered and imposed water stress conditions                       | 69 |
| 5.3.1.10.         | Effect of PBZ on abscisic acid content in chickpea genotypes under well watered and imposed water stress conditions                               | 69 |

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## **ABBREVIATIONS**

|                |                                             |
|----------------|---------------------------------------------|
| ABA            | Absciscic acid                              |
| Car            | Carotenoid                                  |
| CAT            | Catalase                                    |
| Chl            | Chlorophyll                                 |
| DMSO           | Dimethyl Sulphoxide                         |
| DTNB           | 5,5-dithiobis-(2-nitrobenzoic acid)         |
| EDTA           | Ethylene diamine tetra acetic acid          |
| GR             | Glutathione reductase                       |
| GSSG           | Oxidised glutathione                        |
| HI             | Harvest Index                               |
| DTE            | Drought tolerance efficiency                |
| MSI            | Membrane stability index                    |
| NADPH          | Reduced nicotinamide dinucleotide phosphate |
| NBT            | Nitro blue tetrazolium chloride             |
| P <sub>N</sub> | Net photosynthesis rate                     |
| PBZ            | Paclobutrazol                               |
| POX            | Peroxidase                                  |
| RWC            | Relative water content                      |
| SOD            | Superoxide dismutase                        |
| TBARS          | Thiobarbituric acid reactive substance      |
| TCA            | Trichloroacetic acid                        |
| TFA            | Total free amino acid                       |
| TSP            | Total soluble protein                       |
| TSS            | Total soluble sugar                         |
| TG             | Tetra- guaiacol                             |
| WUE            | Water use efficiency                        |

# 1. INTRODUCTION

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India is one of the major pulse growing countries in the world, at present about 25 to 26 million hectares of land is under pulses cultivation in India producing about 17 million tons of pulses annually (IIPR, 2013). Among the pulses, Chickpea (*Cicer arietinum* L.) is the third most important protein rich pulse crop by virtue of N<sub>2</sub> fixation, with global production of 10.9 million tons from an area of 11.5 million ha and a productivity of 0.91 t ha<sup>-1</sup> (ICRISAT, 2013). Chickpea seeds contain 23% protein, 64% carbohydrates, 5% fat, 6% crude fiber, 6% soluble sugar, and 3% ash (William and Singh, 1987). South and South-East Asia region contributes about 90% to the global chickpea production, and India is the principal chickpea producing country (83% share in the region) (<http://www.icrisat.org>). It occupies 15.3% of the total pulses area and contributes 14.6% to total pulse production. Under optimum growing conditions, the yield potential of chickpea is 5-6 t/ha (Ahmad *et al.*, 2005) which is much higher than the current global yield average of 911 kg. Two market classes of chickpea, namely *desi*, and *kabuli*, are currently grown in these areas. *kabuli* chickpea varieties fetch premium price in market. However, *kabuli* type chickpea varieties are relatively more sensitive to abiotic stress than *Desi* ones.

Since chickpea is conventionally cultivated in marginal and rainfed areas (Rao *et al.*, 2002). Therefore, the major constraint in chickpea production is water deficit stress. Water deficit adversely affects the crop growth and yield. It causes 40-50 % reduction in yield globally (Ahmad *et al.*, 2005). In our country, irrigation is available only for 40% of the cultivated area and the remaining 60% depends on scanty rains. Moderate water deficit conditions are observed 1-2 times in 5 years and severe water deficit conditions occur once in 8-9 years. In this crop, yield losses might be the result of intermittent water deficit during the vegetative phase or due to water deficit during reproductive development or due to terminal water deficit at the end of the crop cycle (Serraj *et al.*, 2004). Water deficit stress is one of the major factors limiting chickpea production in arid and semi arid regions.

Water deficit stress is distinguished by attenuation of water content, diminished leaf water potential and turgour loss, closure of stomata and decrease in cell enlargement and growth. It causes numerous physiological and biochemical changes in plants like reduced leaf size, stem extension, root proliferation, reduced water use efficiency (Farooq *et al.*, 2009), alteration in metabolic activities (Lawlor and Cornic, 2002), inhibition of enzymatic activities (Ashraf *et al.*, 1995), ionic imbalance and disturbances in solute accumulation (Khan *et al.*, 1999) or a combination of all these factor.

Water deficit stress reduces plant growth by affecting photosynthesis, respiration, the membrane stability index (MSI) and nutrient metabolism (Jaleel *et al.*, 2008). The morphological and physiological changes in response to water deficit stress can be used to help identify resistant genotypes or produce new varieties of crops for better productivity under water deficit stress (Nam *et al.*, 2001). The reactions of plants to water deficit stress depend on the intensity and duration of stress as well as the plant species and its stage of growth (Parameshwarappa and Salimath, 2008). In water deficit stress conditions, plants close their stomata to avoid further water loss. Decreasing internal CO<sub>2</sub> concentration (C<sub>i</sub>) and inhibition of ribulose-1, 5-bisphosphate carboxylase/oxygenase enzyme activity and ATP synthesis lead to a decrease of net photosynthetic rate under water deficit stress (Dulai *et al.*, 2006). Reduced inhibition of photosynthesis under drought stress is of great importance for water deficit tolerance (Zlatev and Yordanov, 2004).

The effect of water deficit stress on CO<sub>2</sub> assimilation rate, transpiration rate and water use efficiency (WUE) has been investigated in many crops such as *Zea mays* (Ashraf *et al.*, 2007), *Brassica napus* L. (Kausar *et al.*, 2006) and mungbean genotypes (Ahmed *et al.*, 2002). Another plant response to water deficit stress is change in photosynthetic pigment content. Photosynthetic pigments play important roles in harvesting light. The content of both chlorophyll *a* and *b* changes under water deficit stress (Farooq *et al.*, 2009). The carotenoids play fundamental roles and help plants to resist water deficit stress (Jaleel *et al.*, 2009). Water deficit stress inhibits Chl *a/b* synthesis and decreases the content of Chl *a/b* binding proteins, leading to reduction of the light-harvesting pigment protein associated with photosystem II (Sayed, 2003). The effects of water deficit stress on chlorophyll and carotenoid content have been investigated in cotton (Mssacci *et al.*, 2008) and *Catharanthus roseus* (Jaleel *et al.*, 2008). Water deficit stress affects photosystem efficiency (Fv/Fm) and decreases the electron transport rate (ETR) and the effective quantum yield of photosystem II photochemistry (Y) (Ahmed *et al.*, 2002). The Fv/Fm ratio is a parameter which allows detection of any damage to PSII and possible photoinhibition (Ahmed *et al.*, 2002). The effects of water deficit stress on MSI, RWC and leaf water potential have also been investigated in many studies (Siddique *et al.*, 2000; Jinmin and Huang, 2001; Terzi and Kadioglu, 2006; Bayoumi *et al.*, 2008). It is believed that leaf water potential and Relative water content (RWC) are reliable parameters for quantifying the plant water deficit stress response (Siddique *et al.*, 2000; Bayoumi *et al.*, 2008). A common adverse effect of water deficit stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al.*, 2009). Plant productivity under water deficit is strongly related to processes of dry matter partitioning and temporal biomass distribution (Kage *et al.*, 2004). Water deficit mainly influences yield by limiting seed numbers by either influencing the amount of dry matter produced at the time of

flowering by directly influencing pollen or ovule function, which leads to decreased seed-set. Secondly, water deficit influences seed filling mainly by limiting the assimilate supply, leading to smaller seed size and lower yields.

Water deficit stress is a global issue to agricultural crops and sustainable food production (Shao *et al.*, 2006). Currently, there is an extensive research concern to accomplish stress tolerance in crop plants without compromising with their yield. In chickpea, crop yield enhancement through conventional breeding is difficult because of a narrow genetic base (Udupa *et al.*, 1999). Moreover, it is time consuming process and rely on available genetic variability (Zhu, 2002). An alternative approach that attracts more attention in this direction is the application of bioregulators which can be used to induce the stress tolerance in crops without negotiating grain yield under field conditions. At present, there is a greater research interest in identifying and employing bioregulatory molecules, which can be used to achieve the stress tolerance in crops under field conditions.

Plant growth regulators have been suggested to play important roles in stress responses and adaptation (Sharma *et al.*, 2005). They maintain proper balance between source and sink for enhancing crop yield (Gupta *et al.*, 2007). Water deficit stress alters the level of endogenous plant hormones (Morgan, 1990). Under stress conditions, levels of cytokinin and gibberellic acid are decreased and content of abscisic acid is increased (Boucaud and Unger, 1976; Nayer *et al.*, 2005) which in turn, suggested that growth reduction under stress conditions could be the result of the stress induced changes in membrane permeability and water uptake due to altered endogenous hormonal levels (Karmoker and Van Steveninck, 1979).

Triazoles are mediated by their ability to alter the balance of important plant hormones including gibberellic acid, abscisic acid (ABA) and cytokinins (Kamounsis *et al.*, 1999). Triazoles interfere with three steps of ent-kaurene oxidation pathway and inhibit the formation of entkaurenol, ent-kaurenal, and ent-kaurenoic acid (Izumi *et al.* 1985). These microsomal oxidation reactions are catalyzed by kaurene oxidase and interference with the different isoforms of this enzyme could lead to inhibition of gibberellins (GA) biosynthesis and abscisic acid (ABA) catabolism (Rademacher 1997). Triazoles induce a variety of morphological and biochemical responses in plants, including inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses (Jaleel *et al.*, 2007). Protection of plants from apparently unrelated stress by triazole is also mediated by a reduction in free radical damage and increases in the antioxidant potential which have an efficient free-radical scavenging system that enables them to detoxify active oxygen (Koprya and Gwozdz, 2003).

Amongst the various triazoles, Paclobutrazol (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol] has plant growth regulating properties. The growth regulating properties of paclobutrazol are mediated by changes in the balance of important plant hormones including the Gibberellins, ABA and cytokinins (Fletcher and Hotstra, 1990). Paclobutrazol (PBZ) affects the isoprenoid pathway, and alters the levels of plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution, and increasing cytokinin levels (Kamounsis and Chronopoulou, 1999). When gibberellins synthesis is inhibited, more precursors in the terpenoid pathway accumulate and are shunted to the production of abscisic acid. One of the functions of abscisic acid is to cause stomates to close, reducing water loss from leaves through transpiration. Paclobutrazol has also been proved as an agent in stress amelioration in medicinal plants (Jaleel *et al.*, 2007). PBZ has been used to provide plant protection against abiotic stresses such as chilling (Lin *et al.*, 2006), water stress (Banon *et al.*, 2001; Beroova and Zaltev, 2003; Zhu *et al.*, 2004), flooding (Webb and Fletcher, 1996; Lin *et al.*, 2006) and salinity (Elaidy *et al.*, 1992; El-Khashab *et al.*, 1997; Ozmen *et al.*, 2003; Saeed, 2005; Kishor *et al.*, 2009). Paclobutrazol was reported to induce water stress tolerance in some plants such as *Arachis hypogaea* L. (Leguminosae, Papilioideae) and *Phillyrea angustifolia* L. (Oleaceae) (Fernandez *et al.*, 2006; Sankar *et al.*, 2007). The induction of water stress tolerance results from some biochemical changes, which induces the chlorophyll a and chlorophyll b contents in Potato leaf (Tekalign and Hammes, 2004). The proline content also increased with increasing water stress tolerance (Jaleel *et al.*, 2007 and Srivastav *et al.*, 2010). Higher water stress tolerance is also caused because of an increase of antioxidants, a decrease of electrolyte leakage and lipid peroxidation. Lipid peroxidation is well known as an indicator to evaluate cell membrane damage (Gopi *et al.*, 2007; Jaleel *et al.*, 2007; Srivastav *et al.*, 2010). Some morpho-physiological changes, such as reduction of plant height and leaf size, increase of leaf thickness and stomata density are also known to occur (Sankhla *et al.*, 1985; Burrows *et al.*, 1992).

Based on these studies, present thesis research work was undertaken to investigate the protective role of paclobutrazol in water deficit stress amelioration in chickpea (*Cicer arietinum* L.) with following objectives:

#### **Objectives:**

- **To identify optimum dose of paclobutrazol for foliar and drenching applications under water deficit condition**
- **To analyze physiological and biochemical alterations for understanding the paclobutrazol induced mechanism of water deficit stress tolerance**

## 2. BACKGROUND

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### 2.1. INTRODUCTION

Chickpea is the most important crop representing about 27% of the land area under pulse, which contributes 33% of the total pulse production in India. It is the third most important food legume of the world grown in 11.5 m ha with 10.91 million ton production ([Http://apps.fao.org](http://apps.fao.org)). Two market classes of chickpea, namely *desi*, and *kabuli*, are currently grown in these areas. *kabuli* chickpea varieties fetch premium price in market. However, *kabuli* type chickpea varieties are relatively more sensitive to abiotic stress than *Desi* ones. Modern agriculture is affected by abiotic stresses such as water (deficit & flooding), temperature, light, salt salinity and nutrient. Water deficit stress is one of the major abiotic stresses which adversely affects crop growth and yield. Chickpea is traditionally cultivated in marginal and rainfed areas (Rao *et al.*, 2002). Therefore, the major constraint in chickpea production is water deficit and that causes 40-50 % reduction in yield globally (Ahmad *et al.*, 2005).

The adverse effects of water deficit stress on plant growth and development may be due to ionic imbalance, alteration in different metabolic activities of plants, inhibition of various enzymatic activities, disturbances in solute accumulation, photosynthetic parameters, membrane stability index, leaf water potential, activities of the enzymes nitrate reductase and carbonic anhydrase, chlorophyll and relative water contents with a concomitant increase in proline content and the activities of antioxidant enzymes (CAT, POX and SOD) (Blum , 2011).

In chickpea, crop yield enhancement through conventional breeding is difficult because of a narrow genetic base (Udupa *et al.*, 1999). An alternative approaches that attract more attention in this direction is the application of some external plant growth regulating molecules which can be used to achieve the stress tolerance in crops without compromising yield under field conditions. Investigations have shown that natural plant growth regulators take part in the physiological response of plants subjected to water deficit stress (Sairam *et al.*, 1981, Gilley and Fletcher 1997).

Triazole compounds have plant growth regulating features and are called as plant multiprotectants, because of their innate ability to induce abiotic tolerance by increasing antioxidant enzymes and molecules in oxidative stressed plants. In addition, they can protect plants against various stresses, including drought, low and high temperatures, UV-B radiation, air pollutants, fungal pathogens, and flooding. Paclobutrazol (PBZ), which was discovered in 1976, is one of the members of triazole family having growth regulating (retarding) property. Paclobutrazol, is one of the most potent plant growth retardants that has been shown to suppress shoot growth (plant height, internode elongation and leaf area) effectively. This compound inhibits gibberellin synthesis by blocking the conversion of ent-kaurene to ent-kaurenoic acid (Dalziel and Lawrence, 1984). Besides the morphological effects, paclobutrazol is also reported to cause several physiological alterations that are generally correlated with optimized yield formation in various crop plants. The most prominent changes include: retardation of senescence with enhanced concentrations of chlorophyll, protein and mineral elements in the plant tissues, alteration of hormonal balance, improved nutrient uptake from soil, increased resistance to environmental stress, enhanced carbohydrate synthesis, promoted flowering and fruit set and stimulated translocation of assimilates towards seeds (Davis *et al.*, 1986,1988). The broad spectrum plant growth regulating properties of triazoles have been reported to mediate through alteration of balance of plant hormones i.e., GA, ABA, cytokinin and ethylene (Jaleel *et al.*, 2008).

#### **2.1.1. MODE OF ACTION**

Paclobutrazol interferes with gibberellins biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P 450-dependent oxygenase (Zhu *et al.*, 2004; Rady and Gaballah, 2012). Therefore, paclobutrazol is mainly used as growth retardants and stress protectants (Gopi *et al.*, 2009). In addition, it tends to be much more effective than many other plant growth regulators at relatively low rate of applications (Rady and Gaballah, 2012). In addition to interfering with gibberellin production, paclobutrazol is known to affect the synthesis of the hormone abscisic acid. Abscisic acid is also synthesized via the terpenoid pathway (Fig. 1). Unlike the inhibiting effect on gibberellin synthesis, treatment with paclobutrazol promotes the production of abscisic acid much like it promotes the production of phytol. When gibberellins synthesis is

inhibited, more precursors in the terpenoid pathway accumulate and are shunted to the production of abscisic acid.

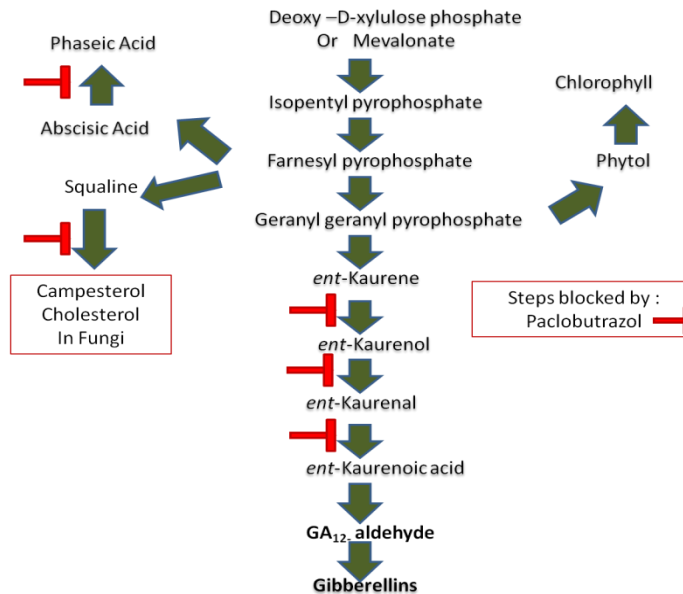


**Figure 1:** Terpenoid pathway for biosynthesis of gibberellins, abscisic acid, phytol, and steroids, and path for degradation of abscisic acid. Steps blocked by paclobutrazol indicated with 

Paclobutrazol also interferes with the normal breakdown of abscisic acid. The combined effect on both the production and breakdown processes results in enhanced concentrations of abscisic acid in leaves. One of the functions of abscisic acid is to cause stomates to close, reducing water loss from leaves through transpiration.

Improved water relations in plants could arise from a combination of increased abscisic acid contents that physiologically reduce stomatal opening, reduced shoot growth resulting in less leaf and stem surface area for transpiration, more fine roots to absorb water, and structural changes in leaves that provide physical barriers to moisture loss. The improvement of water relations in paclobutrazol treated plants is an important secondary benefit of using PBZ.

Chickpea, being a leguminous crop, is sensitive to water stress and so far, no information about the response of chickpea to paclobutrazol under water deficit stress condition is available. Therefore, the present study was conducted to investigate the protective role of paclobutrazol in water stress amelioration in chickpea.

## **2.2. WATER DEFICIT STRESS CAUSED DAMAGE AND PROTECTIVE ROLE OF PACLOBUTRAZOL**

### **2.2.1. Physiological Responses of Plants to Paclobutrazol under Water Deficit Stress Condition (Research Paper I)**

#### **2.2.1.1. GROWTH**

Water stress influences plant growth at various levels (Blumwald *et al.*, 2004). The quantity and quality of plant growth depend on cell-division enlargement, and differentiation, and all of these events are affected by water stress (Correia *et al.*, 2001). Cell division and cell growth are the two primary processes involved in plant growth. In general, cell division is considered to be less sensitive to drought when compared with cell enlargement or growth. However, both cell expansion and cell division can be influenced by relatively mild drought stress, even before photosynthesis or respiration is affected. Maintenance of cell turgor plays an important role in cell growth. Leaf area expansion is often limited under drought stress, such that the expansion and development of the transpiration surface is drastically decreased. Leaf expansion is among the most sensitive growth processes to drought (Alves and Setter, 2004). This sensitivity is expressed in terms of smaller cells and reductions in the number of cells produced by leaf meristems (Randall and Sinclair, 1988; Tardieu *et al.*, 2000). Alves and Setter (2004) showed that reduction in both cell expansion and production of cells contributed to a loss in leaf area depending on the developmental stage at which the leaf was stressed. In leaves that were no longer engaged in cell division, diminished cell expansion affected leaf area by reducing mature cell size, whereas, in younger leaves, inhibition of cell division resulted in fewer cells per leaf (Alves and Setter, 2004). Drought stress can also influence total leaf area through its effect on initiation of new leaves, which is decreased under drought stress. Continued drought stress can accelerate leaf senescence (de Souza *et al.*, 1997) and lead to death of leaf tissue, resulting in leaf drop, particularly old and mature leaves. Triazoles have been

reported to inhibit the plant growth, decrease internodal elongation, thicker leaf tissues and increased root to shoot ratio (Jaleel *et al.*, 2007-09, Rajalekshmi, 2009). Paclobutrazol has been reported to reduce plant growth, height, internodal distance with thicker and darker leaves in pepper and tomato (Rahman *et al.*, 1989). Reduced height is a consequence of triazole induced GA inhibition exemplified by reduced internodal elongation (Fletcher, 2000). Paclobutrazol has been reported to suppress elongation growth in plants by influencing cell division and cell enlargement processes due to its antigibberellin activity (Grossman *et al.*, 1988). Its treated plants were found shorter; more branched and produce more seeds in rape (Zhou *et al.*, 1993) and chinese potato (Kishorekumar, 2006). In addition to this, Paclobutrazol treatment in Chinese potato alters the thickness of leaf (by elongating palisade cells), upper and lower epidermis, number of palisade and spongy cells per unit area, number of chloroplast per palisade, number of stomata in upper and lower epidermis, stomatal pore length, width and also anatomical characteristics (Kumar *et al.*, 2006) and induces a suit of morphological adaptations (i.e. Increased root to shoot ratio, stomatal density, decrease leaf area reduction, fine roots etc) that might allow the *Phillyrea angustifolia* to tolerate drought after transplanting (Fernandez *et al.*, 2006). Its application also reduces light compensation point of plant growth under 550  $\mu\text{mole/m}^2/\text{s}$  light intensity in *Pachira aquatica* (Li *et al.*, 2009). PBZ application improved the growth potential of *Catharanthus roseus* plants grown under saline conditions (Jaleel *et al.*, 2007). Internal carbon dioxide concentration and leaf thickness was found to be high in PBZ treated *Catharanthus roseus* (Jaleel *et al.*, 2007). In oilseed brassicas, paclobutrazol was reported to reduce plant height, increased resistance to lodging, and enhanced branching and improved yield contributing characters with a consequent enhancement in seed yield (Setia *et al.*, 1996). Meanwhile, the reduction of growth and dry weights in cucumber plants treated with paclobutrazol might be attributed to decrease of IAA and GA<sub>3</sub>, revealed by previous study on triazoles (Wang and Chen, 1997). PBZ was also reported to increase root growth in American elm seedling (Watson *et al.*, 2001).

#### **2.2.1.2. WATER RELATION**

As soil dries under drought stress, hydraulic conductivity of soil decreases, and the rate of water movement toward root and absorption are slow to completely replace the water lost from the plant during the daytime because of transpiration. Thus, drought results in

lower plant water potential. When leaf water potential is low, it causes the stomata to close, which causes decreased transpiration which in turn leads to increased water potentials. However, if drought persists, the water potential will continue to decrease and reach a zero turgor. Paclobutrazol was reported to increase dry matter production and also hasten the relative growth rate of the silique wall, and seed in brassica (Setia *et al.*, 1996). PBZ application increased leaf water potential of drought stressed American elm seedlings (Watson *et al.*, 2001), reduced the transpiration rate of tomato seedlings (Still *et al.*, 2004).

Triazole treatments decreased stomatal conductance in *Amorphophallus* plants. Triazole treatments increased WUE in *Amorphophallus* plants. PBZ increased the WUE in *Pseudotsuga menziesii* and *Pinus cornata* seedlings (Vanden *et al.*, 1996). Triazole induced partial closure of stomata and increased inter cellular CO<sub>2</sub> concentration and it may be the reason for increased water use efficiency (WUE) in treated plants. The increase in moisture content can be attributed to the ability of triazoles to partially close the stomata by increasing ABA content and consequently reducing transpiration and water loss from plant leaves (Sreethar, 1991). The reduction in water consumption after PBZ treatment has been attributed to reduced leaf area (Asamoah and Atkinson, 1985; Atkinson and Crisp, 1983) and to changes in plant architecture (Wample and Culver, 1983). In addition, other authors distinguished an antitranspiration effect of PBZ, which had a clear influence on water relations and related physiological and biochemical changes (Asare-Boamah *et al.*, 1986; George and Nissen, 1992).

#### **2.2.1.3. RELATIVE WATER CONTENT**

Relative water content (RWC) of leaves is an important character which is directly related to soil water content (Sarker *et al.*, 1999). RWC has been reported as an important indicator of water stress in leaves (Merah, 2001). Stress exposed plants immediately lower down RWC of their leaves (Grover *et al.*, 2004). Sinclair and Ludlow, 1985 proposed that leaf RWC is a better indicator of water status than water potential.

Paclobutrazol has been found to accelerate stomatal response, enhanced water retention and increased survival under drought in jack pine (Mashall *et al.*, 1990), oak (Percival *et al.*, 2007). PBZ reduced transpiration rates, increased relative water content and significantly increase yield in crop plants under moisture stress (Sairam *et al.*, 1995; Gadi *et*

*al.*, 2001; Aly *et al.*, 2011). Reduced transpiration rate was reported to increase the moisture content in the *Amorphophallus campanulatus* plants (Manivannan *et al.*, 2007). Paclobutrazol application significantly reduced water consumption compared with control in *Arbutus unedo* (Navarro *et al.*, 2007). The lower water consumption in paclobutrazol treated plants was the result of combination of mechanism which reduced transpiration loss (Navarro *et al.*, 2007) and increased resistance (Asare –Biamah *et al.*, 1986). Paclobutrazol treated plants show higher value of relative water content in *Curcuma alismatifolia* Gagnep (Jungklang *et al.*, 2012), triticale (Berova and Zlatev, 2003) and cucumber seedling (Baninasab *et al.*, 2011).

#### **2.2.1.4. ANATOMICAL CHARACTERISTICS:**

Tissues exposed to environment with low water availability have generally shown reduction in cell size, increase in vascular tissue, 3-4 layers of spongy parenchyma cells with large intercellular cavities and thickening of palisade cells and cell wall thickness (Pitman *et al.*, 1983; Gauerfel *et al.*, 2009 and Makbul *et al.*, 2011). Water stressed leaves have smaller stomata than well watered leaves (Larcher, 1995). The vascular tissue area was decreased by low moisture (Ristic and Cass, 1991). Ennajeh *et al.*, 2010 reported the increase in stomatal density and thickness of upper and lower epidermis in water stressed leaves of olive.

PBZ increased the thickness of epicuticular wax layer in rose cultivars (Jenks *et al.*, 2001) and maize (Sopher *et al.*, 1999). No. of cells in palisade mesophyll per unit area also increased to a larger extent in PBZ treated *Catharanthus roseus* (Jaleel *et al.*, 2007). Induction of additional layers of palisade parenchyma was observed with PBZ treatment in *chrysanthemum* (Burrows *et al.*, 1992). PBZ increased the palisade cell length and width in potato plants (Tekalign *et al.*, 2005). Potato plants treated with PBZ exhibited dense and spongy mesophyll tissue (Tekalign *et al.*, 2005). Increased diameter and length of fibrous roots, enhanced lateral root formation were observed in PBZ treated *Catharanthus roseus* (Jaleel *et al.*, 2007). PBZ treatments reduced the diameter of xylem vessels in *Catharanthus roseus*, however phloem sieve tubes had an increased diameter as compared to control in PBZ treated *Catharanthus roseus* plants (Jaleel *et al.*, 2007). PBZ reduced the proportion of xylem and increased that of phloem and cortex and increased xylem density in maize seedlings (Sopher *et al.*, 1999). It decreases cell no. and length, so PBZ treated plants

characteristically have smaller leaf (Fletcher, 2000). Increased root length in PBZ treated plants is associated with larger parenchyma cells and promotion of radial cell expansion (Fletcher, 2000). Paclobutrazol stimulated radial elongation of cells and it increased silique diameter in brassica (Setia *et al.*, 1996), increased radial elongation of peach root parenchyma cells (Williamson *et al.*, 1986), increased the leaf thickness in rape plant due to elongated palisade cells (Zhou *et al.*, 1993) and wheat leaves (Sopher *et al.*, 1999)

#### **2.2.1.5. CHLOROPLAST ULTRA STRUCTURE:**

The major damage to the chloroplast caused by water stress consists of structural changes resulting from excessive swelling, distribution of thylakoids and the appearance of lipid droplets (Ristic and Cass, 1991). Alterations in chloroplast ultra structure caused by water stress share many common features with development of senescence manifested as appearance of large plastoglobuli, thylakoid swelling and dilation (Kolodziejek *et al.*, 2003). In well watered plants, the plastoglobuli were small and strongly osmophilic, but with increasing water stress they become large and less osmophilic. With increasing water stress, the number of large crystals similar to crystals of Rubisco decreased and ultimately was absent in severe stressed plants (Vappavoovi *et al.*, 1984).

#### **2.2.1.6. PHOTOSYNTHETIC PIGMENTS**

Even a short-term water deficiency changes the total content of chlorophylls (Sestak *et al.*, 1986) contained within protein–pigment complexes of thylakoid membranes (which are the first structures to be damaged under stress conditions (Synkova., 2000)). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species ( Smirnoff, 1995). Drought stress produced changes in the ratio of chlorophyll ‘a’ and ‘b’ and carotenoids (Anjum *et al.*, 2003; Farooq *et al.*, 2009). A reduction in chlorophyll content was reported in drought stressed cotton (Massacci *et al.*, 2008), Horse chestnut and *Catharanthus roseus* (Jaleel *et al.*, 2008). The chlorophyll content decreased to a significant level at higher water deficits in sunflower plants (Kiani *et al.*, 2008) and *Vaccinium myrtillus* (Tahkokorpi *et al.*, 2007).

PBZ application has been reported to increase level of photosynthetic pigments in oak (Percival *et al.*, 2007), *Setaria italica* (Bisht *et al.*, 2007), *Amorphophallus campanulatus* (Gopi *et al.*, 2009) and cucumber seedling (Baninasab *et al.*, 2011). Similarly other triazoles - propiconazole and hexaconazole were also found to enhance level of

photosynthetic pigments in *Plecaranthus sp.* (Rajalekshmi *et al.*, 2009) and triadimefon & hexaconazole in tapioca (Gomathinayagam *et al.*, 2008). Triazoles application has also been found to improve chlorophyll fluorescence, total foliar chlorophyll, carotenoids concentrations in horse chestnut (Percival, 2008). The increase in chlorophyll content with triazole treatment is attributed to the ability of triazoles to increase the cytokinin level and thereby the stimulation of chlorophyll biosynthesis (Fletcher *et al.*, 2000). Paclobutrazol enhanced chlorophyll, carotenoid and soluble protein in *Triticum aestivum* (Nouriyani *et al.*, 2012) and photosynthetic pigment per unit leaf area in *Zea mays* (Khalil *et al.*, 1995). Its application also enhanced the content of photosynthetic pigment under water stress in *Festuca arundinacea* and *Lolium perenne* (Shahrokhi *et al.*, 2011). Paclobutrazol significantly increased chlorophyll a, b and carotenoids in wheat cultivars (Aly *et al.*, 2011). Paclobutrazol increased carotenoid content in *Catharanthus* (Jaleel, 2006) *Raphanus sativa* (Sankari *et al.*, 2006), *Sesamum indicum* (Abraham *et al.*, 2008) and *Amorphophallus campanulatus* (Gopi *et al.*, 2009). Paclobutrazol induced a suit of physiological adaptation to tolerate drought more especially, increased total leaf content of carotenoids (Lutein, betacarotene, alpha carotene, neoxanthin) and xanthophylls (Antheraxanthin, zeaxanthin, violaxanthin) and chlorophylls in Evergreen oak (Percival *et al.*, 2007). In addition paclobutrazol strengthen leaf membrane integrity, increased leaf photosynthetic efficiency and light induced carbon dioxide fixation before at the termination of drought treatment. PBZ showed increased chlorophyll and carotenoids which helped in resistance of black spruce to high light and thermal stress (Mahoney *et al.*, 1998). Paclobutrazol enhanced the total chlorophyll content of the silique wall and seed in brassica (Setia *et al.*, 1996). Paclobutrazol treated plants had more total chlorophyll, decreased Chl a:b ratio and enhanced Hill's reaction activity of chloroplasts, which are indicative of increased photosynthetic and metabolic activities (Fedtke *et al.*, 1973, 1974).

#### **2.2.1.7. PHOTOSYNTHESIS:**

Drought stress is known to inhibit photosynthetic activity in tissues due to an imbalance between light capture and its utilization (Foyer and Noctor, 2000). Stomatal limitation was generally accepted to be the main determinant of reduced photosynthesis under drought stress (Cornic, 2000). This has been attributed to decreases in both photosynthetic rate and internal CO<sub>2</sub> concentration, which finally inhibits total

photosynthetic metabolism. Drought stress inhibits photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe *et al.*, 1998). Both stomatal and non-stomatal limitation was generally accepted to be the main determinant of reduced photosynthesis under drought stress (Farooq *et al.*, 2009). Paclobutrazol enhanced net photosynthesis rate in triticale (Berova and Zlatev, 2003).

Paclobutrazol was also reported to increase photosynthetic rate, prolonged leaf longevity and increased green pod area in mustard (Zhou *et al.*, 1993). Paclobutrazol enhanced the total chlorophyll, decreased chl a/b ratio and hill activity of chloroplast in Brassicca (Setia *et al.*, 1996) and enhanced net photosynthetic rate, transpiration rate, stomatal resistance in *Setaria italica*, grown under field condition (Bisht *et al.*, 2007). In Horse chestnut, chlorophyll fluorescence (Fv/Fm), photosynthetic rate were higher in trees treated with paclobutrazol (Percival *et al.*, 2008). PBZ reduced the chilling induced photoinhibition by protecting PS II in water melon seedlings (Baninasab *et al.*, 2009). The higher chlorophyll content in the leaves, leading to higher photosynthesis might have increased total dry weight in the triazole treated carrot as reported in the triadimefon treated cucumber seedlings (Feng *et al.*, 2003). Maintenance of relatively high fluorescence ratio in PBZ treated plants under low temperature stress had been observed (Pinhero and Fletcher, 1994). Paclobutrazol increased activity of RUBP-carboxylase, and photosynthetic rates in peanut (Yan and Pan, 1992). PBZ treatment significantly increased the Fv/Fm ratio in cucumber leaves (Baninasab *et al.*, 2011). The increased intercellular CO<sub>2</sub> concentration and stomatal conductance may be the reasons for the increased Photosynthesis in triazole treated *Amorphophallus* plants (Manivannan *et al.*, 2007; Gopi *et al.*, 2009). Triazole treatment also increased photosynthesis in *Withania somnifera* seedlings (Jaleel *et al.*, 2008).

#### **2.2.1.8. MEMBRANE STABILITY INDEX (MSI)**

Agarie *et al.*, 1995 used the cell membrane stability as a drought tolerance test. It was observed that electrolyte leakage showed an increase with increasing water deficit. Paclobutrazol had protective effect on membrane damage in *Mangifera indica* under saline stress conditions (Srivastava *et al.*, 2010). PBZ decreased electrolyte leakage in carrot (Gopi *et al.*, 2007). The inhibition of electrolyte leakage is correlated with the proved ability

of triazoles in maintaining the membrane integrity (Fletcher *et al.*, 2000) of plants. Triazole altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane (Burden *et al.*, 1987). This change in sterol composition may induce changes in cell membrane that may be reflected in increased membrane stability, acclimatization and frost hardiness as observed in white spruce (Sailevora *et al.*, 1997). PBZ reduced electrolyte leakage in wheat seedlings (Krans *et al.*, 1995; Aly *et al.*, 2011) and Horse chestnut (Percival *et al.*, 2008) during drought stress. Seedlings of winter rape and tomato treated with uniconazole and PBZ, respectively, had much less electrolyte leakage than the control seedlings following high-temperature stress (Zhou and Leul, 1999; Still and Pill, 2004).

#### **2.2.1.9. GRAIN YIELD AND DRY MATTER PARTITIONING:**

Drought mainly influences yield by limiting seed numbers by either influencing the amount of dry matter produced by the time of flowering or by directly influencing pollen or ovule function, which leads to decreased seed-set. Secondly, drought influences seed filling mainly by limiting the assimilate supply, leading to smaller seed size and lower yields.

Application of triazoles was also reported to enhance grain yield (Fletcher *et al.*, 2000). PBZ has also been reported to enhance yield in mustard (Setia *et al.*, 1996) and barley (Rady *et al.*, 2012). In tomato, plant treated with paclobutrazol beared significantly higher number of fruit per plant, however, it could not increase the fruit yield as compared to control (Rahman and Khan, 1989). However, Mohamed *et al.*, 2011 reported the significant enhancement in fruit yield and water use efficiency of tomato. Paclobutrazol was reported to enhance the yield of field grown *Camelina sativa* by enhancing the number of siliques per plant, photosynthetic gas exchange, higher chlorophyll content, photosynthetic activity for a longer period and so facilitated higher photo assimilation (Kumar *et al.*, 2012). However, there was no effect of paclobutrazol on fruit yield of pepper (Rahman and Khan, 1989). Content of root alkaloid ajmaline got increased significantly under PBZ treatment in *Catharanthus roseus* (Jaleel *et al.*, 2007). It enhanced no. of seeds per pod and seed weight in *Brassica napus* (Zhou *et al.*, 1993). The no. and mass of flower buds in PBZ treated tomato plants essentially increased (Berova *et al.*, 2000). Application of PBZ on plants increased both early fruit yield and index of economic earliness in *Lycopersicon esculentum* (Berova *et al.*, 2000) increased the ability of partitioning of assimilates to tuberous organs as

observed in potato and *Gladiolus* (Steinitz et al., 1991). PBZ increased starch content in carrot (Gopi et al., 2007). This stimulated root growth and starch content of the tuber may be related to the increased partitioning of assimilates towards the root than shoot as observed by (Wang et al., 1985). Paclobutrazol has been reported to improve shoot and root weight, fresh and dry weight in cucumber seedling subjected to high temperature stress (Baninasab et al., 2011). Paclobutrazol induced precocious flowering in *Populus deltoids* (Yuceer et al., 2002). It also enhanced the number of mature seeds per silique, increased seed size in brassica, increased dry matter of silique wall and seed were observed (Setia et al., 1996). Paclobutrazol was reported to alter sink strength within the plant resulting in redistribution of assimilates to meristematic regions, other than the shoot apices, and also improved the flow of assimilates towards reproductive structures (Setia et al., 1996). Treatment of paclobutrazol caused an overall increase in dry matter per plant and partitioning coefficient (percent ratio of silique dry weight to plant dry matter) in *B. juncea* and *B. carinata* (Setia et al., 1995). Effect of PBZ on seed yield improvement (including oil yield) might be linked to improved CO<sub>2</sub> assimilation physiology, enhanced sink activity, better partitioning coefficient, quantitative enhancement of yield determining growth traits, altered phenology, better plant canopy and rooting in *Camelina sativa* (Kumar et al., 2012). PBZ increased fruit set and increased no. of berries per cluster in grapes (Assem, 1986). The increase in yield was a result of higher number of clusters and of greater mean weight (Christov et al., 1995). Its role as anti-gibberellin is considered sufficient for the induction of higher yield and for improvement of the quality in cucurbits and wheat (Hedden and Graebe, 1985; Reynolds and Wardle, 1990).

## **2.2.2. Biochemical Responses of Plants to Paclobutrazol under Water Deficit Stress Condition (Research Paper II)**

### **2.2.2.1. GROWTH HORMONES**

Plants respond to water deficit and adapt to drought stress through various physiological and biochemical changes (Monneveux and Belhassen 1996; Emam and Seghatoeslami, 2005). These mechanisms include changes in endogenous phytohormones levels such as abscisic acid (ABA), Gibberellin<sub>3</sub> (GA<sub>3</sub>), Indole acetic acid (IAA), zeatin (ZT), or cytokinin (CKs) (Wang et al., 2008). Abscisic acid is defined as a stress

phytohormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that help plant survival over the stresses (Zhang *et al.*, 2006). The accumulation of ABA due to water stress has been reported in plants such as rice (Yang *et al.*, 2001), cucumber (Pustovoitova *et al.*, 2004) and bean (Domash *et al.*, 2006). Absciscic acid plays a critical role in regulating corn water status through guard cells and growth (Luan, 2002; Zhu, 2002). The ABA that is produced under stress conditions in dehydrated roots in drying soil is transported to the xylem and regulates stomatal opening and leaf growth in the shoots (Zhang *et al.*, 2006).

An increase in ABA content in response to water-deficit stress may arise from an increase in ABA biosynthesis and/ or a decrease in ABA breakdown (Cutler and Krochko, 1999; Zeevaart, 1999). In *Arabidopsis thaliana* seedlings, Huang *et al.* (2008) showed that drought enhanced both ABA biosynthesis and catabolism, resulting in an increase in ABA and catabolites. Likewise, drought-treated plants of *Laurus azorica* showed an increase in leaf ABA concentrations respect to that of the control (Sánchez-Díaz *et al.*, 2008). On the other hand, exogenous application of ABA enhances the tolerance of plants or plant cells to drought (Lu *et al.*, 2009). In relation to endogenous ABA, different reports showed that drought tolerant cultivars have more ABA than susceptible ones (Perales *et al.*, 2005; Veselov *et al.*, 2008; Thameur *et al.*, 2011). The increase in ABA endogenous level under drought induces the stomatal closure. This fact constitutes one of the first external symptoms of water deficit, and is recorded as the increase of stomatal resistance or the decrease of its inverse (stomatal conductance). Stomatal closure take place to minimize the water loss by transpiration, and ABA plays a fundamental role in this process. Thus, stomatal resistance is used as a reference to compare the intensity of water deficit in different species and growth conditions (Medrano *et al.*, 2002). Guard cells continuously sense information from the surrounding environment, biotic and abiotic, as well as long distant signals coming from the roots. Stomatal closing under drought is a response to increasing levels of endogenous ABA synthesized in the roots as a result of water deprivation in the soil (Kim *et al.*, 2010).

The change in IAA level under drought stress is very inconsistent. Drought stress decreased IAA concentration in the leaves of wheat (Xie *et al.*, 2003; Bano and Yasmeen, 2010) or corn (Wang *et al.*, 2008). However, some studies have indicated that the adaptation to drought could enhance the IAA concentration in squash (Sakurai *et al.*, 1985)

and cucumber (Pustovoitova *et al.*, 2004). A very moderate drought stress increased the IAA concentration; however, as stress intensified the IAA concentration was decreased. This decrease may have been due to the reduction in the IAA production or increase in its degradation (Davenport *et al.*, 1980). Decrease in the IAA concentration might have also been as a result of increase in the ABA concentration (Bano and Yasmeen, 2010). Also, understanding about changes in cytokinin and gibberellins concentrations in plants under drought stress is rare (Yang *et al.*, 2001; Xie *et al.*, 2003). Bano and Yasmeen (2010) reported that drought stress resulted in maximum decrease in GA concentration in wheat leaves. The ZT and GA<sub>3</sub> concentrations rapidly decreased in corn leaves subjected to drought stress conditions (Wang *et al.*, 2008). Drought stress decreased GA<sub>3</sub>, and this reduction was dependent on drought severity. Drought stress decreased the GA<sub>3</sub> concentration; the magnitude of decrease was dependent on the stress severity. The decrease in the levels of this phytohormone may be due to decreased GA<sub>3</sub> synthesis (Xie *et al.*, 2004) or increase in the destruction of GA<sub>3</sub> by enhancing the activity of oxidase (Davenport *et al.*, 1980). Also, this decrease might be as a result of increase in the ABA concentration, as Bano and Yasmeen (2010) with application of ABA observed that the GA<sub>3</sub> concentration of wheat spikes was decreased. Many researchers have reported that drought stress decreased GA<sub>3</sub> concentration in leaves (Xie *et al.*, 2003; Wang *et al.*, 2008; Bano and Yasmeen, 2010).

Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Fletcher *et al.*, 2000). Paclobutrazol has been reported to inhibit gibberellin biosynthesis in plant by inhibiting activity of ent-kaurene oxidase and cytochrome P450 oxidase, thus blocking the oxidation of ent-kaurene to ent-kaurenoic acid (Daziel and Lawrence., 1984). Its treatment has also been reported in apple and wheat to reduce endogenous abscisic acid level by about one third and prevent the marked accumulation of water stress induced abscisic acid that occur in untreated seedling (Wang *et al.*, 1987, Buta and Spaulding, 1991). It has also been shown that paclobutrazol stimulate accumulation of ABA in the leaves (Asare-Boamah *et al.*, 1986). Fletcher *et al.*, 2000 proposed that triazoles stimulate cytokinin synthesis and that enhances chloroplast differentiation, chlorophyll biosynthesis and prevent chlorophyll degradation. Further, Paclobutrazol was reported to significantly enhance zeatin and zeatin riboside (Zhu *et al.*, 2004). In wheat, Paclobutrazol altered the endogenous level of phytohormones i.e. level of GA<sub>3</sub>, IAA was decreased and

ABA was increased (Aly *et al.*, 2011). Increased root growth of triazoles is also associated with increased level of endogenous cytokinin (Fletcher and Arnold., 1986). PBZ considerably delayed the onset of senescence in grape vines and treated plants retained photosynthetically active leaves longer period (Hunter *et al.*, 1994). PBZ delayed senescence and extended period of 'stay-green' in Camelina (Kumar *et al.*, 2012). An increased levels of cytokinins and polyamines over the senescence promoting hormones ABA and ethylene has been reported in plants treated with PBZ. PBZ delayed the onset of senescence in Camelina leaves by enhanced endogenous levels of cytokinins promoted chlorophyll formation, increased activity of certain antioxidant enzymes and retard senescence. A longer 'staygreen' condition might have concurrently enhanced the duration of leaf photosynthesis in PBZ treated plants by keeping the leaves photosynthetically active for a longer time which in turn might have contributed to better plant productivity in Camelina (Kumar *et al.*, 2012).

#### **2.2.2.2. ANTIOXIDANTS**

Down regulation of photosystem II (PSII) activity results in an imbalance between the generation and utilization of electrons, apparently resulting in changes in quantum yield. These changes in the photochemistry of chloroplasts in the leaves of drought-stressed plants result in the dissipation of excess light energy in the PSII core and antenna, thus generating active oxygen species ( $O_2^-$ ,  $^1O_2$ ,  $H_2O_2$ , OH), which are potentially dangerous under drought stress conditions (Peltzer *et al.*, 2002). . The sequence of events in the plant tissue subjected to drought stress are: (1) increased production of ROS and of oxidized target molecules; (2) increases in the expression of genes for antioxidant functions; (3) increases in the levels of antioxidative systems and antioxidants; and (4) increased scavenging capacity for ROS, resulting in tolerance against the drought stress (Mano, 2002). Mechanisms of ROS detoxification exist in all plants and can be categorized as enzymatic superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), and non-enzymatic antioxidant molecules like ascorbic acid (ASA),  $\alpha$ -tocopherol and reduced glutathione (GSH) (Prochazkova *et al.*, 2001).

#### 2.2.2.2.1. ENZYMATIC ANTIOXIDANTS

Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX). SOD catalyses the dismutation of superoxide anion radicals ( $O^{2-}$ ) with great efficiency resulting in the production of  $H_2O_2$  and  $O_2$  (Smironoff, 1993; Winston, 1990) which improves the scavenging systems of cell and reduces the accumulation of free radicals. Catalase enzyme is an important antioxidant system that catabolises hydrogen peroxide, a precursor of reactive oxidants (Larson et al., 1988) and reacts with  $H_2O_2$  directly to form water and oxygen (Smironoff, 1993; Winston, 1990). POX is one of the major systems for the enzymatic removal of  $H_2O_2$  in plants. The increased activities of POX in plants suggest the protective role of the enzyme stress.

PBZ application has been reported to significantly enhance antioxidant enzymes (SOS, CAT, POX, APX) activity in various plant species e.g. oak (Percival, 2007), *Arachis hypogea* (Sankar et al., 2007), *Daucus carota* (Gopi et al., 2007), *Sesamum indicum* (Somasundaram et al., 2009), horse chestnut (Percival, et al., 2008), *Festuca arundinacea* and *Lolium perenne* (Maha shahrokhi et al., 2011), tomato (Mohamed et al., 2011) . Similarly other triazoles like hexaconazole and triadimefon application were noted to enhance antioxidant enzymes activity in *Manihot esculenta* (Gomathinayagam, 2009), and wheat (Sairam et al., 1989; 1991; 1995; Aly and Latif, 2011). Hexaconazole and triadimefon have also been reported as enhancer for non-enzymatic antioxidant potentials of *Manihot esculenta* (Gomathinayagam et al., 2008). It was reported to protect wheat leaves from damage caused by free radical generated in wheat (Gilley et al., 1997). Paclobutrazol application induces the level of enzymatic antioxidants like SOD, CAT, APX and non enzymatic antioxidants like ascorbic acid, reduced glutathione,  $\alpha$ -tocopherol in *Dioscorea rotundata* (Jaleel et al., 2007 ). It minimizes the negative effects of salinity with evidence of less membrane damage by upregulating the endogenous production of antioxidant enzymes like SOD, CAT, and POX (Srivastava et al., 2010). By means of increased antioxidant activities, PBZ protect the *Vigna unguiculata* plants from salt stress (Manivannan et al., 2008). The APX activity increased in the triazole treated carrot plants when compared to the control (Gopi et al., 2007). An increase in APX activity has also been reported in all parts of *C. roseus* plants with triadimefon treatment (Jaleel et al., 2006). The reduction of lipid

peroxidation in paclobutrazol-exposed Patumma plants treated with paclobutrazol that increased the antioxidant potential system (Jungklang *et al.*, 2003).

#### **2.2.2.2.2. NON ENZYMATIC ANTIOXIDANTS**

The non-enzymatic antioxidant contents play major roles in maintaining the balance between free-radicals production and elimination (Jaleel *et al.*, 2007).  $\alpha$ -tocopherol and ascorbic acid are two important non-enzymatic antioxidants in higher plants (Foyer *et al.*, 1993). They are concentrated in the chloroplasts and the cytosol and protect the photosynthetic apparatus under stress by scavenging excess reactive oxygen species (ROS) (Smirnoff *et al.*, 1995). Ascorbic acid has the capacity to eliminate different ROS including singlet oxygen, superoxide and hydroxyl radicals (Foyer *et al.*, 1993).  $\alpha$ -tocopherol is synthesized in the chloroplasts and closely associated with the thylakoid membrane of the chloroplasts. The thylakoid membrane, which contains substantial unsaturated lipids, is one of the major sites of oxidative damage through lipid peroxidation. This may result in an increase of  $\alpha$ -tocopherol which quenches oxygen radicals within the membrane and terminates chain reaction that cause oxidative damage. Increased  $\alpha$ -tocopherol and ascorbic acid content is observed in *Vigna unguiculata* plants treated with propiconazole (a triazole compound) under water deficit condition (Manivannan *et al.*, 2007).

An increase in ascorbic acid and reduced glutathione content due to PBZ treatment is the basis of the stress protection ability of PBZ (Fletcher *et al.* 2000). Reduced glutathione (GSH) plays an important role in the response of plants to environmental stresses, including oxidative stress, due to the generation of active oxygen species (Szalai *et al.*, 2009). Increased ascorbic acid and reduced glutathione and  $\alpha$ -tocopherol contents were reported due to PBZ application in lemon (Jain *et al.*, 2002) and *Dioscorea rotundata* (Jaleel *et al.*, 2007). Both PBZ and Hexaconazole treatment significantly increased the GSH content to an appreciable level in carrot (Gopi *et al.*, 2007). Higher GSH content was reported under triadimefon treatment in *C. roseus* (Jaleel *et al.*, 2006).

#### **2.2.2.3. TOTAL SOLUBLE PROTEINS**

The content of proteins in plant leaves decreases with the onset of water deficiency. The diminution of the protein pool was, most likely, caused by suppressed synthesis and augmented catabolism (due to increased hydrolytic activity). These changes

reached their maximum during leaf wilting (Sestak *et al.*, 1986). The key enzyme of photosynthesis ribulose 1, 5-*bis*- phosphate carboxylase-oxygenase (Rubisco; EC 4.1.1.39) is worthy of special consideration. Its amount in the leaves accounts for 50% of their protein content. Moreover, Rubisco is the most abundant protein on earth. In the leaves of barley plants that survived drought, both the content of Rubisco and the total amount of soluble proteins are decreased (Popova, 1989). PBZ treatment increased protein content of the leaves and tubers in carrot (Gopi *et al.*, 2007). The increased protein content under triazole treatment can be correlated to the increased production of cytokinins by triazoles (Davis *et al.*, 1988).

#### **2.2.2.4. TOTAL FREE AMINO ACIDS:**

Total amino acid content increased as the stress condition prolonged and it increased significantly due to ABA treatment in chickpea (Kumar *et al.*, 2001). Yadav *et al.*, 1996 and Gorbani *et al.*, 1998 also reported increased free amino acid content in chickpea cultivars under moisture stress condition. PBZ increased the amino acid content in carrot (Gopi *et al.*, 2007). Compatible solutes accumulation in the cytoplasm is considered as a mechanism to contribute stress tolerance.

#### **2.2.2.5. PROLINE**

Proline plays a pivotal role in cytoplasmic tolerance in many plant species and hence in the resistance of whole plant to severe plant stresses (Bandurska, 2000). Proline is one of the most important compatible osmolytes in drought stressed plants. Proline accumulation helps to maintain turgor and promotes continued growth under moisture stress condition. Proline content of carrot leaves and tubers increased under triazole treatment. Triazole induced a transient raise in ABA content (Davis *et al.*, 1988) and this increased ABA content due to triazole treatment could be the reason for increased proline and amino acid content of triazole treated carrot plants. Proline may act as a non-toxic osmotic solute preferentially located in the cytoplasm or as an enzyme protectant, stabilizing the structure of macromolecules and organelles. Proline as an osmoprotectant compound plays a major role in osmoregulation and osmotolerance (Jaleel *et al.*, 2007). The increased proline in Patumma plants is one mechanism for water stress amelioration. Lower amounts of proline in the treated plants compared to the control imply that paclobutrazol treatment can induce

water stress tolerance in the plants (Jungklang *et al.*, 2003; Turkan *et al.*, 2005; Aly *et al.*, 2011). Increased level of proline resulted less membrane damage of *Mangifera indica* leaves (Srivastava *et al.*, 2010), *Festuca arundinacea* and *Lolium perenne* (Shahrokhi *et al.*, 2011) and Horse chestnut (Percival *et al.*, 2008).

#### **2.2.2.6. TOTAL SOLUBLE SUGARS**

During the course of drought stresses active solute accumulation of compatible solutes such as carbohydrates is claimed to be an effective stress tolerance mechanism (McKersie and Leshem, 1994). The tolerant variety accumulated more soluble carbohydrate than the sensitive one (Mafakheri *et al.*, 2011). Paclobutrazol enhanced the levels of total soluble sugars, starch and proteins in silique wall and seed. Increased carbohydrate levels in silique wall during early stages of growth mean that there is increased energy to support seed development. Paclobutrazol was reported to stimulate carbohydrate metabolism in brasicca (Setia *et al.*, 1996). PBZ treatments significantly enhanced the accumulation of dry matter in stem when compared to control in *Camelina sativa* (Kumar *et al.*, 2012).

#### **2.2.2.7. LIPID PEROXIDATION**

Lipid peroxidation is the system most commonly ascertained to oxidative damage and hence is regarded as an indicator of stress. As one of the end products of lipid peroxidation, the malonyldialdehyde (MDA) content reflects the degree of the peroxidation of membrane lipids. Significant enhancement in the concentration of lipid peroxide was reported in drought stressed rice seedlings (Sharma and Dubey, 2005). Drought was substantially increased the malonaldehyde content in green bean genotypes (Yaser *et al.*, 2008). Decreased lipid peroxidation in PBZ treated plants is correlated with the proved ability of triazoles in maintaining the membrane integrity (Fletcher *et al.*, 2000). The increased malonyldialdehyde (MDA) with drought stress in wheat seedlings, but uniconazole reduced MDA accumulation of stressed soybean (Zhang *et al.*, 2007).

### 3. MATERIALS AND METHODS

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The study was conducted in the Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi. It is located at latitude of 28°N and longitude of 77°E, and about 250 m above mean sea level). The intention of this study was identification of the optimum dose of paclobutrazol suitable for water deficit stress condition and to analyse the physiological and biochemical alterations induced by paclobutrazol as a mechanism of water deficit stress tolerance. Therefore, for the accomplishment of task, during *Rabi* (2013-2014) two experiments were carried out. Initially, a preliminary experiment was conducted for optimising the paclobutrazol dose followed by 2<sup>nd</sup> main experiment was carried out to study the paclobutrazol induced physiological and biochemical alterations in chickpea in relation to water deficit stress tolerance. The plants were grown in pot culture inside Net House of Division of Plant Physiology, IARI, New Delhi-110012. All recommended agronomic practices were followed to raise the healthy chickpea crop. Recommended basal dose of fertilizers in the form of urea, SSP and MOP were applied.

#### 3.1. PLANT MATERIAL

For the present study, two genotypes i.e. *Kabuli* type (PUSA 1108, Water deficit stress sensitive) and *Desi* type (PUSA 362, Water deficit stress tolerant) were used.

#### 3.2. WEATHER DATA

The data of maximum, minimum temperature, mean rainfall, relative humidity, sunshine hours and evaporation during the cropping season from November 2013 to April, 2014 is illustrated Fig. 1.

#### 3.3. EXPERIMENTAL DETAILS

Previous studies reported that Paclobutrazol can be applied either foliar or drenching methods. Thus, Preliminary experiment was designed and conducted to identify optimum dose of paclobutrazol dose for foliar and drenching applications.

Water stress was imposed at vegetative stage in both *Kabuli* and *Desi* type varieties by restricting water supply until the appearance of severe symptoms of wilting. Before

imposition of water stress plants were treated exogenously with varying dose paclobutrazol (Sigma-Aldrich) for foliar (0, 30, 60, 90, 120 and 150 ppm) and drenching (0, 60, 120, 180, 240 and 300 ppm) applications. During preliminary experiment observations were recorded on photosynthetic pigments, photosynthesis, MSI, RWC. Based on the data of preliminary experiment of both foliar and drenching applications optimum dose was identified by curve fitting. Main experiment was conducted in factorial CRD using both *Kabuli* and *Desi* type varieties. Experiment had two main treatments (Well watered & Water deficit stress). Further, each main treatment was divided into four sub treatments (Water spray, Drenching with water, foliar spray with PBZ and Drenching with PBZ). Water deficit stress was imposed by withholding water supply before reproductive stage. Before imposition of water deficit stress plants were treated exogenously with identified optimum doses of paclobutrazol (PBZ). Optimum doses used for foliar spray and drenching were 60 and 120 ppm respectively.

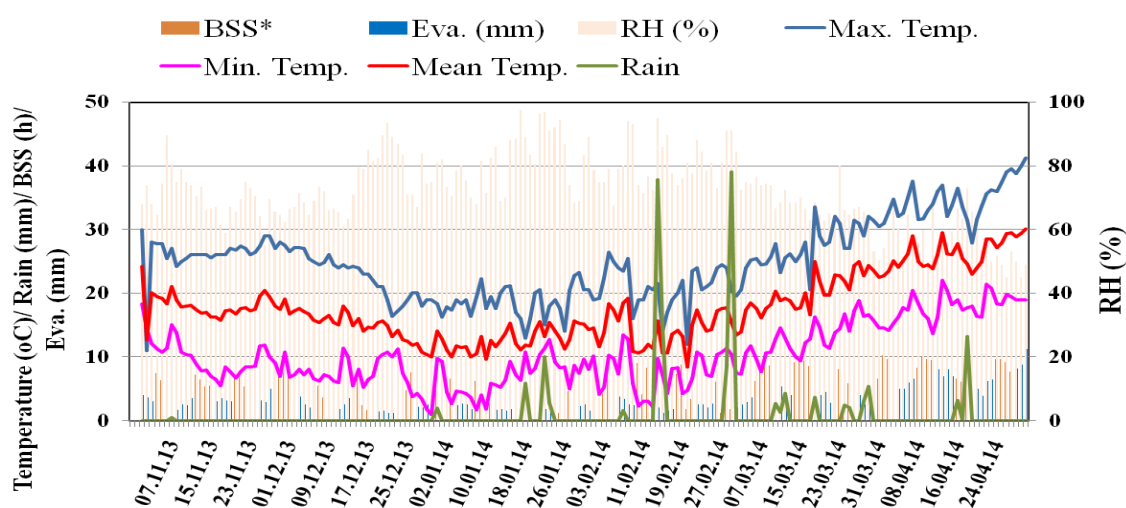


Fig. 1: Weather records during the course of investigation

### 3.4. SAMPLE COLLECTION

During preliminary experiment, water deficit stress treatment was imposed at vegetative stage for dose optimization of paclobutrazol (PBZ). At vegetative stage plant samples were taken at zero and 14 days after withholding water. After sampling, water deficit stress was terminated and further sampling was done at 7<sup>th</sup> day during recovery period. While during main experiment water deficit stress treatment was imposed at reproductive stage. Plant samples were collected at 0<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days after withholding/ restricting water supply. After sampling, water deficit stress was terminated by irrigating plants and

subsequent plant sampling during recovery period was done on 7<sup>th</sup> and 14<sup>th</sup>. Post harvest observations were recorded at harvest.

### 3.5. SOIL MOISTURE CONTENT

Soil moisture content was estimated periodically during imposed water stress treatment at vegetative stage by gravimetric method. Soil sample was taken from pot at 10 cm depth using augur. For each sample 10 g fresh weight of soil was recorded using a weighing balance. Then the sample was kept in the oven at 105<sup>0</sup>C, and dried till constant weight was achieved. The soil moisture content was calculated by the following formula (Faulkner *et al.*, 1989) and expressed in percentage (%). Fully saturated soil at 100 % capacity (FC) had 30 % moisture. By using this relation of field capacity with soil moisture FC (%) was estimated at different water deficit stressed period.

$$\text{Soil moisture content} = ((\text{Fresh weight} - \text{Dry weight}) / \text{Dry weight}) \times 100$$

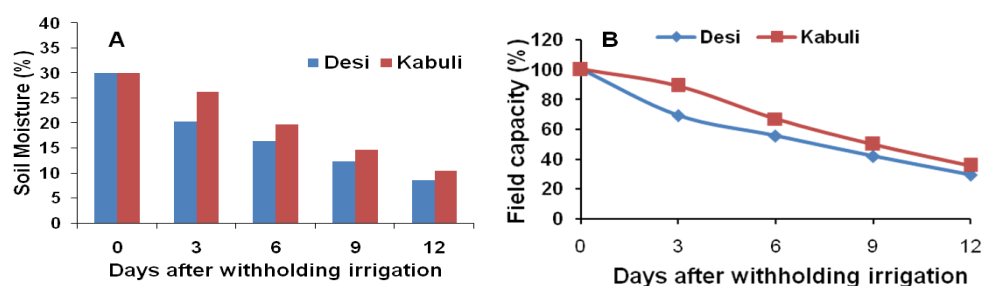


**Fig.2:** Soil moisture content (A) and field capacity (B) recorded during course of water deficit treatment of Preliminary experiment

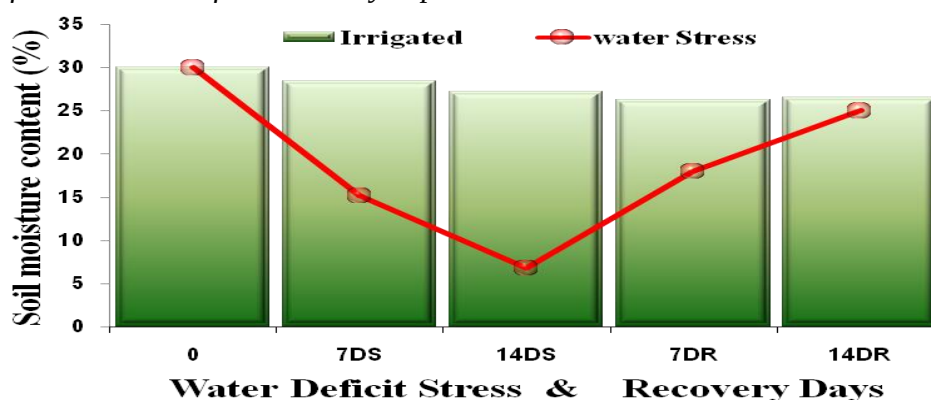


**Fig.3:** Soil moisture content recorded during course of water deficit treatment of Main experiment.

### 3.6. PHYSIOLOGICAL PARAMETERS

The following physiological parameters were studied in the present experiment.

#### 3.6.1. Relative Water Content

Leaf relative water content (RWC) was estimated by recording the turgid weight of 0.5 g fresh leaf samples by keeping in water for 4 h, followed by drying in hot air oven at 70 °C till constant weight was achieved (Weatherley, 1950).

$$\text{RWC} = [(\text{Fresh wt.} - \text{Dry wt.}) / (\text{Turgid wt.} - \text{Dry wt.})] \times 100$$

#### 3.6.2. Membrane Stability Index

Membrane stability index (MSI) was estimated according to the method described by Premachandra *et al.*, (1990). For estimation of membrane stability index 100 mg leaf material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40 °C for 30 min in a metabolic water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge ( $C_1$ ). Second set was boiled at 100 °C on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge ( $C_2$ ). Membrane stability index (MSI) was calculated as:

$$\text{MSI} = [1 - (C_1/C_2)] \times 100$$

#### 3.6.3. Chlorophyll and Carotenoid content

Chlorophyll and carotenoid content were estimated as per the method described by Hiscox and Israelstam, 1979. The procedure for estimation of chlorophyll content in plants is based on the absorption of light by chlorophyll extracts prepared by incubating the leaf tissues in DMSO (dimethyl sulfoxide). DMSO renders plasmalemma permeable thereby, causing the leaching of the pigments (Hiscox and Israelstam, 1979). The absorbance of the known volume of solution containing known quantity of leaf tissue at two respective wavelengths (663 and 645) was determined for chlorophyll content and at 480 nm for total carotenoid contents. Chlorophyll a, chlorophyll b and total chlorophyll content were estimated using the formula given by Arnon, (1949) while carotenoid content was determined by following the formula given by Lichtenthaler and Welburn, 1983. Thirty mg fresh leaf samples were added to the test tubes containing 4 ml DMSO. Tubes were kept in dark for 4 h at 65 °C. Then the samples were taken out cooled at room temperature and the absorbance was recorded at 663, 645 and 480 nm using DMSO as blank and was expressed as  $\text{mg g}^{-1}$  dry wt.

$$\text{Chlorophyll 'a'} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V/W \times 1000$$

$$\text{Chlorophyll 'b'} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times V/W \times 1000$$

$$\text{Total chlorophyll} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times V/W \times 1000$$

$$\text{Total carotenoids} = (A_{480} + (0.114 \times A_{663}) - (0.638 - A_{645})) \times V/W \times 1000$$

Where,

$A_{663}$  = Absorbance values at 663 nm

$A_{645}$  = Absorbance values at 645 nm

$A_{480}$  = Absorbance values at 480 nm

W = Weight of the sample in g

V = Volume of the solvent used (ml)

### **3.6.4. Photosynthetic pigment separation using thin layer chromatography (TLC)**

Separation of pigments by TLC was done according to the method described by Pocock *et al.*, 2004 with minor modifications.

#### **3.6.4.1. Extraction of leaf pigments**

Fresh leaves (5g) were grinded in a pestle and mortar with 20 ml acetone, 3 ml petroleum ether and calcium carbonate. The homogenate was filtered through whatman filter paper in to a separating funnel and to it 10% NaCl and petroleum ether was added. The mixture was shaken carefully and was separated in a separating funnel. The upper layer was collected and washed three to four times with double distilled water. The final extract was evaporated in a cool and dark place and the volume was made up to 2ml using acetone.

#### **3.6.4.2. Application of extract to the TLC plate**

The TLC plate was kept in the oven for 3-4 hours at 90-100 °C in order to remove any traces of moisture present on it. A line was drawn on the plate (1.5 cm above bottom) and the extract was applied on it. The spot was dried thoroughly. Then the plate was kept in the TLC chamber consisting of petroleum ether, acetone and distilled water based solvent system as described elsewhere (Pocock *et al.*, 2004) with little modifications. The chromatogram was removed when the solvent went 15 cm above from the origin and it was immediately photographed. For the identification of the different photosynthetic pigments, bands of pigments from TLC plate were scratched and eluted using acetone by centrifuging at 3000g for 10 minutes. Then the spectra of each band were drawn with the help of UV-Visible

spectrophotometer. Thus, based on the spectra and  $R_f$  value photosynthetic pigments were identified.

### 3.6.5. Photosynthesis and chlorophyll fluorescence associated parameters



Rate of photosynthesis was measured using portable Infrared Gas Analyzer (IRGA), model LI-6400XT Model (Li-COR Ltd., Lincoln, Nebraska, USA). The rate of photosynthesis, respiration, stomatal conductance, transpiration rate, chlorophyll fluorescence and  $(f_v/f_m)$  were measured by operating the IRGA in the closed mode. The rate of respiration was measured by adapting the leaves to darkness for 10 minutes.

All the parameters were determined at different growth stages between 10 to 11.30 am by providing artificial light source of intensity  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The parameters were expressed in the following units; rate of photosynthesis ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ ). The actual flux of photons ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) driving photosystem II (PS II) can be inferred from chlorophyll fluorescence measurements. Canopy photosynthesis ( $\text{CP}_N$ ) was estimated by multiplying photosynthesis rate with leaf area index (LAI) i.e.  $\text{CP}_N = P_N \times \text{LAI}$ .

### 3.6.6. Growth parameters:

At all the stages growth sampling was done and shoot length, root length, number of main branches, total leaf number, total leaf area, shoot dry weight, root dry weight, leaf dry weight and total dry weight were recorded. Growth rate, specific leaf area, specific leaf weight, dry matter partitioning and leaf area ratio were also calculated as described elsewhere (Gardner *et al.*, 1985).

#### Shoot length

Plants were harvested by uprooting the whole plants from the pot culture and separated into leaf, root and stem. The shoot length was measured using a scale.

#### Number of main branches

The number of main branches was counted and clearly noted down.

**Leaf area**

Leaf area was measured by using a standard LI-COR leaf area meter (Model LI-3100) and was expressed as  $\text{cm}^2 \text{ plant}^{-1}$ .

**Number of leaves**

Total number of leaves from each plant was collected and was counted manually.

**Leaf, stem and root dry weights**

All the plant parts i.e. leaves, root and stem from each plant were collected cleaned and were dried in hot air oven at 70 °C for 4 hours and then at 60 °C till constant weight were reached and dry weight recorded by balance (electronic digital model TS 400D).

**Specific leaf area (SLA)**

Specific leaf area was calculated using following formula

$SLA = LA/LW$  where,

LA= leaf area

LW= leaf weight

**Specific leaf weight (SLW)**

Specific leaf weight was calculated using following formula

$SLW = LW/LA$  where,

LW= leaf weight

LA= leaf area

**Dry matter partitioning (%)**

Dry matter partitioning was calculated using following formula

$DP = (\text{organ dry weight}) / (\text{total dry weight}) \times 100$

**Leaf area ratio (LAR)**

Leaf area ratio was calculated using following formula

$LAR = LA/W$  where,

LA= leaf area

W= total dry matter

**3.6.7. Elucidation of chloroplast ultra structure by transmission electron he microscopy**

Fresh leaves of chickpea were collected and cut properly (2 mm), washed and then fixed in 2% para-formaldehyde, 1% glutaraldehyde, 0.1M sodium cacodylate buffer (pH 6.9), for 2 h at room temperature



(Gunawardena *et al.*, 2001). The segments were washed in the buffer, and post-fixed in 1% aqueous osmium tetroxide for 1h, before being stained in 0.5% uranyl acetate at 4<sup>0</sup>C overnight. Tissue sections were dehydrated in a graded ethanol series (30 min each) and then embedded through ethanol : spur resin mixtures ( 3:1, 1:1, 1:3) for 1h each. They were finally embedded in 100% Spur resin and polymerized at 60<sup>0</sup> C for 9h in flat silicon rubber moulds. Ultra thin sections (60-80 nm) were cut on a Reichert Jung Ultracut E microtome (Leica, Milton Keynes, UK), collected on Formavar-coated grids (200-mesh copper hexagonal mesh: Agar scientific, Stansted, UK), tinted in lead citrate and examined with a transmission electron microscope (Philips CM-10) operated at 60-80 kV.

### **3.7. BIOCHEMICAL PARAMETERS**

#### **3.7.1. Lipid peroxidation**

Lipid peroxidation was estimated as the thiobarbituric acid reactive substances, according to the method of Heath and Packer, 1968.

#### **Reagents**

1. Trichloroacetic acid solution: Trichloroacetic acid (TCA) solution (0.1%) was prepared by dissolving 0.1 g TCA in double distilled water and volume was made up to 100 ml with double distilled water.
2. Thiobarbituric acid reagent : Thiobarbituric acid (TBA) 0.5 g was dissolved in 20% trichloro acetic acid (TCA) (prepared by dissolving 20 g TCA in 100 ml distilled water) and volume was made up to 100 ml with 20% TCA solution.

#### **Estimation**

Leaf samples (0.5 g) were homogenized in 10 ml 0.1% trichloro-acetic acid (TCA). The homogenate was centrifuged at 15,000 g for 15 min. To 1.0 ml aliquot of the supernatant 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at



95 °C for 30 min in the water bath and then cooled under room temperature. After centrifugation at 10,000 g for 10 min the absorbance of the supernatant was recorded at 532 nm. The TBARS content was calculated according to its extinction coefficient, i.e.,  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ . The values for non-specific absorbance at 600 nm were subtracted (Heath and Packer, 1968).

### 3.7.2. Estimation of total soluble sugars

Total soluble sugars were estimated by anthrone reagent method (McCready *et al.*, 1950).

#### Reagents

1. Sulphuric acid (95%): To 95.9 ml of sulphuric acid 4.1 ml of distilled water was added to make upto 100 ml.
2. Anthrone reagent: Two hundred mg anthrone was dissolved in 100 ml of ice cold 95% sulphuric acid. Fresh reagent was prepared each time.

One gram of leaf samples were plunged in 80% hot ethanol and preserved. There were three replications for each determination.

#### Extraction of sugars

For sugar analysis, one gram of leaf tissue was grinded in a pestle and mortar containing 10 ml (80%) hot ethanol and the homogenate was centrifuged at 10000 rpm for 10 minutes. The supernatant was taken separately and the residue was resuspended in 10 ml (80%) hot ethanol and was again centrifuged at 10000 rpm for 10 minutes. This step was repeated for a total of three times and all the supernatant were pooled together and final volume was made up to 50 ml using 80% hot ethanol.

#### Determination of Total sugars

To 0.2 ml of sugar sample, 4 ml solution of anthrone reagent was added and the mixture was heated on a boiling water bath for 5 min followed by cooling. The optical density of green to dark green colour was read at 630 nm in UV-visible spectrophotometer (model

Specord Bio-200, AnalytikJena, Germany). A blank and two freshly prepared glucose standards were also included with each set of samples. The total sugar content was calculated from the standard curve of glucose ( $y = 0.004x - 0.005$ ;  $R^2 = 0.997$ ) and was expressed as mg/g dry weight.

### 3.7.3. Estimation of Proline

Proline content was estimated as per the method described by Bates *et al.*, 1973.

#### Reagents

1. Acid Ninhydrin: 2.48 g of ninhydrin was dissolved in 60ml glacial acetic acid and 40 ml of 6N phosphoric acid.
2. Sulphosalicylic acid (3%)
3. Toluene
4. Glacial acetic acid

#### Estimation

Leaf samples (0.5g) were homogenized in 10 ml 3% sulphosalicylic acid and were filtered through whatman filter paper. Two ml of this filtrate was mixed with 2ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was heated at 100 °C in a water bath for 1 hour. The reaction was stopped by removing the tubes from hot water bath and placing them in ice bath. Toluene (4ml) was added to the mixture and vortexed for 15-20 seconds. The chromophore was aspirated from the aqueous phase. Then the absorbance of toluene phase was measured at 520 nm. A blank was run and its absorbance was subtracted from the sample absorbance. The proline content was calculated from a standard curve using L-Proline as standard ( $y = 0.007x - 0.013$ ;  $R^2 = 0.990$ ) and was expressed as  $\mu\text{mol/g}$  fresh weight.

### 3.7.4. Total free amino acids

Total free amino acids were estimated following the method as described by Lee and Takahashi, 1966.

#### Extraction

One gram of plant material was weighed and grinded in a mortar-pestle with 5ml of ethanol. The crushed material was centrifuged and the supernatant was carefully transferred. The residue was again ground with 3ml of ethanol and treated as above. The extraction was

done three times to obtain total free amino acids. The final volume was made up to 10ml with ethanol and was used for estimation of the total free amino acids.

### Reagents

1. Citrate buffer: 0.5M (pH 5.6)
2. Ninhydrin: 1% solution in 0.5M citrate buffer (pH5.6)
3. Glycerol: 55% solution of glycerol was prepared in distilled water.

### Estimation

Following reagents were taken in test tubes in duplicate.

0.2ml ninhydrin solution.

0.2ml citrate buffer

1.2ml glycerol

0.1ml of sample solution (in control, sample solution was not added)

The volume of the mixture was made up to 5ml by adding distilled water. Test tubes were then heated in a boiling water bath for 30 minutes, cooled in running tap water and gently shaken. The absorbance was read using a spectrophotometer at 570 nm. A blank or control was also taken simultaneously for background correction. The quantity of total free amino acids was computed using a standard curve prepared from glycine ( $y = 0.009x - 0.019$ ;  $R^2 = 0.986$ ) and was expressed as mg/g fresh weight.

### 3.7.5. Estimation of Total Soluble Proteins

Total soluble protein was estimated following the method of Lowry *et al.*, (1951).

### Reagents

1. Alkaline copper reagent

Solution 'A': 2% sodium carbonate solution was prepared in 0.1 N sodium hydroxide.

Solution 'B': 0.5% copper sulphate solution was prepared in 1% potassium sodium tartrate.

50ml of Solution 'A' and 1ml of Solution 'B' were mixed together thoroughly to make the reagent.

2. Folin-ciocalteau-phenol reagent

3. Phosphate buffer
4. Bovine serum albumin (for standard curve)

### **Extraction of protein**

Leaf sample (1g) was weighed and ground well in 10ml of phosphate buffer with a pestle and mortar and was centrifuged at 20000 g for 15 minutes. The supernatant was taken for protein estimation and the pellets were discarded.

### **Estimation of protein**

0.1ml of the above extract was taken in a test tube and 1 ml of alkaline copper reagent was added to it. The mixture was stirred gently and allowed to stay at room temperature for 15 minutes. Then 0.2 ml of diluted 1:1 (v:v) folin-ciocalteau-phenol reagent was added to it and shaken well. It was kept for 30 minutes incubation at room temperature in dark, and blue colour was developed. The volume was made up to 5 ml with double distilled water. The absorbance was recorded at 660 nm wavelength. A blank was run, in which 0.2 ml of distilled water was added, instead of enzyme extract.

### **3.7.6. Estimation of Catalase Activity**

The total catalase activity was estimated following the method described by Aebi *et al.*, 1984.

#### **Reagents**

1. Hydrogen Peroxide (75mM): Hydrogen peroxide (30%, 775  $\mu$ L) was dissolved in double distilled water and the volume was made upto 100 ml.
2. Phosphate buffer (100mM, pH 7.0)

#### **Preparation of Enzyme Extract**

Leaf samples (1g) were freeze-dried in liquid nitrogen to prevent proteolytic activity and were homogenized with 10 ml extraction buffer containing 0.1 M phosphate buffer, pH 7.5 and 0.5 mM EDTA. Homogenate was passed through 4 layers of cheese cloth and the filtrate was centrifuged at 15000g for 20 minutes and the supernatant was used as enzyme.

#### **Enzyme Assay**

The reaction mixture was prepared with the following reagents

1. Potassium phosphate buffer 50 mM (1.5 ml of 100 mM buffer, pH 7.0)
2. Enzyme (50  $\mu$ L)
3. Water (950  $\mu$ L)

Then hydrogen peroxide (0.5 ml) was added to start the reaction and the decrease in absorbance was recorded for 1 minute at 240nm and enzyme activity was calculated as per extinction coefficient of hydrogen peroxide ie.  $36.5 \text{ M}^{-1} \text{ cm}^{-1}$ . Enzyme activity was expressed as  $\mu$ mol hydrogen peroxide reduced per minute per mg protein.

### **3.7.7. Estimation of Peroxidase activity**

The total peroxidase activity was estimated following the method described by Castillo *et al.*, 1984.

#### **Reagents**

1. Phosphate buffer (100mM, pH 6.1)
2. Hydrogen Peroxide (12mM): Hydrogen peroxide (30%, 124  $\mu$ L) was dissolved in double distilled water and the volume was made upto 100 ml.
3. Guaiacol (96mM): Analytical grade guaiacol (1075  $\mu$ L) was dissolved in distilled water and the volume was made up to 100 ml.

#### **Preparation of Enzyme Extract**

Leaf samples (1g) were freezed in liquid nitrogen to prevent proteolytic activity and were homogenized with 10 ml extraction buffer containing 0.1 M phosphate buffer, pH 7.5 and 0.5 mM EDTA. Homogenate was passed through 4 layers of cheese cloth and the filtrate was centrifuged at 15000g for 20 minutes and the supernatant was used as enzyme.

#### **Enzyme Assay**

The reaction mixture was prepared with the following reagents

1. Potassium phosphate buffer 50 mM (1.0 ml of 100 mM buffer, pH 6.1)
2. Guaiacol 16mM (0.5 ml of 96 mM)
3. Hydrogen peroxide 2mM (0.5 ml of 12 mM)
4. Enzyme (0.1 ml)
5. Water (0.4 ml) and the total volume were made up to 3 ml.

Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and enzyme activity was calculated as per extinction coefficient of its oxidation product, tetra-guaiacol, ie.  $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ . Enzyme activity was expressed as  $\mu\text{mol}$  tetra-guaiacol formed per minute per mg protein.

### **3.7.8. Estimation of superoxide dismutase (SOD) activity**

The assay is based formation of blue coloured formazone by nitro-blue tetrazolium and  $\text{O}_2^{\cdot -}$  radical, which absorbs at 560 nm, and the enzyme (SOD) decreases this absorbance due to reduction in the formation of  $\text{O}_2^{\cdot -}$  radical by the enzyme (Dhindsa *et al.*, 1981).

#### **Reagents:**

1. Methionine (200 mM)
2. Nitroblue tetrazolium chloride (NBT) (2.25 mM)
3. EDTA (3.0 mM)
4. Riboflavin (60  $\mu\text{M}$ )
5. Sodium carbonate (1.5 M)
6. Phosphate buffer (100 mM, pH 7.8):

Sol A: Potassium dihydrogen phosphate 6.80 g was dissolved in water and the volume was made up to 500 ml with double distilled water.

Sol B: Di-potassium hydrogen phosphate 8.71 g was dissolved in water and the volume was made up to 500 ml with double distilled water.

8.5 ml of sol. A and 91.5 ml of sol. B was mixed and final pH was adjusted with the help of pH meter.

Grinding media: (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA) in case of SOD, CAT, GR and POX: EDTA 0.0186 g was dissolved in phosphate buffer 0.1 M, pH 7.5 (made by mixing 16 ml of sol. A and 84 ml of sol. B and final pH was adjusted with the help of pH meter) and volume was made to 100 ml with the buffer.

#### **Preparation of enzyme extract**

Enzyme extract for superoxide dismutase was prepared by first freezing the weighed amount of leaf samples (1 g) in liquid nitrogen to prevent proteolytic activity followed by grinding with 10 ml extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM.

Homogenate was passed through 4 layers of cheesecloth and filtrate was centrifuged for 20 min at 15 000 g and the supernatant was used as enzyme.

#### **Enzyme assay**

Superoxide dismutase activity was estimated by recording the decrease in optical density of formazone made by superoxide radical and nitro-blue tetrazolium dye by the enzyme. Three ml of the reaction mixture was prepared by mixing the following reagents:

1. 13.33 mM methionine (0.2 ml of 200mM)
2. 75  $\mu$ M nitroblue tetrazolium chloride (NBT) (0.1 ml of 2.25 mM)
3. 0.1 mM EDTA (0.1 ml of 3mM)
4. 50 mM phosphate buffer (pH 7.8) (1.5 ml of 100 mM)
5. 50 mM sodium carbonate (0.1 ml of 1.5M)
6. 0.1 ml enzyme
7. 0.9 to 0.95 ml of water (to make a final volume of 3.0 ml)

Reaction was started by adding 2  $\mu$ M riboflavin (0.1 ml) and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal colour, served as control. Switching off the light and putting the tubes into dark stopped the reaction.

The absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbency reading to 50 % in comparison with tubes lacking enzyme. The enzyme activity was expressed as units per mg protein per hour.

#### **3.7.9. Estimation of glutathione reductase activity**

The total Glutathione reductase activity was estimated following the method described by Smith *et al.*, 1988. Glutathione reductase catalyze the reduction of oxidized glutathione (glutathione disulphide) (GSSG) to reduced glutathione (GSH) by using reductant NADPH. The assay of the enzyme is based on the formation of a red coloured complex by reduced glutathione with 5,5-dithiobis-2-nitrobenzoic acid (DTNB), which absorbs at 412 nm (Smith *et al.*, 1988).

## Reagents

1. 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (3.0 mM) in phosphate buffer (10 mM, pH 7.5)
2. Oxidized glutathione (GSSG) (20 mM)
3. NADPH (2.0 mM):
4. Phosphate buffer (200 mM, pH 7.5):

Sol A: Potassium dihydrogen phosphate 6.80 g was dissolved in water and the volume was made up to 500 ml with double distilled water.

Sol B: Dipotassium hydrogen phosphate 8.71 g was dissolved in water and the volume was made up to 500 ml with double distilled water.

15 ml of sol.A and 85 ml of sol. B were mixed and final pH was adjusted.

## Preparation of enzyme extract

Leaf samples (1g) were freezed in liquid nitrogen to prevent proteolytic activity and were homogenized with 10 ml extraction buffer containing 0.1 M phosphate buffer, pH 7.5 and 0.5 mM EDTA. Brie was passed through 4 layers of cheese cloth and the filtrate was centrifuged at 15000g for 20 minutes and the supernatant was used as enzyme.

## Enzyme assay

The reaction mixture was prepared with the following reagents:

66.67 mM potassium phosphate buffer (pH 7.5) and 0.33 mM EDTA (1 ml of 0.2 M buffer containing 1 mM EDTA)

1. 0.5 mM DTNB in 0.01 M potassium phosphate buffer (pH 7.5) (0.5 ml of 3.0 mM)
2. 66.67  $\mu$ M NADPH (0.1 ml of 2.0 mM)
3. 666.67 $\mu$ M GSSG (0.1 ml of 20 mM)
4. 0.1 ml enzyme extract

Distilled water to make up a final volume of 3.0 ml

Reaction was started by adding 0.1 ml of 20.0 mM GSSG (oxidized glutathione). The increase in absorbance at 412 nm was recorded spectro-photometrically and enzyme activity was calculated as per extinction coefficient of oxidised glutathione ie.  $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$ . Enzyme activity was expressed as  $\mu$ mol oxidised glutathion formed per minute per mg protein.

### 3.7.10. Estimation of abscisic acid (ABA) by HPLC

ABA in the leaf sample was estimated by using HPLC, following the methods of Zeevart, 1980.

#### Preparation of sample:

Leaf samples (1 g) collected, were frozen in liquid nitrogen, pulverized and stored at -20 °C until used. Frozen samples were extracted three times with 10 ml of 80 % v/v acetone (80 ml acetone, 1 ml glacial acetic acid and 100 mg of 2, 6 di-tert-butyl 4-methyl phenol in a total volume of 100 ml) and collected in



a 100 ml volumetric flask. The tissue residue was then homogenized with pestle and mortar with acetone (80 % v/v). The homogenate was filtered through Whatman No. 1 filter. The filtrate along with the extracts made earlier was transferred to the boiling flask of rotary flash vacuum evaporator for removing the acetone. As the acetone was evaporated, the lipid soluble material was deposited on the walls of the boiling flask (round bottomed). This was dissolved in 1% acetic acid solution and the amber coloured aqueous solution was transferred into small vials (10 ml). Before injecting the sample into HPLC, the samples were filtered with 0.45 mm Millipore filter using 2.5 ml plastic syringe.

#### Conditions of assay

Detector: A variable wavelength 150 UV-visible detector.

Injector: Rheodyne injector (20 µl loop).

Column/stationary phase: The column used is µBondapak TM/C18 P/N 841/6 S/N. The stationary phase consisted of a Lichrosorb C-18.

Mobile phase: 1 % acetic acid in 95 % methanol solution attached to the pump.

Flow rate: 2.5 ml/min

Wavelength: Wavelength was set at 265 nm before injecting the sample.

Instrument was set on injection mode and 20 µl of standard ABA (10 ppm)/or sample solution was injected into it and then the instrument was switched on to operation mode. Data on retention time and area of the peak recorded by the integrator was collected. The peak

belonging to ABA was identified based on its specific retention time, using the data registered by the standard ABA.

The concentration of ABA present in the sample was calculated by calculating the area under the peak by the formula as described in the principal of the instrument ( $A = \frac{1}{2} b \times h$ ), and comparing it with the standard curve prepared with known concentrations of ABA. Standard curve was prepared by plotting concentrations on X-axis and respective areas (of the peak) of the standard ABA on Y-axis.

### **3.8. YIELD AND ITS ATTRIBUTES**

The following parameters were recorded at the time of harvest, viz., plant height, root length, number of main branches, number of secondary branches, pods per plant, shoot weight, leaf weight, pod weight, root weight, total biomass per  $m^2$ , 10 pod weight, 10 pod seed weight, seeds per 10 pods, total seed weight, 100 seed weight, dry matter partitioning and harvest index. Drought susceptibility index (DSI) and drought tolerance efficiency were calculated according to the formulas described by (Fischer and Wood, 1981).

#### **Number of pods per plant**

The total number of pods from three plots were counted, averaged and expressed as number of pods per plant

#### **Pod weight per plant**

The weight of the pods from three plots were recorded, averaged and expressed as the pod weight.

#### **100-seed weight (g)**

One hundred seeds were counted from the sample and their weights were recorded in grams.

#### **Seed yield per plant (g)**

The seeds from dried pods of three plots were separated and weighted to obtain total grain weight and expressed as g per  $m^2$ .

#### **Dry matter partitioning (%)**

Dry matter partitioning was calculated using following formula

$$DP = (\text{organ dry weight}) / (\text{total dry weight}) \times 100$$

#### **Harvest index (%)**

Harvest index was calculated using following formula

$$HI = [\text{Economic yield} / \text{Biological Yield}] \times 100$$

**Drought susceptibility index (DSI)**

Drought susceptibility index was calculated using following formula

$$DSI = (1 - Y_0/Y_1)/DI$$

Where,  $Y_0$  = yield of individual treatment under drought stress

$Y_1$  = yield of individual treatment under no stress

$$DI = (1 - Y_r/Y_i)$$

$Y_r$  = mean yield of all the treatments under drought stress

$Y_i$  = mean yield of all the treatments under no stress

**Drought Tolerance Efficiency**

Drought Tolerance Efficiency was calculated using following formula

$$\text{Drought Tolerance Efficiency} = (\text{Yield under stress} / \text{Yield under no stress}) \times 100$$

**3.9. STATISTICAL ANALYSIS**

Both the experiments were carried out in factorial CRD. Statistical analysis of the data was done following the methods of analysis of variance (ANOVA) (Panse and Sukhatme, 1967). The critical difference was calculated at 5% probability level.

# 4. RESEARCH PAPER I

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## Physiological Responses of Chickpea (*Cicer arietinum* L.) genotypes to Paclobutrazol under Water deficit Stress Condition

Soumya, P. R. and Pramod Kumar

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110012

### ABSTRACT

Water deficit is one of the major constraints to the productivity of crops worldwide. Water deficit is also a major limitation in chickpea production, since this crop is traditionally cultivated in marginal and rainfed areas. Paclobutrazol (PBZ) has been reported to induce abiotic stress tolerance. Therefore, initially by conducting a preliminary experiment optimum dose of paclobutrazol for foliar and drenching applications in chickpea @ 60 ppm and 120 ppm respectively were identified. Subsequently, in order to investigate the possibility of application of PBZ in improving growth, biomass partitioning and yield under water deficit stress conditions present main experiment was carried out using two contrasting chickpea varieties viz. *desi* (Pusa 362) and *kabuli* (Pusa 1108). Water deficit stress was imposed by withholding water at reproductive stage. Observations were recorded on relative water content (RWC), membrane stability index (MSI), various growth parameters, chloroplast ultrastructure, photosynthetic pigments, rate of photosynthesis, stomatal conductance, transpiration, chlorophyll fluorescence parameters, yield and its attributes. The results showed decrease in RWC, MSI, chlorophyll and carotenoid contents, rate of photosynthesis stomatal conductance, transpiration, Fv/Fm ratio, growth parameters, harvest index, drought tolerance efficiency, dry matter stress tolerance index, yield and its attributes under imposed water stress condition. Interestingly, application of PBZ in general, maintained higher values of all these parameters, retained the integrity chloroplast structure under imposed water deficit stress and also stimulated faster recovery after the water deficit stress termination in both chickpea varieties. In addition to this, PBZ drenching treatment exhibited better efficacy than foliar application. Present findings emphasized the possibility of application of PBZ in improving growth, biomass partitioning and yield by protecting photosynthetic machinery under water deficit stress in chickpea at the physiological level and thereby improving productivity.

**Key words:** Chickpea, drenching, paclobutrazol, photosynthesis, water deficit stress, yield

### 4.1. INTRODUCTION

Chickpea as a third major grain legume in the world has an important role in poor people's diet. The cultivated chickpea (*Cicer arietinum* L.) is grown on 11 million hectares with 9.77 million ton production (<http://faostat.fao.org>). However, water deficit poses a serious threat to its production, as the major chickpea growing areas are in the arid and semi-arid zones and about 90 % of world's chickpea is grown under rainfed conditions (Kumar and Abbo, 2001). In chickpea, yield losses might be the result of intermittent drought during the vegetative

phase, due to drought during reproductive development or due to terminal drought at the end of the crop cycle (Serraj *et al.*, 2004). Water deficit affects many morphological features and physiological processes associated with plant growth and development (Toker and Cagiran, 1998). These changes include reduction of water content (RWC), diminished leaf water potential and turgor loss, closure of stomata and a decrease of cell enlargement and plant growth. Drought stress reduces plant growth by affecting photosynthesis, respiration, the membrane stability index (MSI) and nutrient metabolism (Jaleel *et al.*, 2008). Severe drought stress also inhibits the photosynthesis of plants by affecting chlorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe *et al.*, 1998).

Yield enhancement in chickpea through conventional breeding is difficult because of a narrow genetic base (Udupa *et al.*, 1999). An alternative approach in this direction is the use of external bioregulatory molecules, which can be used to achieve the stress tolerance in crops under field conditions. Paclobutrazol (PBZ) belongs to triazoles group, has plant growth regulating properties. Its growth regulating properties are mediated by altering in the balance of important plant hormones including the gibberellins, ABA and cytokinins (Fletcher *et al.*, 1990). Paclobutrazol (PBZ) affects the isoprenoid pathway and alters the levels of plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution, and increasing cytokinin levels (Kamounsis and Chronopoulou-Sereli, 1999). When gibberellins synthesis is inhibited, more precursors in the terpenoid pathway accumulate and are shunted to the production of abscisic acid. One of the functions of abscisic acid is to cause stomates to close, reducing water loss from leaves through transpiration. Paclobutrazol has also been proved as an agent in stress amelioration in medicinal plants (Jaleel *et al.*, 2007). PBZ has been used to provide plant protection against abiotic stresses such as chilling (Lin *et al.*, 2006), water stress (Banon *et al.*, 2001; Berova and Zaltev, 2003; Zhu *et al.*, 2004), flooding (Webb and Fletcher, 1996; Lin *et al.*, 2006) and salinity (Kishor *et al.*, 2009). Recently, paclobutrazol was also reported to induce water deficit stress tolerance in some plants such as *Arachis hypogaea* L. (Leguminosae, Papilioideae) and *Phillyrea angustifolia* L. (Oleaceae) (Fernandez *et al.*, 2006; Sankar *et al.*, 2007). Since rainfed chickpea often experiences water deficit therefore, need was felt to investigate the possibility of water deficit stress amelioration through PBZ application. The aim of this study was to analyze the PBZ induced physiological changes for understanding

the paclobutrazol induced mechanism of water deficit stress tolerance under water deficit in chickpea.

## 4.2. MATERIAL AND METHODS

For the present study, two experiments, one preliminary and another main experiment were conducted using two chickpea varieties viz. *Desi* type (Pusa 362) , *Kabuli* type (Pusa 1108). The seed material was obtained from Division of Genetics, IARI, New Delhi. The former variety is relatively tolerant while latter is water deficit susceptible. Preliminary experiment was carried out for optimizing paclobutrazol dose for foliar and drenching applications. Water deficit stress was imposed at vegetative stage in both *Kabuli* and *Desi* type varieties by restricting water supply until the appearance of severe symptoms of wilting. Before imposition of water stress plants were treated exogenously with varying dose of paclobutrazol for foliar (0, 30, 60, 90,120 and 150 ppm) and drenching (0, 60, 120, 180, 240 and 300 ppm) applications.

During *Rabi* season (2013-2014) main pot experiment was conducted in factorial CRD using aforementioned two chickpea varieties to study the growth, photosynthesis, biomass partitioning and yield response of chickpea to paclobutrazol under water deficit stress conditions. In one half of the total number of pots optimal soil moisture conditions were maintained by watering the plants. While in the remaining half set of pots water stress was imposed by withholding water until the severe symptoms of wilting were visible at reproductive stage. Before imposition of water stress, plants of both sets were treated with PBZ foliar spray @ 60 ppm and its drenching application @120 ppm and for controls plants were treated with water spray & drenching with water.

### 4.2.1. SOIL MOISTURE CONTENT

Soil moisture content was estimated periodically during imposed water stress treatment by gravimetric method. Soil samples were taken from pot at 10 cm depth using augur. For each samples 10 g fresh weight of soil was recorded using a weighing balance. Then the samples were kept in the oven at 105<sup>0</sup>C, and dried till constant weight was achieved. The soil moisture content was calculated by the following formula (Faulkner *et al.*, 1989) and expressed in percentage (%) (Fig. 2-3).

#### **4.2.2. SAMPLE COLLECTION**

During preliminary experiment, water deficit stress treatment was imposed at vegetative stage for dose optimization of paclobutrazol (PBZ). At vegetative stage plant samples were taken at zero and 14 days after withholding water. After sampling, water deficit stress was terminated and further sampling was done at 7<sup>th</sup> day during recovery period. While during main experiment water deficit stress treatment was imposed at reproductive stage. Plant samples were collected at 0<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days after withholding/ restricting water supply. After sampling, water deficit stress was terminated by irrigating plants and subsequent plant sampling during recovery period was done on 7<sup>th</sup> and 14<sup>th</sup>. Post harvest observations were recorded at harvest.

#### **4.2.3. Physiological parameters**

Observations were recorded on growth parameters (Gardner *et al.*, 1985), Relative water Content (Weatherley, 1950), Membrane stability index (Premachandra, Saneoka and Ogata. 1990), Chlorophyll (Hiscox and Israelstam, 1979; Arnon, 1949), carotenoid content (Lichtenthaler and Welburn, 1983), Pigment separation by TLC (Pocock *et al.*, 2004), transmission electron microscopy (Gunawardena *et al.*, 2001) and photosynthesis, respiration, chlorophyll fluorescence & associated parameters were measured using portable Infrared Gas Analyzer (IRGA), model LI-6400XT Model (Li-COR Ltd., Lincoln, Nebraska, USA).

#### **4.2.4. Yield and yield attributes**

The following parameters were recorded at the time of harvest, viz., plant height, root length, number of main branches, number of secondary branches, pods per plant, shoot weight, leaf weight, pod weight, root weight, total biomass per plant 10 pod weight, 10 pod seed weight, total seed weight, 100 seed weight, dry matter partitioning and harvest index. Drought susceptibility index (DSI) and drought tolerance efficiency were calculated according to the formulas described by (Fischer and Wood, 1981).

### **4.3. RESULTS**

#### **4.3.1. Physiological parameters**

The results obtained on various physiological parameters are presented below:

##### **4.3.1.1. Optimization of Paclobutrazol (PBZ) dose for foliar and drenching application**

Varying dose of PBZ (30 ppm, 60 ppm, 90 ppm, 120 ppm, 150 ppm) and water spray as a control were used for exogenous foliar application at vegetative stage in two contrast chickpea varieties i.e. Pusa 1108 and PUSA 362 under imposed water deficit condition for the identification of optimum dose in terms of RWC, MSI, photosynthetic rate and photosynthetic pigments. RWC, MSI, photosynthetic rate, chl a, chl b, total chlorophyll and carotenoids were increased with increasing dose of PBZ up to 60 ppm and decreased thereafter during imposed water deficit and after water stress termination (during recovery) in both the varieties. Maximum values of RWC, MSI, photosynthetic rate, chl a, chl b, total chlorophyll and carotenoids were recorded with the foliar application of PBZ @ 60 ppm.

Similarly, for optimizing PBZ dose, varying dose of PBZ (60 ppm, 120 ppm, 180 ppm, 240 ppm, 300 ppm) and water drenching as a control were used for drenching application at vegetative stage in both aforementioned varieties under imposed water deficit condition. Similarly in drenching treatments, RWC, MSI, photosynthetic rate, chl a, chl b, total chlorophyll and carotenoids were increased with increasing dose of PBZ up to 120 ppm and further decreased at its higher dose during imposed water deficit and recovery in both the varieties. Maximum values of RWC, MSI, photosynthetic rate, chl a, chl b, total chlorophyll and carotenoids were reported with the drenching application of PBZ at the rate of 120 ppm. However, under both foliar and drenching applications total carotenoids response of chickpea *desi* variety (Pusa 362) to PBZ dose was obtained relatively less than other physiological parameters. Based on curve fitting chickpea response of both varieties to PBZ in terms of RWC, MSI, photosynthetic rate, chl a, chl b, total chlorophyll and carotenoids are depicted in Figures (Fig. 4.3.1.1. (a-n) and Table 4.3.1.1.(a-b).

#### **4.3.1.2. Response of chickpea varieties to PBZ applications under well watered and imposed water deficit stress conditions**

##### **4.3.1.2.1. Relative water content**

In general, there was no effect of PBZ foliar and drenching treatments on leaf RWC under well watered condition in both the varieties at reproductive stage. However, values of RWC were reported higher with the foliar application of PBZ at 7 days after treatment in *desi* variety and with the drenching application of PBZ at 14 days after treatment in *kabuli* type. Under imposed water deficit condition RWC was decreased with increased duration of water

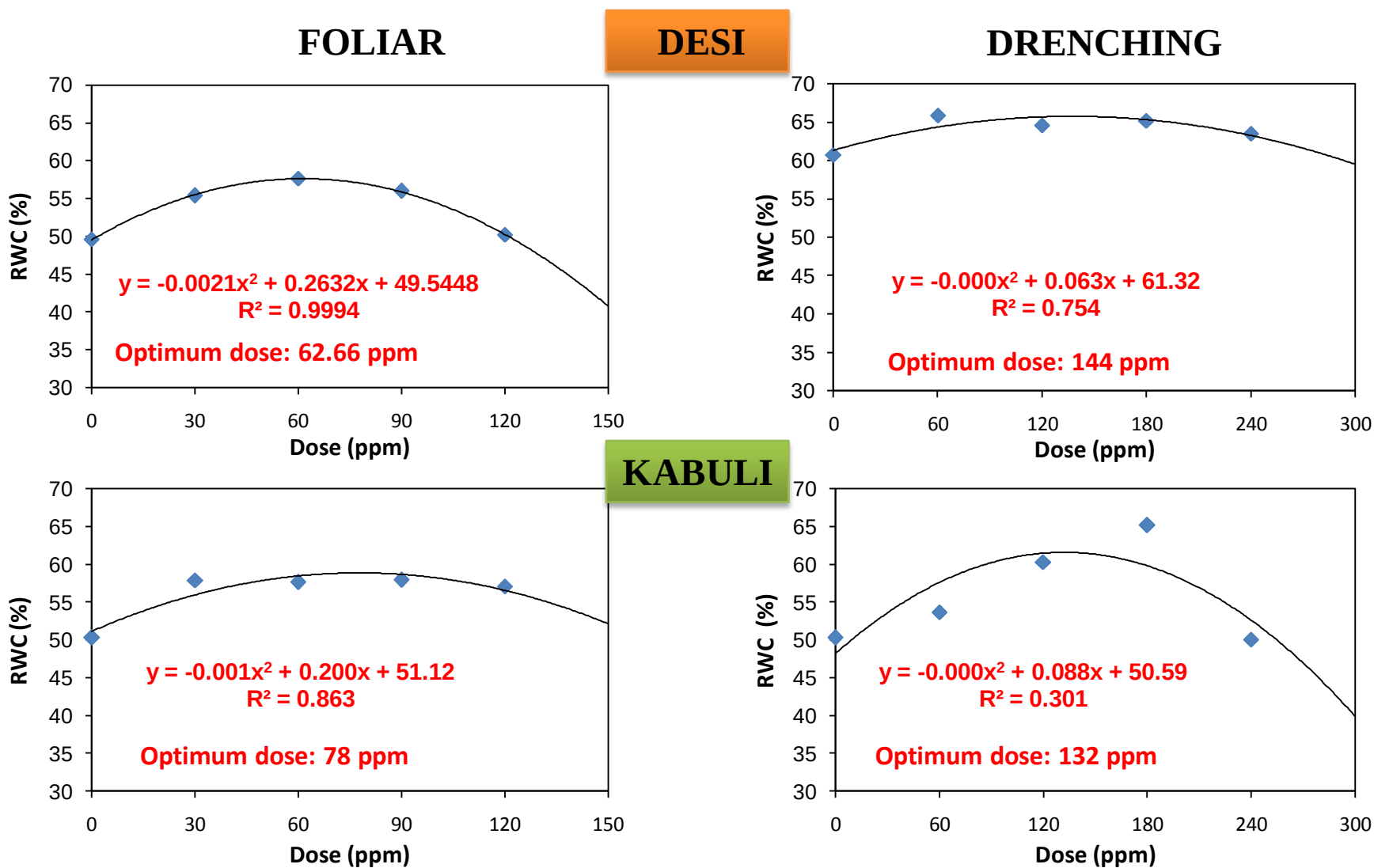
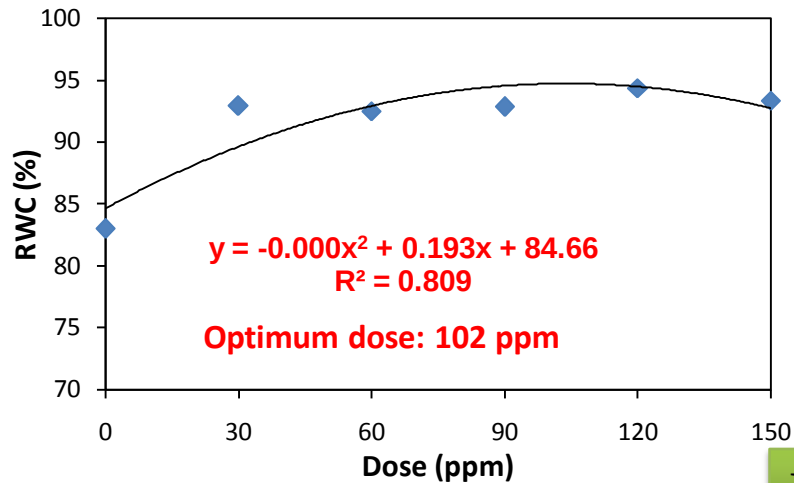


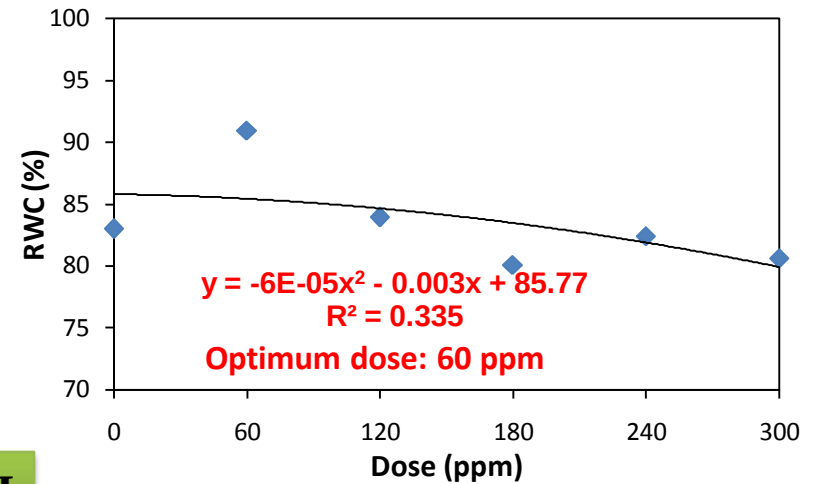
Fig.4.3.1.1.a. Relative water content response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

## FOLIAR

## DESI



## DRENCHING



## KABULI

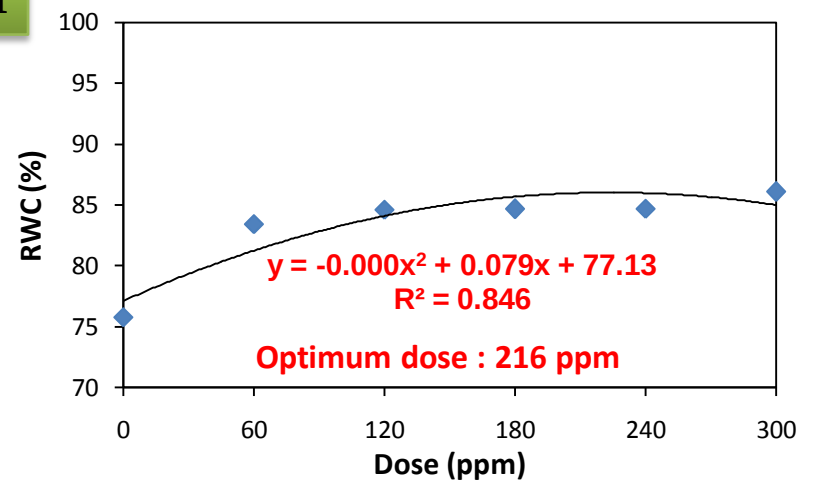
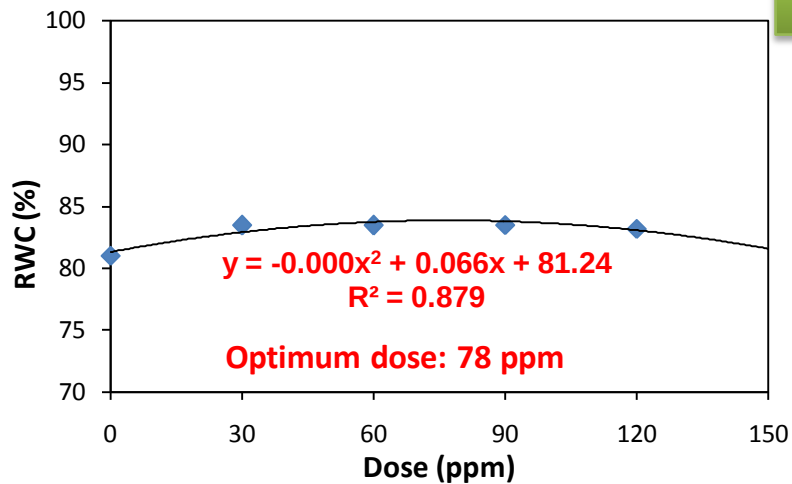
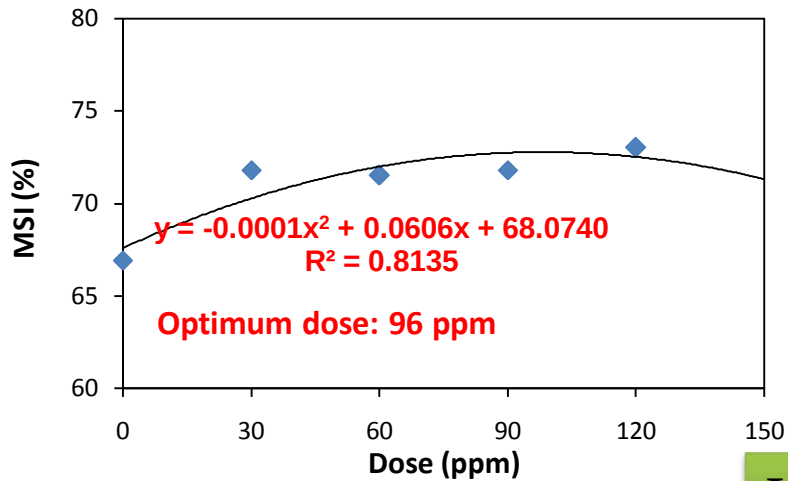


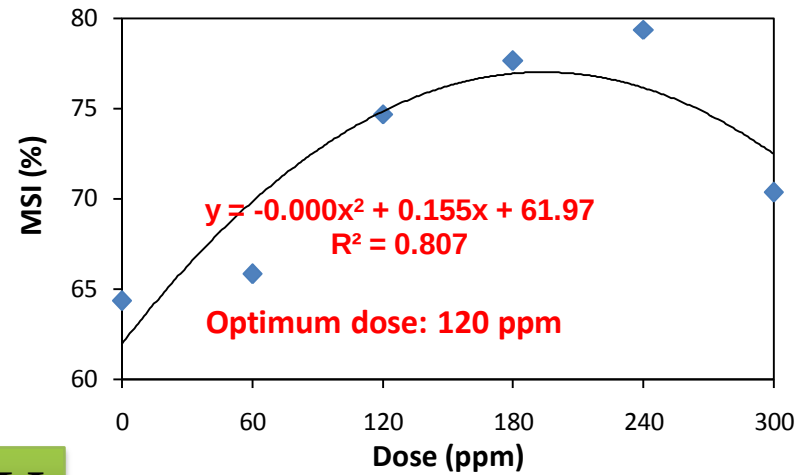
Fig.4.3.1.1.b. Relative water content response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

## DESI

### FOLIAR



### DRENCHING



## KABULI

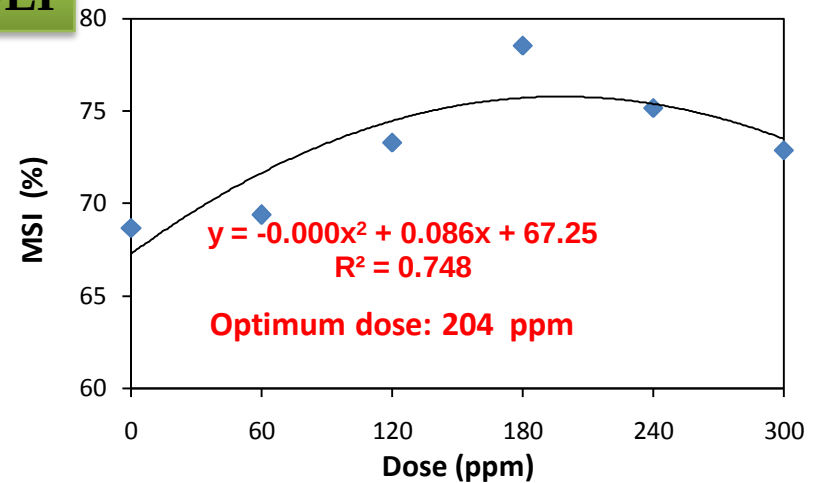
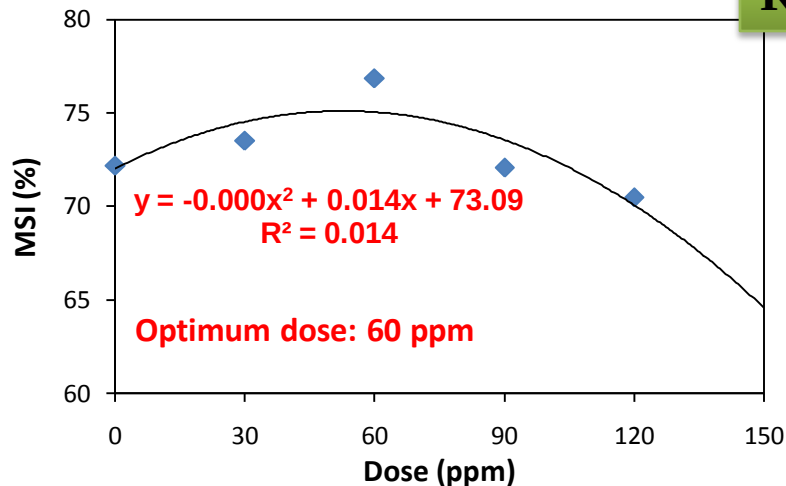
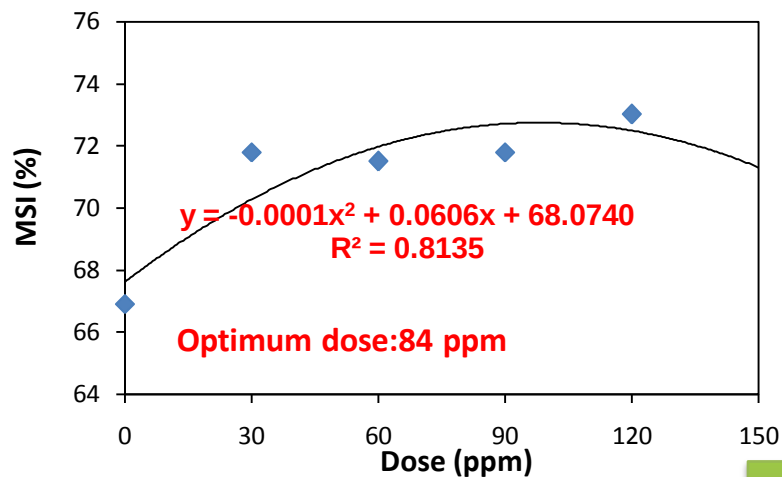


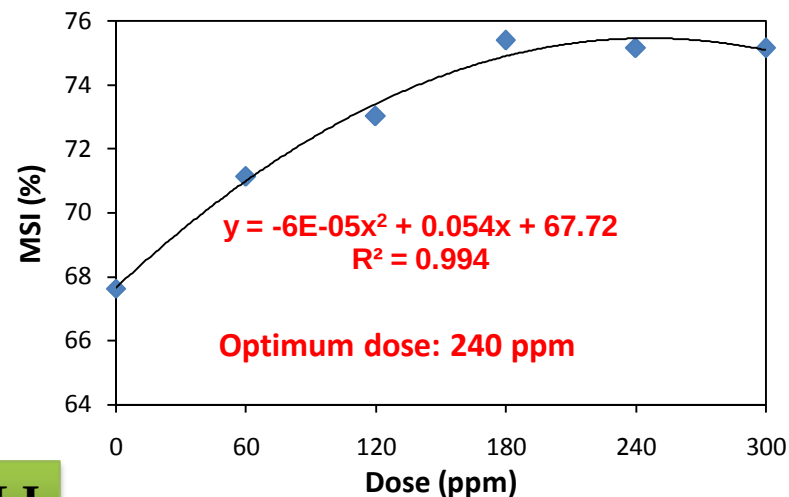
Fig.4.3.1.1.c. Membrane stability index response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

## DESI

### FOLIAR



### DRENCHING



## KABULI

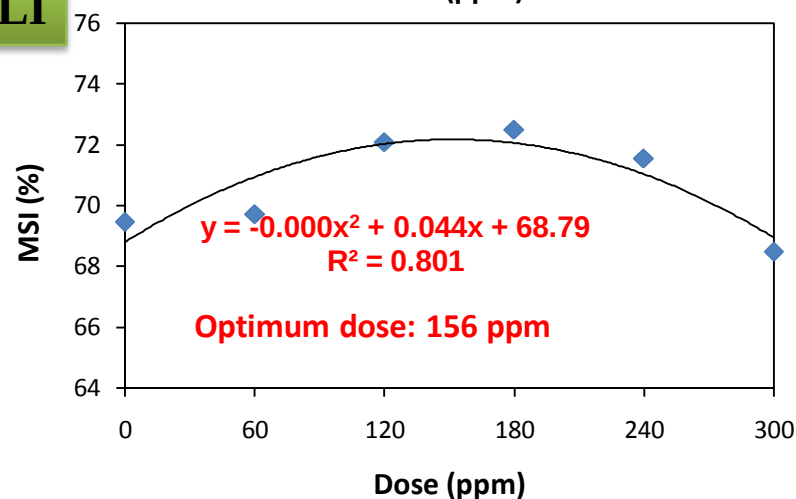
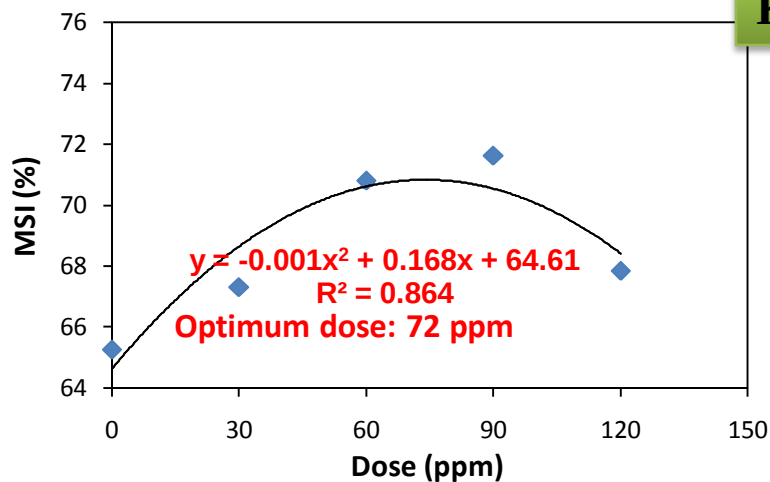


Fig.4.3.1.1.d. Membrane stability index response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

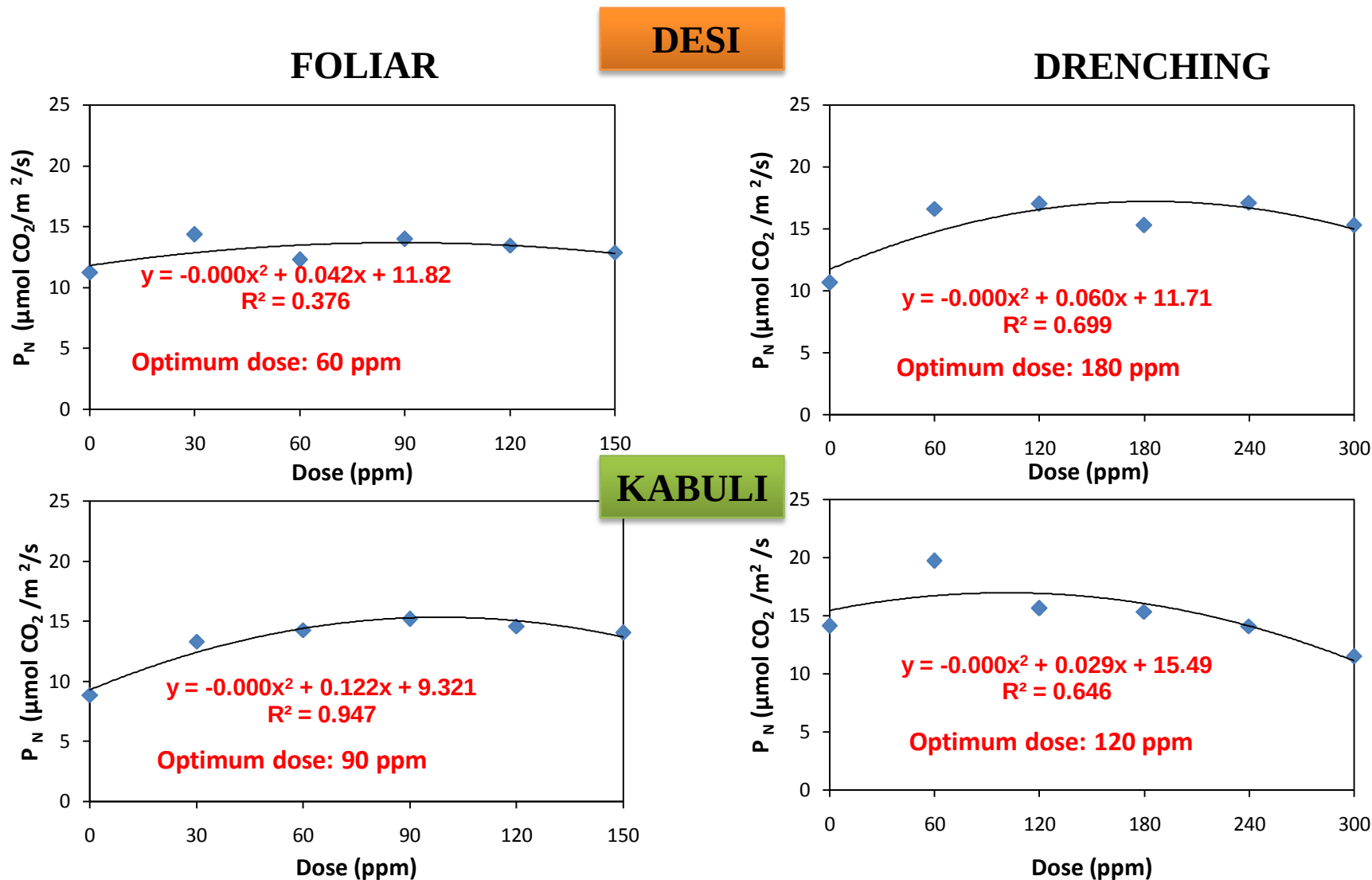
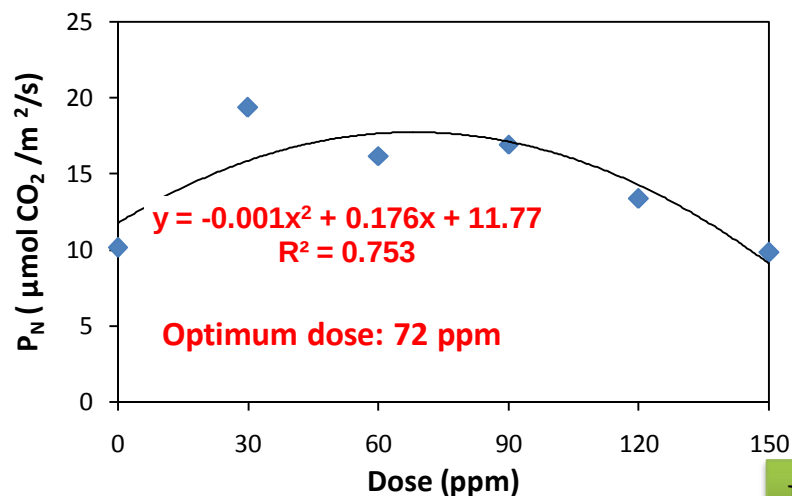


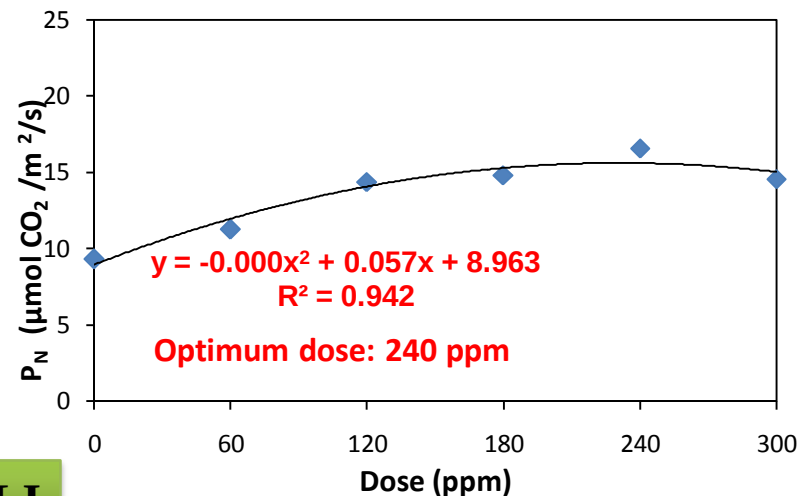
Fig.4.3.1.1.e. Photosynthesis rate response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

## FOLIAR

## DESI



## DRENCHING



## KABULI

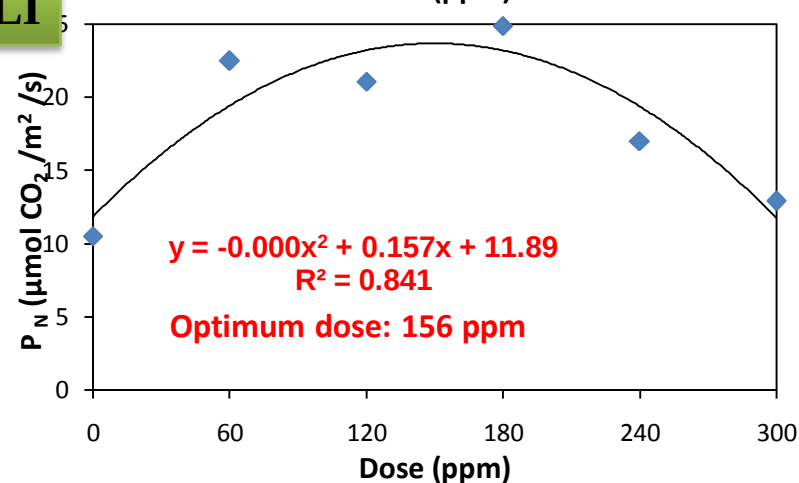
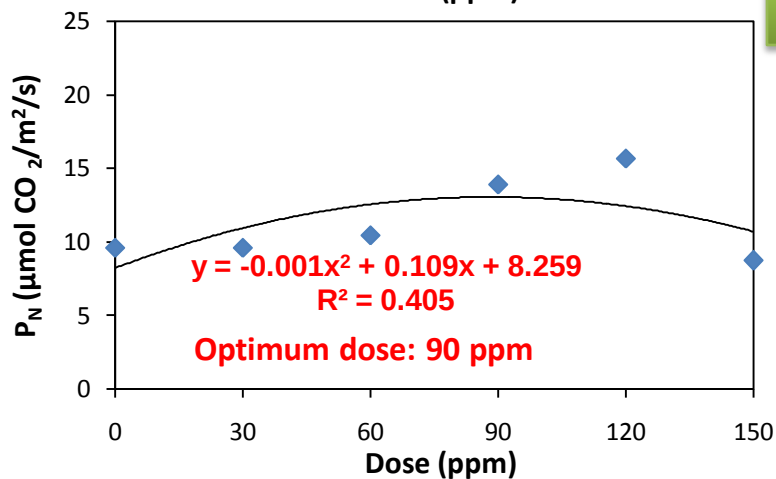


Fig.4.3.1.1.f. Photosynthesis rate response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

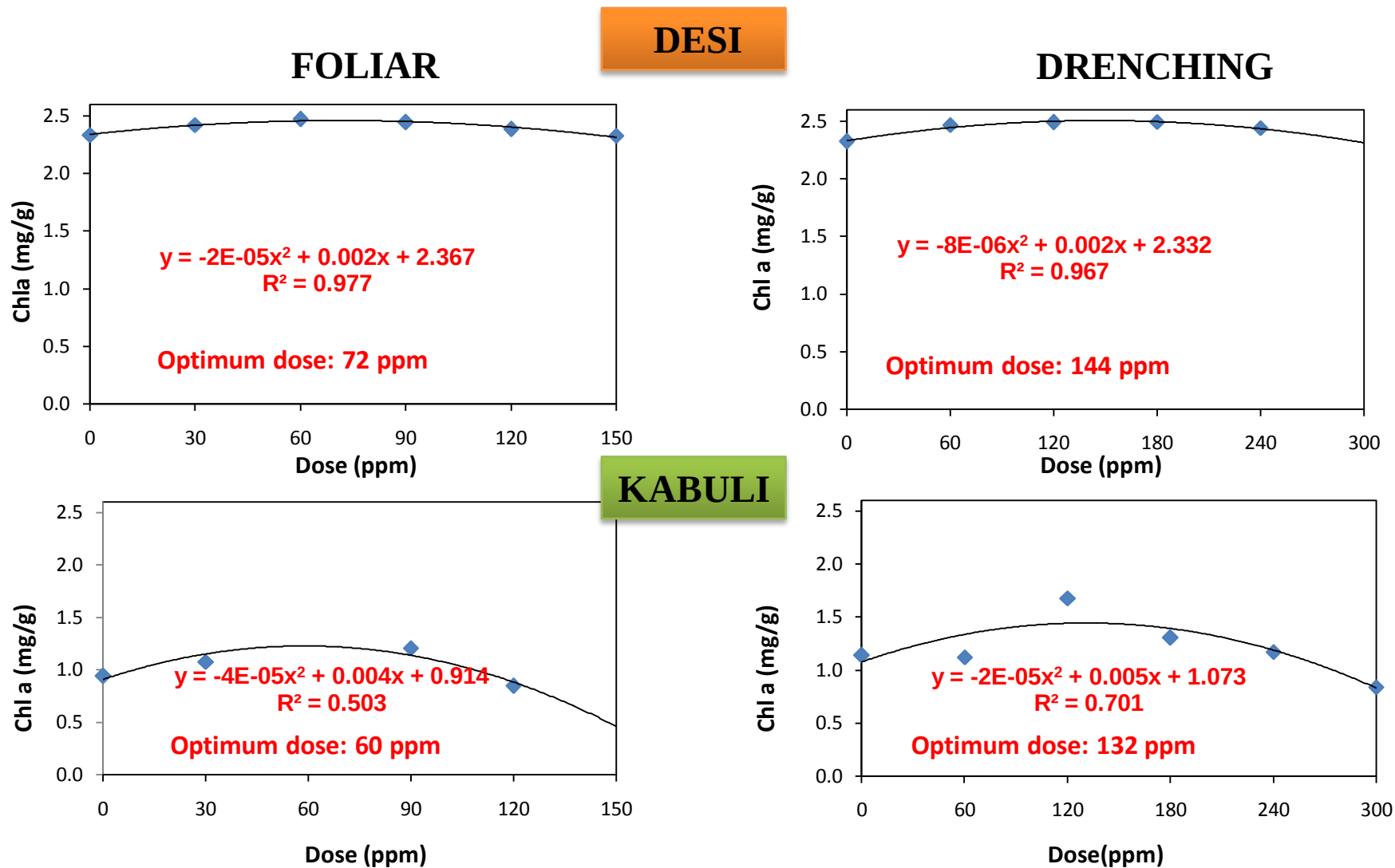
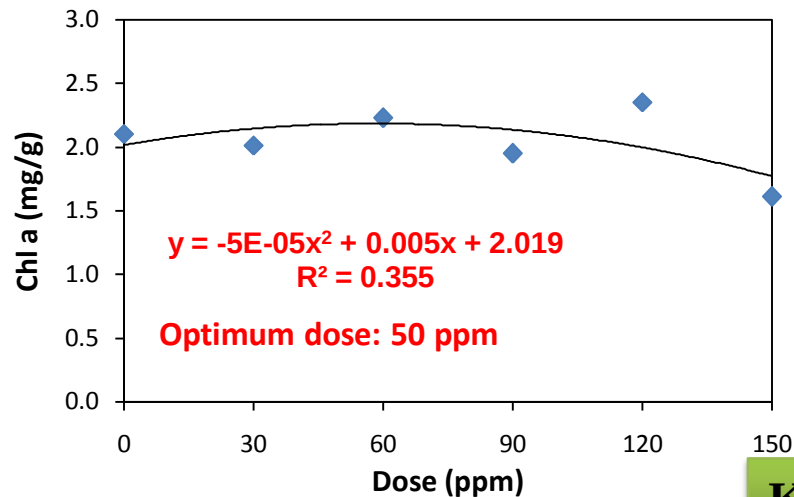


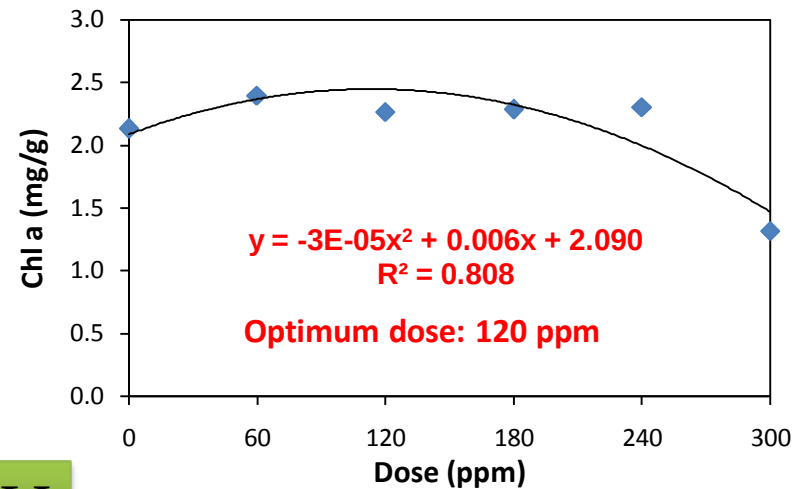
Fig.4.3.1.1.g. Chl a response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

## DESI

### FOLIAR



### DRENCHING



## KABULI

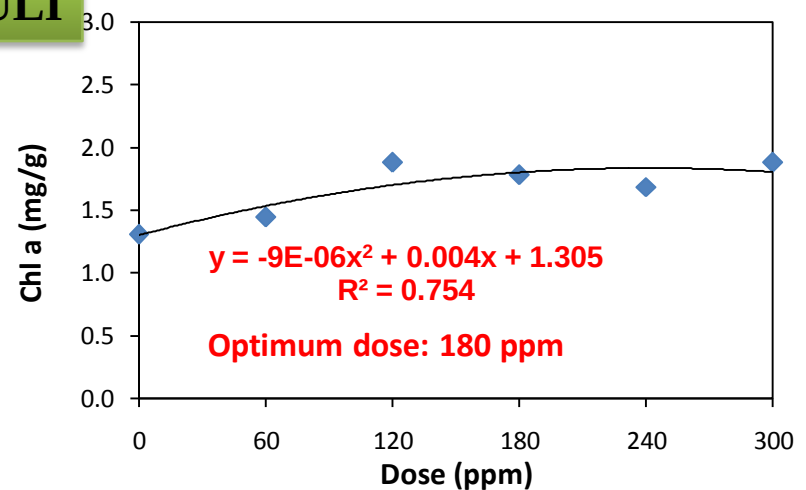
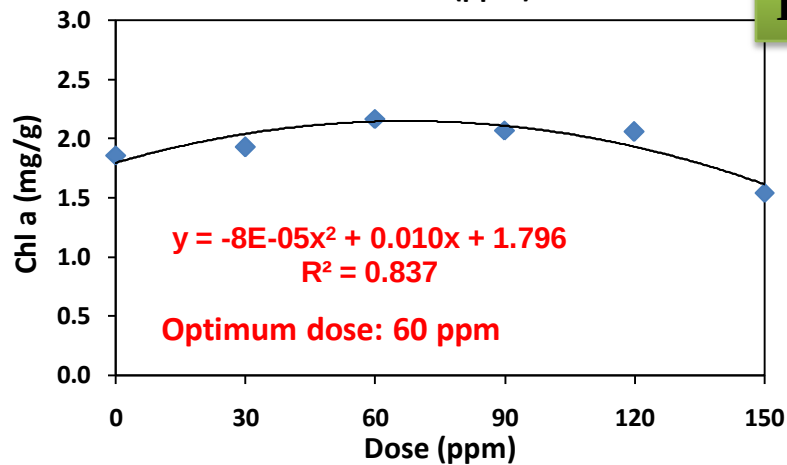
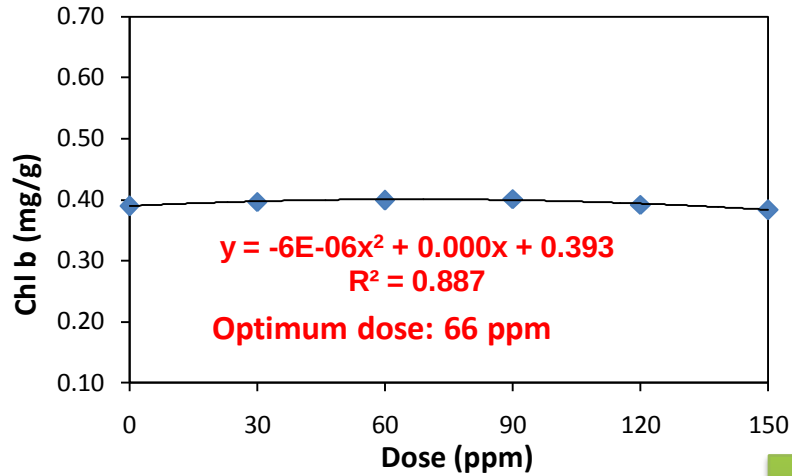


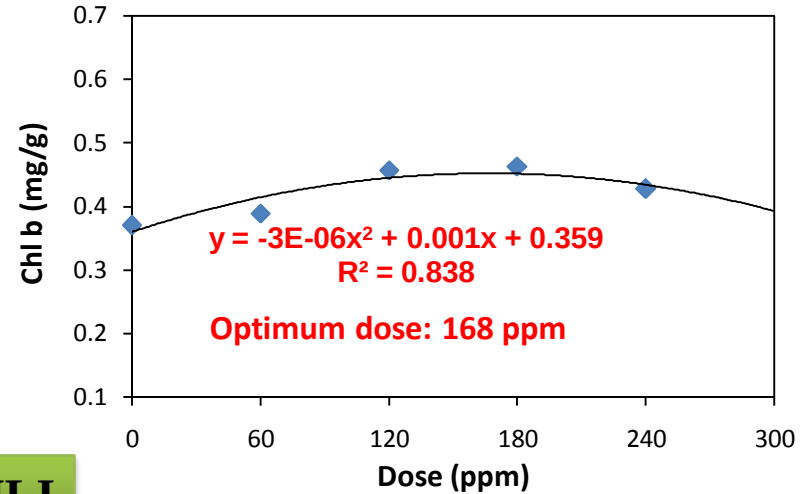
Fig.4.3.1.1.h. Chl a response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

## DESI

### FOLIAR



### DRENCHING



## KABULI

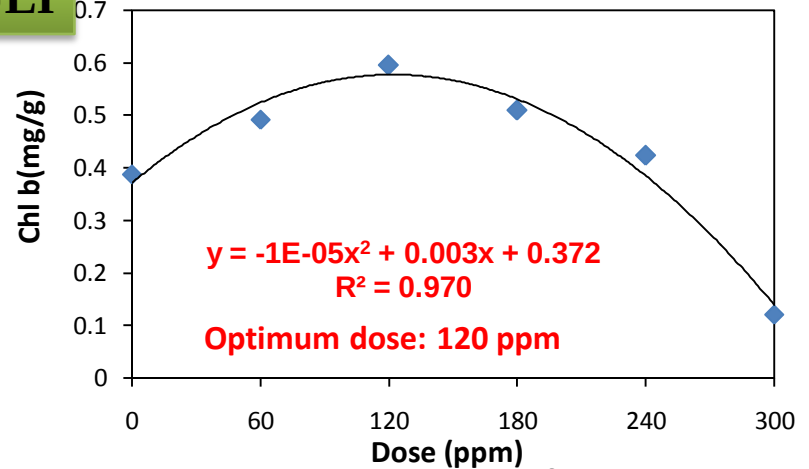
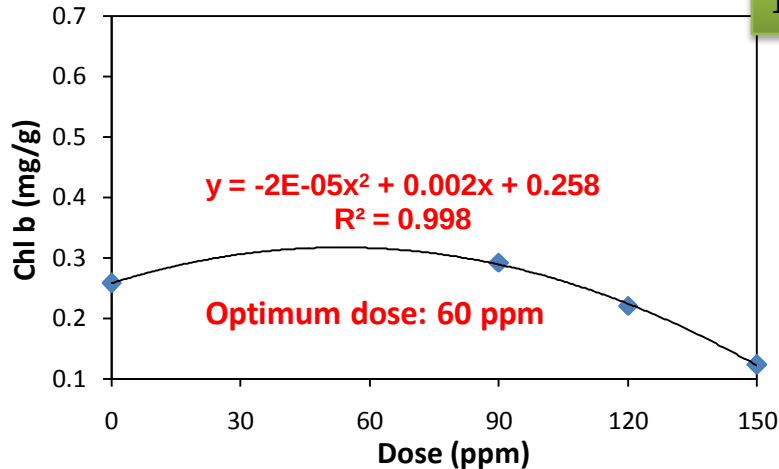
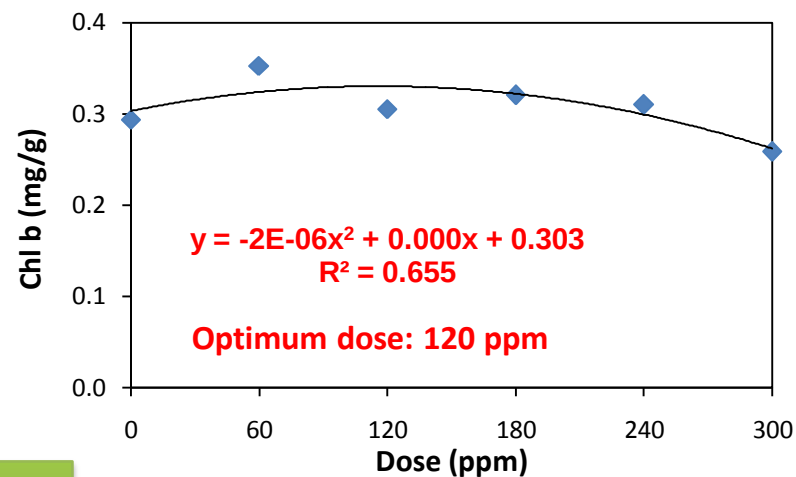
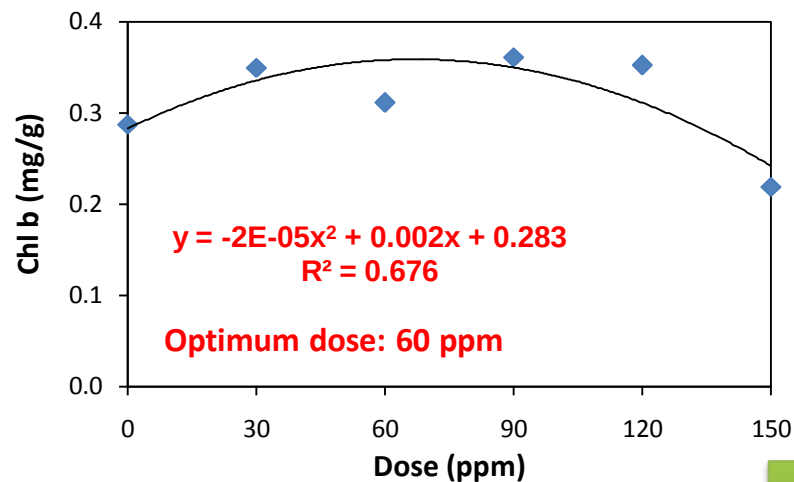


Fig.4.3.1.1.i. Chl b response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

## FOLIAR

## DESI

## DRENCHING



## KABULI

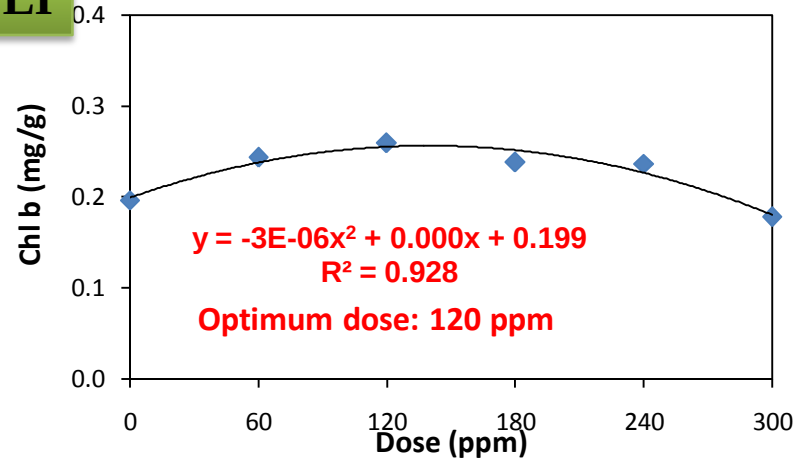
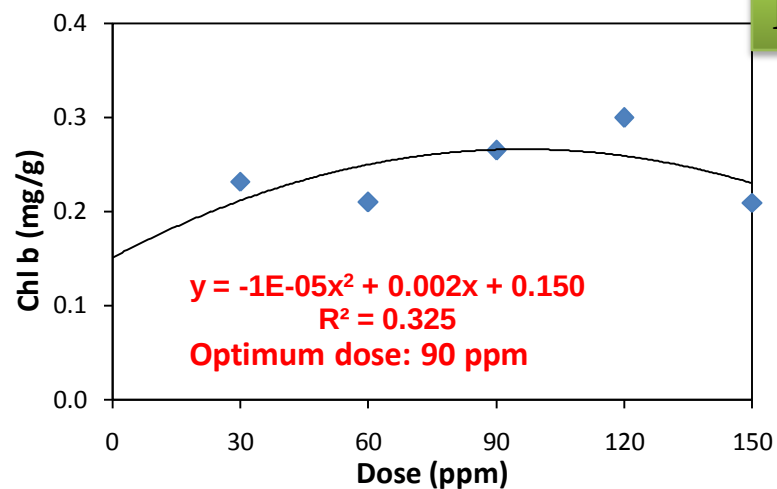
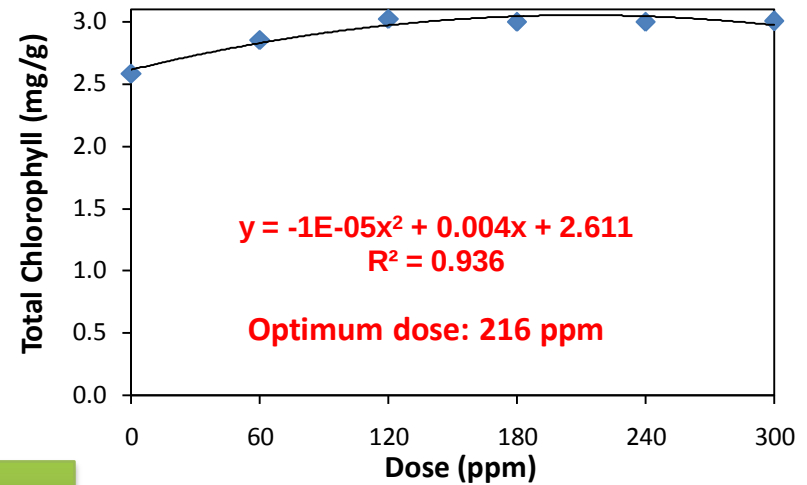
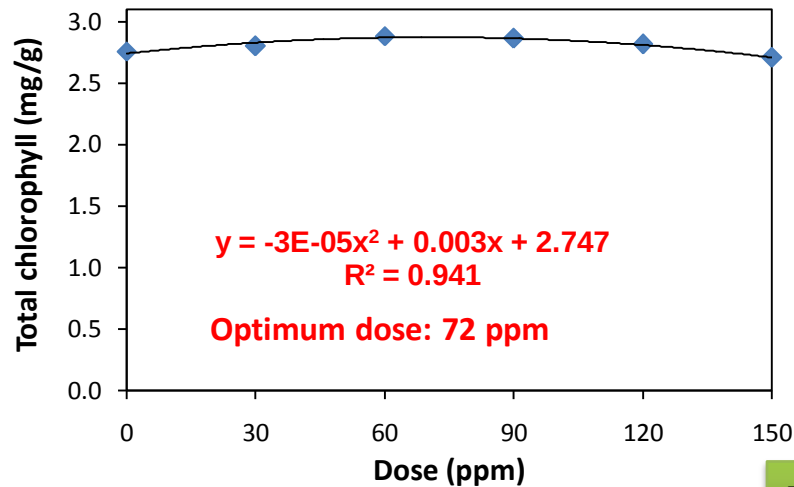


Fig.4.3.1.1.j. Chl b response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

**FOLIAR**

**DESI**

**DRENCHING**



**KABULI**

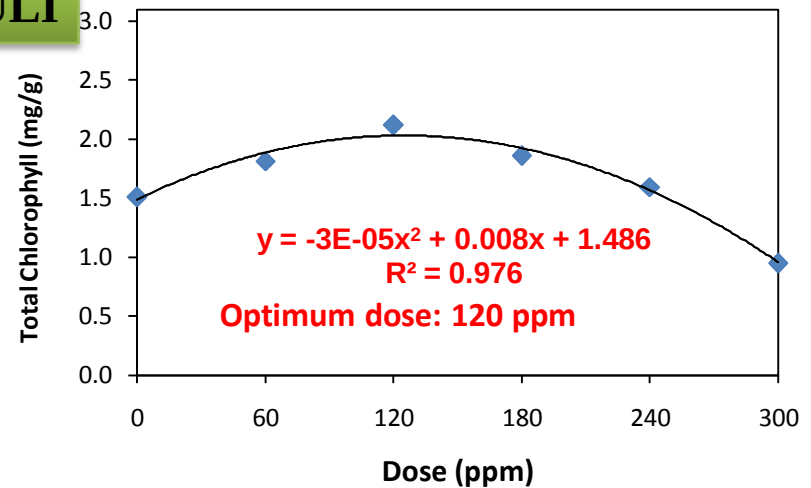
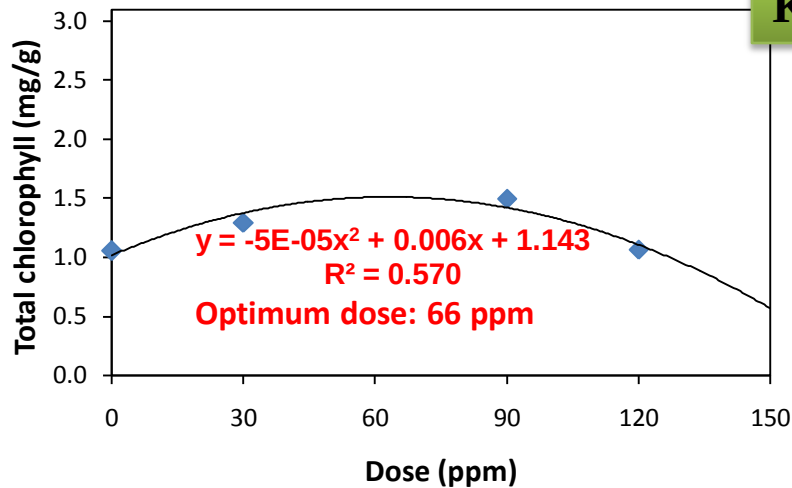


Fig.4.3.1.1.k. Total Chlorophyll response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

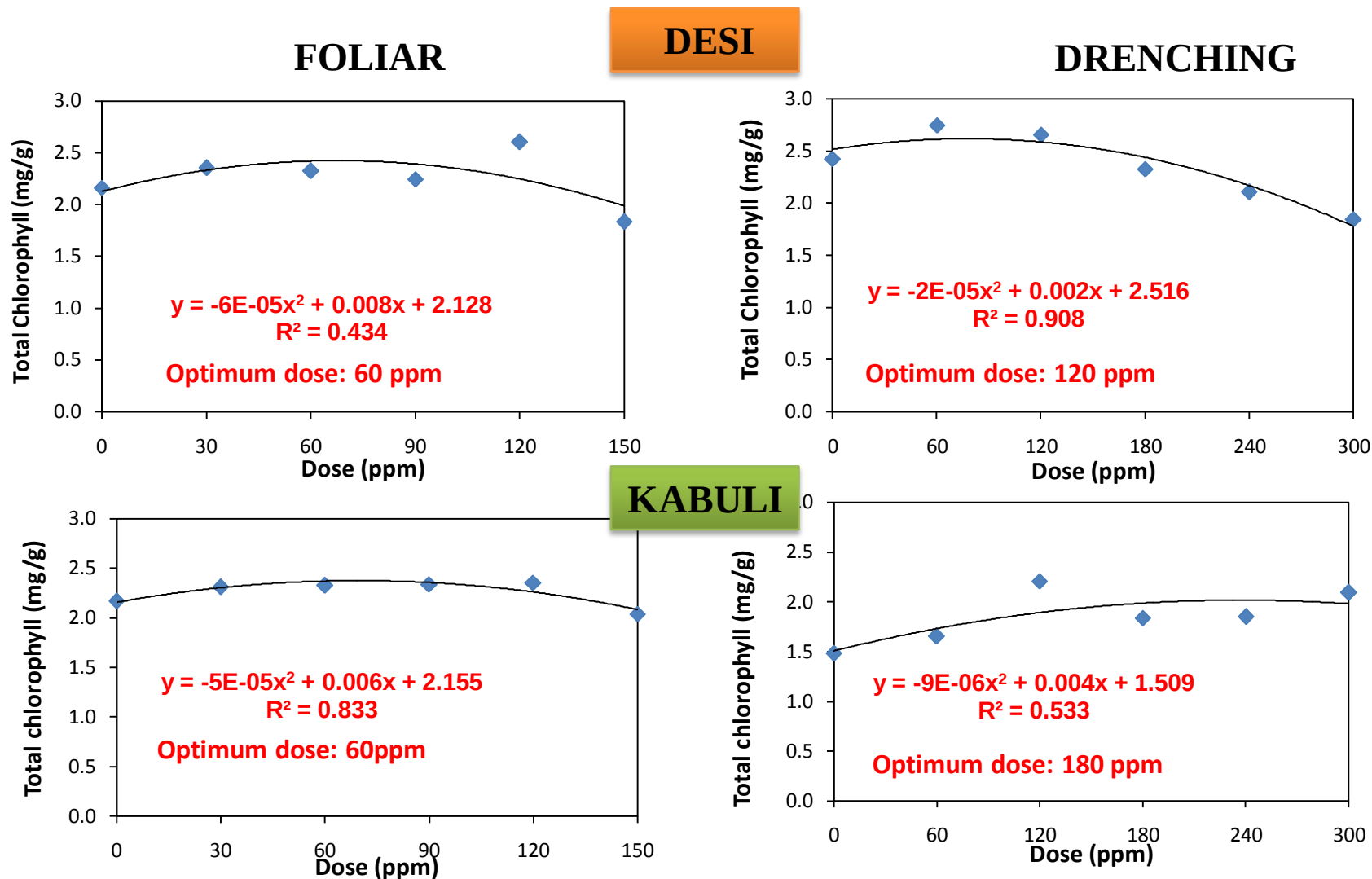
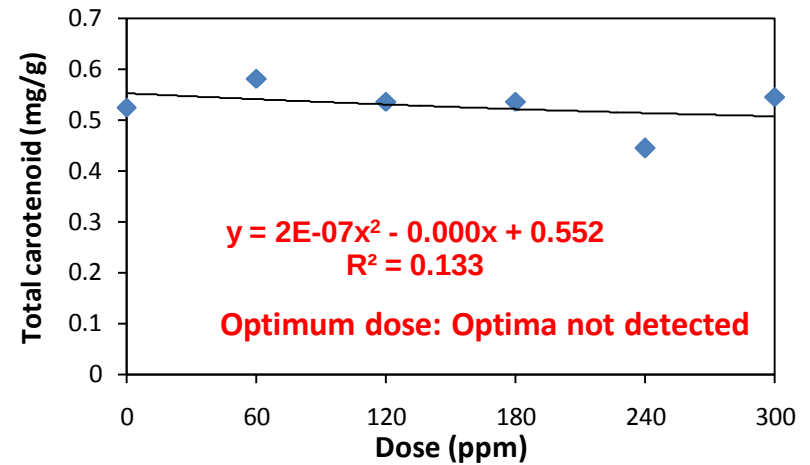
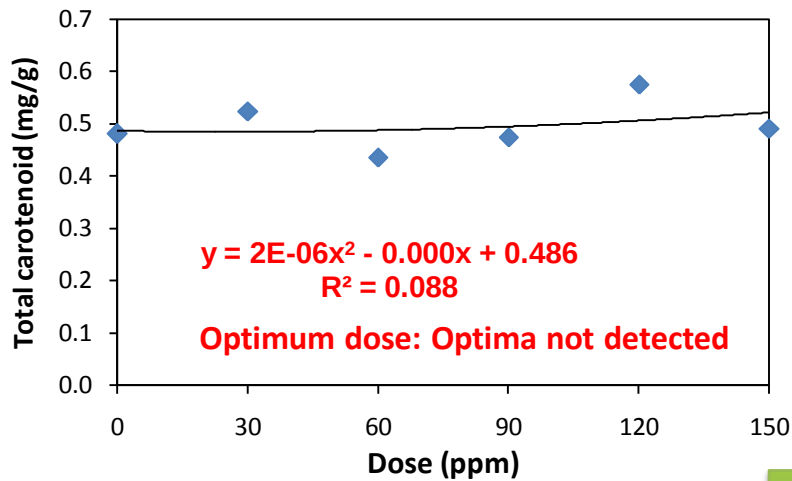


Fig.4.3.1.1.i. Total Chlorophyll response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

**FOLIAR**

**DESI**

**DRENCHING**



**KABULI**

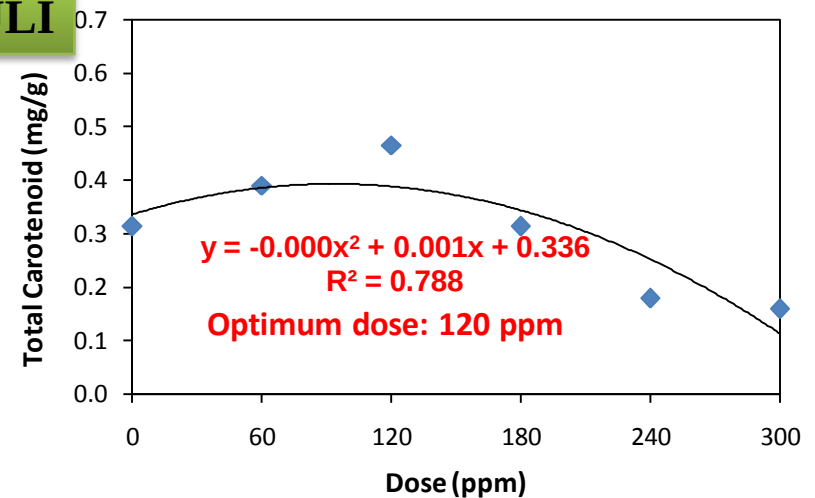
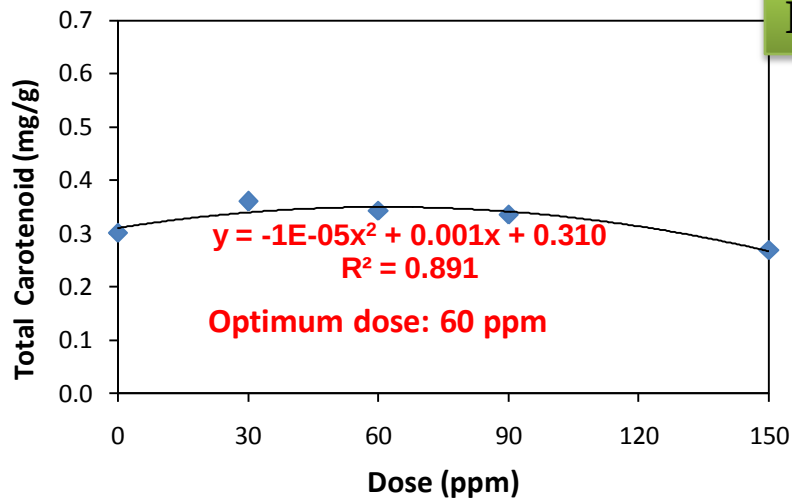


Fig.4.3.1.1.m. Total carotenoids response of *desi* and *kabuli* chickpea varieties to PBZ during imposed water deficit stress condition.

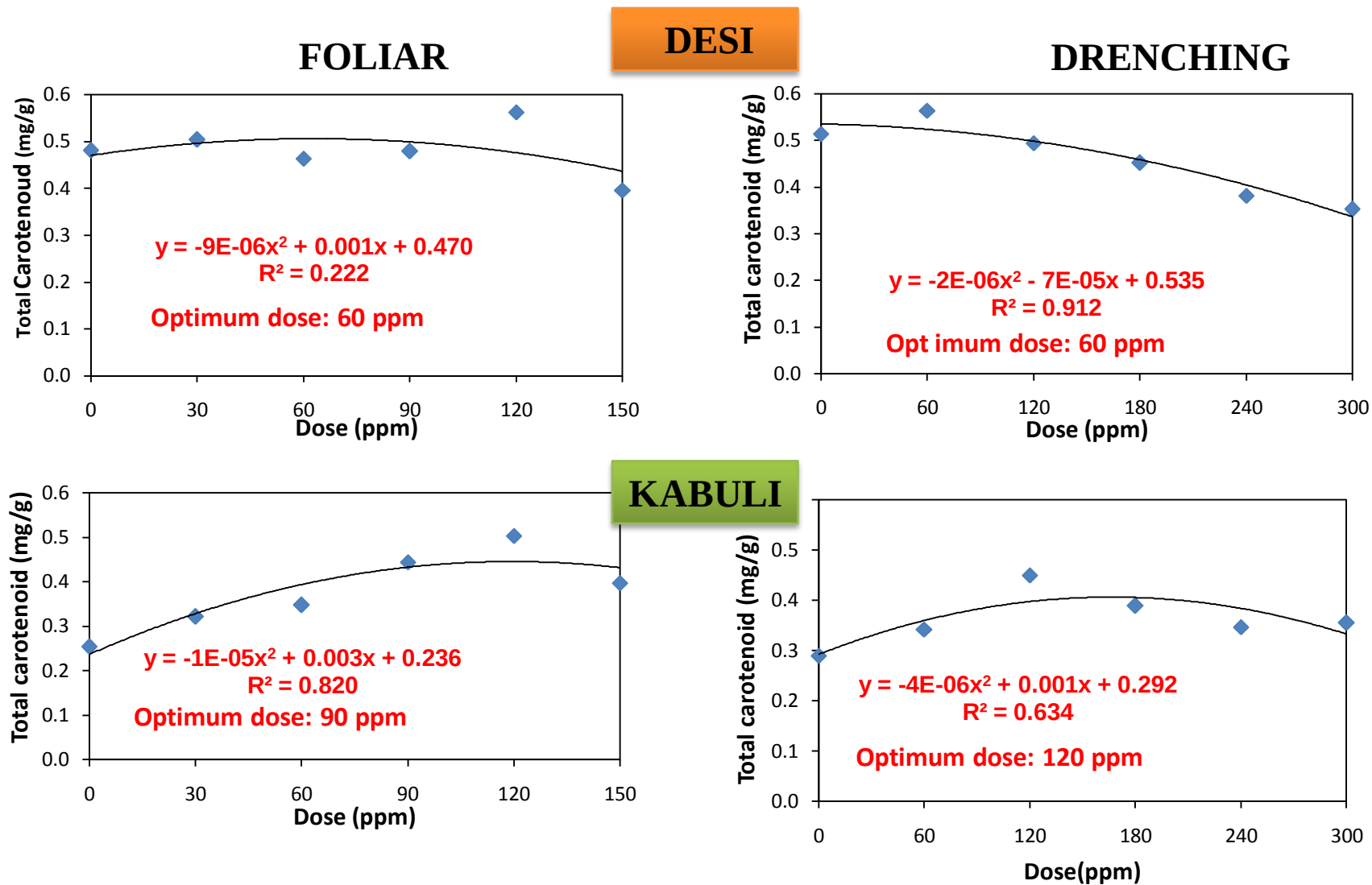


Fig.4.3.1.1.n. Total carotenoids response of *desi* and *kabuli* chickpea varieties to PBZ during recovery.

Table 4.3.1.1.a. Optimization of the foliar application dose of PBZ in Kabuli (Pusa 1108) and Desi (Pusa 362) under water deficit condition

| S. N.  | Param<br>eters<br>used<br>for<br>optimi<br>zing<br>PBZ<br>dose | Under water deficit stress          |                |                                               | After water stress termination (during<br>recovering) |                |                                               |
|--------|----------------------------------------------------------------|-------------------------------------|----------------|-----------------------------------------------|-------------------------------------------------------|----------------|-----------------------------------------------|
|        |                                                                | Regression equation                 | R <sup>2</sup> | Estim<br>ated<br>Opti<br>mum<br>dose<br>(ppm) | Regression equation                                   | R <sup>2</sup> | Estim<br>ated<br>Optim<br>um<br>dose<br>(ppm) |
| Kabuli |                                                                |                                     |                |                                               |                                                       |                |                                               |
| 1      | RWC                                                            | $y = -0.001x^2 + 0.200x + 51.12$    | 0.863          | 78                                            | $y = -0.000x^2 + 0.066x + 81.24$                      | 0.879          | 78                                            |
| 2      | MSI                                                            | $y = -0.000x^2 + 0.014x + 73.09$    | 0.014          | 60                                            | $y = -0.001x^2 + 0.168x + 64.61$                      | 0.864          | 72                                            |
| 3      | P <sub>N</sub>                                                 | $y = -0.000x^2 + 0.122x + 9.321$    | 0.947          | 90                                            | $y = -0.001x^2 + 0.109x + 8.259$                      | 0.405          | 90                                            |
| 4      | Chl a                                                          | $y = -4E-05x^2 + 0.004x + 0.914$    | 0.503          | 60                                            | $y = -8E-05x^2 + 0.010x + 1.796$                      | 0.837          | 60                                            |
| 5      | Chl b                                                          | $y = -2E-05x^2 + 0.002x + 0.258$    | 0.998          | 60                                            | $y = -1E-05x^2 + 0.002x + 0.150$                      | 0.325          | 90                                            |
| 6      | TOTAL<br>Chl                                                   | $y = -5E-05x^2 + 0.006x + 1.143$    | 0.570          | 66                                            | $y = -5E-05x^2 + 0.006x + 2.155$                      | 0.833          | 60                                            |
| 7      | TOTAL<br>Carot.                                                | $y = -1E-05x^2 + 0.001x + 0.310$    | 0.891          | 60                                            | $y = -1E-05x^2 + 0.003x + 0.236$                      | 0.820          | 90                                            |
| Desi   |                                                                |                                     |                |                                               |                                                       |                |                                               |
| 1      | RWC                                                            | $y = -0.0021x^2 + 0.263x + 49.544$  | 0.994          | 62.66                                         | $y = -0.000x^2 + 0.193x + 84.66$                      | 0.809          | 102                                           |
| 2      | MSI                                                            | $y = -0.0001x^2 + 0.0606x + 68.074$ | 0.815          | 96                                            | $y = -0.000x^2 + 0.134x + 67.99$                      | 0.826          | 84                                            |
| 3      | P <sub>N</sub>                                                 | $y = -0.000x^2 + 0.042x + 11.82$    | 0.376          | 60                                            | $y = -0.001x^2 + 0.176x + 11.77$                      | 0.753          | 72                                            |
| 4      | Chl a                                                          | $y = -2E-05x^2 + 0.002x + 2.367$    | 0.977          | 72                                            | $y = -5E-05x^2 + 0.005x + 2.019$                      | 0.355          | 50                                            |
| 5      | Chl b                                                          | $y = -6E-06x^2 + 0.000x + 0.393$    | 0.887          | 66                                            | $y = -2E-05x^2 + 0.002x + 0.283$                      | 0.676          | 60                                            |
| 6      | TOTAL<br>Chl                                                   | $y = -3E-05x^2 + 0.003x + 2.747$    | 0.941          | 72                                            | $y = -6E-05x^2 + 0.008x + 2.128$                      | 0.434          | 60                                            |
| 7      | Total<br>Carot.                                                | $y = 2E-06x^2 - 0.000x + 0.486$     | 0.088          |                                               | $y = -9E-06x^2 + 0.001x + 0.470$                      | 0.222          | 60                                            |

Table 4.3.1.1.b. Optimization of the drenching application dose of PBZ in Kabuli (Pusa 1108) and Desi (Pusa 362) under water deficit condition

| S. N.  | Paramet<br>ers used<br>for<br>optimizi<br>ng PBZ<br>dose | Under water deficit stress       |                |                                               | After water stress termination (during<br>recovering) |                |                                               |
|--------|----------------------------------------------------------|----------------------------------|----------------|-----------------------------------------------|-------------------------------------------------------|----------------|-----------------------------------------------|
|        |                                                          | Regression equation              | R <sup>2</sup> | Estim<br>ated<br>Opti<br>mum<br>dose<br>(ppm) | Regression equation                                   | R <sup>2</sup> | Estim<br>ated<br>Opti<br>mum<br>dose<br>(ppm) |
| Kabuli |                                                          |                                  |                |                                               |                                                       |                |                                               |
| 1      | RWC                                                      | $y = -0.000x^2 + 0.088x + 50.59$ | 0.301          | 132                                           | $y = -0.000x^2 + 0.079x + 77.13$                      | 0.846          | 216                                           |
| 2      | MSI                                                      | $y = -0.000x^2 + 0.086x + 67.25$ | 0.748          | 204                                           | $y = -0.000x^2 + 0.044x + 68.79$                      | 0.801          | 156                                           |
| 3      | P <sub>N</sub>                                           | $y = -0.000x^2 + 0.122x + 9.321$ | 0.947          | 90                                            | $y = -0.000x^2 + 0.157x + 11.89$                      | 0.841          | 156                                           |
| 4      | Chl a                                                    | $y = -2E-05x^2 + 0.005x + 1.073$ | 0.701          | 132                                           | $y = -9E-06x^2 + 0.004x + 1.305$                      | 0.754          | 180                                           |
| 5      | Chl b                                                    | $y = -2E-05x^2 + 0.002x + 0.258$ | 0.998          | 60                                            | $y = -3E-06x^2 + 0.000x + 0.199$                      | 0.928          | 120                                           |
| 6      | TOTAL<br>Chl                                             | $y = -3E-05x^2 + 0.008x + 1.486$ | 0.976          | 120                                           | $y = -9E-06x^2 + 0.004x + 1.509$                      | 0.533          | 180                                           |
| 7      | TOTAL<br>Carot.                                          | $y = -0.000x^2 + 0.001x + 0.336$ | 0.788          | 120                                           | $y = -4E-06x^2 + 0.001x + 0.292$                      | 0.634          | 120                                           |
| Desi   |                                                          |                                  |                |                                               |                                                       |                |                                               |
| 1      | RWC                                                      | $y = -0.000x^2 + 0.063x + 61.32$ | 0.754          | 144                                           | $y = -6E-05x^2 - 0.003x + 85.77$                      | 0.335          | 60                                            |
| 2      | MSI                                                      | $y = -0.000x^2 + 0.155x + 61.97$ | 0.807          | 120                                           | $y = -6E-05x^2 + 0.054x + 67.72$                      | 0.994          | 240                                           |
| 3      | P <sub>N</sub>                                           | $y = -0.000x^2 + 0.060x + 11.71$ | 0.699          | 180                                           | $y = -0.000x^2 + 0.057x + 8.963$                      | 0.942          | 240                                           |
| 4      | Chl a                                                    | $y = -8E-06x^2 + 0.002x + 2.332$ | 0.967          | 144                                           | $y = -3E-05x^2 + 0.006x + 2.090$                      | 0.808          | 120                                           |
| 5      | Chl b                                                    | $y = -3E-06x^2 + 0.001x + 0.359$ | 0.838          | 168                                           | $y = -2E-06x^2 + 0.000x + 0.303$                      | 0.655          | 120                                           |
| 6      | TOTAL Chl                                                | $y = -1E-05x^2 + 0.004x + 2.611$ | 0.936          | 216                                           | $y = -2E-05x^2 + 0.002x + 2.516$                      | 0.908          | 120                                           |
| 7      | Total<br>Carot.                                          | $y = 2E-07x^2 - 0.000x + 0.552$  | 0.133          |                                               | $y = -2E-06x^2 - 7E-05x + 0.535$                      | 0.912          | 60                                            |

stress and recovered after water stress termination. Both PBZ foliar and drenching application during water deficit condition maintained higher RWC values over their respective controls. Furthermore, after water stress termination recovery in terms of RWC was also obtained higher in both PBZ foliar and drenching treated plants. During water deficit condition and thereafter during recovery, drenching application of PBZ maintained the higher value of RWC than foliar application of PBZ (Fig. 4.3.1.2.1. and Table 4.3.1.2.1.).

#### **4.3.1.2.2 Membrane stability index**

Membrane stability index response of both chickpea varieties at reproductive stage under well watered and water stressed conditions showed a trend similar to RWC. MSI gradually decreased with increasing duration of imposed water stress and gradually recovered after water stress termination. In general, plants treated with PBZ maintained the higher values of MSI under water stress and also showed fast recovery after water stress termination. In both the varieties, drenching application of PBZ maintained the higher value of MSI under water stress as well as after water stress termination (Fig. 4.3.1.2.2. and Table 4.3.1.2.2.).

#### **4.3.1.2.3. Growth parameters**

##### **4.3.1.2.3.1. Shoot length**

Reduction in the shoot length of both the varieties was recorded under water deficit conditions. Application of both PBZ treatments showed the non significant alteration in shoot length under both normal and water deficit conditions (Fig. 4.3.1.2.3.1. and Table 4.3.1.2.3.1.).

##### **4.3.1.2.3.2. Root length**

Root length was increased with the increase in the period of water deficit condition. Maximum enhancement in root length was recorded in both *desi* and *kabuli* varieties under both well watered and water deficit conditions with the drenching application of PBZ followed by its foliar application of PBZ. However, during the water deficit period plants treated with foliar application of PBZ had the higher root length than the drenching application and thereafter during recovery plants treated with PBZ drenching had the longer root length (Fig. 4.3.1.2.3.2. and Table 4.3.1.2.3.2.).

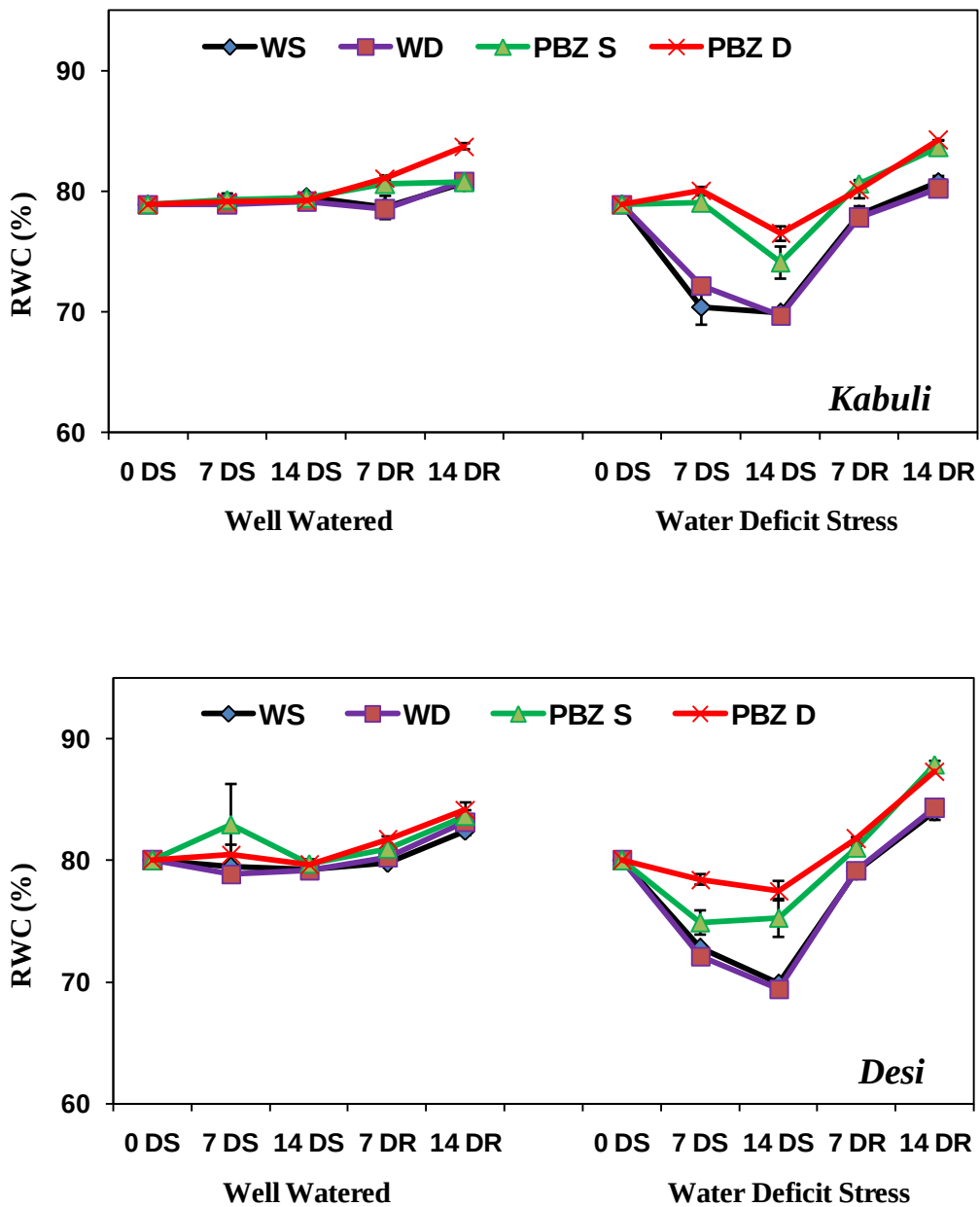


Fig. 4.3.1.2.1. Effect of PBZ on relative water content (RWC) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.1. Effect of PBZ on relative water content (RWC) in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Relative water content (%) |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|----------------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress         |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                     | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 78.90                      | 80.01 | 79.46 | 78.93              | 79.47 | 79.20 | 79.53               | 79.30 | 79.42 | 78.65          | 79.79 | 79.22 | 80.71           | 82.44 | 81.58 |
|                | Water Drenching           | 78.90                      | 80.01 | 79.46 | 78.89              | 78.93 | 78.91 | 79.13               | 79.23 | 79.18 | 78.50          | 80.28 | 79.39 | 80.85           | 83.22 | 82.03 |
|                | PBZ Foliar spray (60 ppm) | 78.90                      | 80.01 | 79.46 | 79.32              | 82.99 | 81.16 | 79.43               | 79.76 | 79.59 | 80.58          | 81.00 | 80.79 | 80.76           | 83.68 | 82.22 |
|                | PBZ Drenching (120 ppm)   | 78.90                      | 80.01 | 79.46 | 79.15              | 80.50 | 79.83 | 79.21               | 79.65 | 79.43 | 81.10          | 81.72 | 81.41 | 83.73           | 84.20 | 83.97 |
|                | Mean                      | 78.90                      | 80.01 | 79.46 | 79.07              | 80.47 | 79.77 | 79.32               | 79.49 | 79.40 | 79.71          | 80.70 | 80.20 | 81.51           | 83.39 | 82.45 |
| Water stressed | Water Foliar spray        | 78.90                      | 80.01 | 79.46 | 70.41              | 72.78 | 71.60 | 69.89               | 69.91 | 69.90 | 78.03          | 79.14 | 78.59 | 80.78           | 84.03 | 82.41 |
|                | Water Drenching           | 78.90                      | 80.01 | 79.46 | 72.19              | 72.14 | 72.16 | 69.68               | 69.45 | 69.57 | 77.81          | 79.23 | 78.52 | 80.21           | 84.33 | 82.27 |
|                | PBZ Foliar spray (60 ppm) | 78.90                      | 80.01 | 79.46 | 79.05              | 74.91 | 76.98 | 74.07               | 75.29 | 74.68 | 80.61          | 81.08 | 80.84 | 83.64           | 87.92 | 85.78 |
|                | PBZ Drenching (120 ppm)   | 78.90                      | 80.01 | 79.46 | 80.10              | 78.46 | 79.28 | 76.48               | 77.53 | 77.01 | 80.16          | 81.80 | 80.98 | 84.22           | 87.35 | 85.79 |
|                | Mean                      | 78.90                      | 80.01 | 79.46 | 75.44              | 74.57 | 75.00 | 72.53               | 73.05 | 72.79 | 79.15          | 80.31 | 79.73 | 82.21           | 85.91 | 84.06 |
|                | SEM                       | 0.283                      |       |       | 0.59               |       |       | 0.362               |       |       | 0.247          |       |       | 0.192           |       |       |
|                | CD at 5 %                 |                            |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                        |       |       | 0.601              |       |       | 0.368               |       |       | 0.252          |       |       | 0.196           |       |       |
|                | Treatment (T)             | N/A                        |       |       | 0.849              |       |       | 0.521               |       |       | 0.356          |       |       | 0.277           |       |       |
|                | Varieties (V)             | 0.288                      |       |       | N/A                |       |       | N/A                 |       |       | 0.252          |       |       | 0.196           |       |       |
|                | W x T =                   | 0.576                      |       |       | 1.201              |       |       | 0.737               |       |       | N/A            |       |       | 0.392           |       |       |
|                | W x V =                   | 0.407                      |       |       | 0.849              |       |       | N/A                 |       |       | N/A            |       |       | 0.277           |       |       |
|                | T x V =                   | 0.576                      |       |       | N/A                |       |       | N/A                 |       |       | 0.504          |       |       | 0.392           |       |       |
|                | W x T x V=                | N/A                        |       |       | 1.699              |       |       | N/A                 |       |       | N/A            |       |       | 0.555           |       |       |

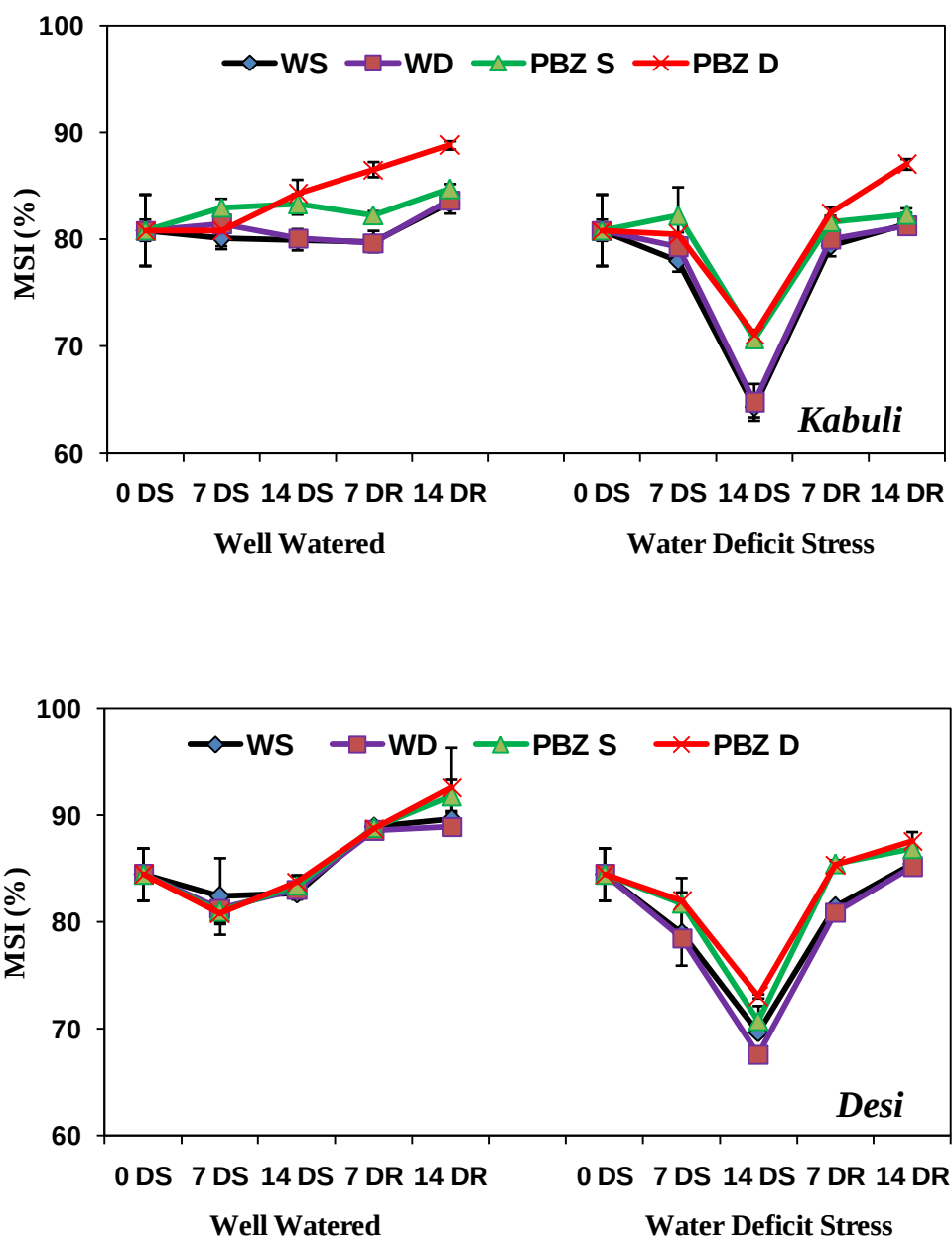


Fig.4.3.1.2.2. Effect of PBZ on membrane stability index (MSI) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.2. Effect of PBZ on membrane stability index (MSI) in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Membrane stability index (%) |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|------------------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress           |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                       | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 80.86                        | 84.45 | 82.65 | 80.09              | 82.40 | 81.24 | 79.97               | 82.69 | 81.33 | 79.80          | 88.99 | 84.39 | 83.43           | 89.65 | 86.54 |
|                | Water Drenching           | 80.86                        | 84.45 | 82.65 | 81.44              | 81.29 | 81.37 | 80.15               | 83.08 | 81.62 | 79.69          | 88.59 | 84.14 | 83.73           | 88.95 | 86.34 |
|                | PBZ Foliar spray (60 ppm) | 80.86                        | 84.45 | 82.65 | 82.97              | 80.98 | 81.98 | 83.29               | 83.45 | 83.37 | 82.30          | 88.85 | 85.58 | 84.73           | 91.78 | 88.25 |
|                | PBZ Drenching (120 ppm)   | 80.86                        | 84.45 | 82.65 | 80.80              | 80.82 | 80.81 | 84.28               | 83.81 | 84.05 | 86.55          | 88.80 | 87.68 | 88.82           | 92.65 | 90.73 |
|                | Mean                      | 80.86                        | 84.45 | 82.65 | 81.32              | 81.37 | 81.35 | 81.92               | 83.26 | 82.59 | 82.09          | 88.81 | 85.45 | 85.18           | 90.76 | 87.97 |
| Water stressed | Water Foliar spray        | 80.86                        | 84.45 | 82.65 | 77.98              | 78.92 | 78.45 | 64.33               | 69.69 | 67.01 | 79.42          | 81.43 | 80.43 | 81.47           | 85.49 | 83.48 |
|                | Water Drenching           | 80.86                        | 84.45 | 82.65 | 79.28              | 78.51 | 78.89 | 64.74               | 67.66 | 66.20 | 79.99          | 80.93 | 80.46 | 81.27           | 85.18 | 83.23 |
|                | PBZ Foliar spray (60 ppm) | 80.86                        | 84.45 | 82.65 | 82.24              | 81.72 | 81.98 | 70.67               | 70.77 | 70.72 | 81.65          | 85.44 | 83.55 | 82.30           | 86.86 | 84.58 |
|                | PBZ Drenching (120 ppm)   | 80.86                        | 84.45 | 82.65 | 80.44              | 81.98 | 81.21 | 71.10               | 73.04 | 72.07 | 82.54          | 85.39 | 83.97 | 87.04           | 87.65 | 87.35 |
|                | Mean                      | 80.86                        | 84.45 | 82.65 | 79.99              | 80.28 | 80.13 | 67.71               | 70.29 | 69.00 | 80.90          | 83.30 | 82.10 | 83.02           | 86.29 | 84.66 |
|                | SEM                       | 0.681                        |       |       | 0.957              |       |       | 0.666               |       |       | 0.254          |       |       | 0.639           |       |       |
|                | CD at 5 %                 |                              |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                          |       |       | 0.975              |       |       | 0.679               |       |       | 0.259          |       |       | 0.651           |       |       |
|                | Treatment (T)             | N/A                          |       |       | 1.379              |       |       | 0.96                |       |       | 0.366          |       |       | 0.92            |       |       |
|                | Varieties (V)             | 1.713                        |       |       | N/A                |       |       | 0.679               |       |       | 0.259          |       |       | 0.651           |       |       |
|                | W x T =                   | N/A                          |       |       | 1.95               |       |       | 1.357               |       |       | 0.517          |       |       | N/A             |       |       |
|                | W x V =                   | N/A                          |       |       | N/A                |       |       | N/A                 |       |       | 0.366          |       |       | 0.92            |       |       |
|                | Tx V =                    | N/A                          |       |       | N/A                |       |       | 1.357               |       |       | N/A            |       |       | 1.301           |       |       |
|                | W x Tx V=                 | 4.844                        |       |       | N/A                |       |       | N/A                 |       |       | 0.732          |       |       | N/A             |       |       |

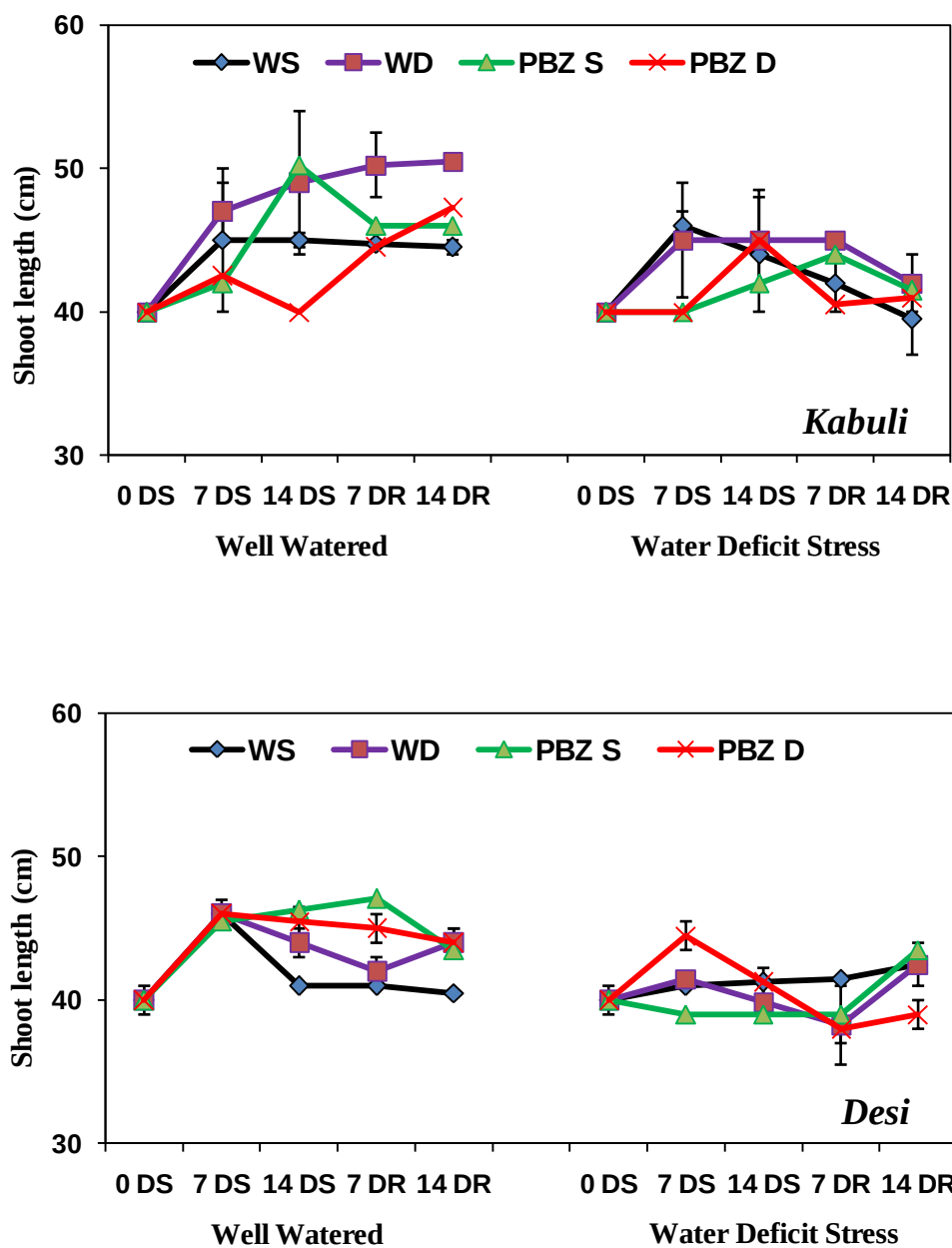


Fig. 4.3.1.2.3.1. Effect of PBZ on shoot length (cm) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.1. Effect of PBZ on shoot length in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Shoot length (cm)  |       |       |                    |       |       |        |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|--------------------|-------|-------|--------------------|-------|-------|--------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress |       |       | 7 day water stress |       |       |        |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli             | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 40.00              | 40.00 | 40.00 | 45.00              | 46.00 | 45.50 | 45.00  | 41.00 | 43.00 | 44.75          | 41.00 | 42.88 | 44.50           | 40.50 | 42.50 |
|                | Water Drenching           | 40.00              | 40.00 | 40.00 | 47.00              | 46.00 | 46.50 | 49.00  | 44.00 | 46.50 | 50.25          | 42.00 | 46.13 | 50.50           | 44.00 | 47.25 |
|                | PBZ Foliar spray (60 ppm) | 40.00              | 40.00 | 40.00 | 42.00              | 45.50 | 43.75 | 50.25  | 46.29 | 48.27 | 46.00          | 47.08 | 46.54 | 46.00           | 43.50 | 44.75 |
|                | PBZ Drenching (120 ppm)   | 40.00              | 40.00 | 40.00 | 42.50              | 46.00 | 44.25 | 40.00  | 45.50 | 42.75 | 44.50          | 45.00 | 44.75 | 47.25           | 44.00 | 45.63 |
|                | Mean                      | 40.00              | 40.00 | 40.00 | 44.13              | 45.88 | 45.00 | 46.06  | 44.20 | 45.13 | 46.38          | 43.77 | 45.07 | 47.06           | 43.00 | 45.03 |
| Water stressed | Water Foliar spray        | 40.00              | 40.00 | 40.00 | 46.00              | 41.00 | 43.50 | 44.00  | 41.25 | 42.63 | 42.00          | 41.50 | 41.75 | 39.50           | 42.50 | 41.00 |
|                | Water Drenching           | 40.00              | 40.00 | 40.00 | 45.00              | 41.50 | 43.25 | 45.00  | 39.88 | 42.44 | 45.00          | 38.25 | 41.63 | 42.00           | 42.50 | 42.25 |
|                | PBZ Foliar spray (60 ppm) | 40.00              | 40.00 | 40.00 | 40.00              | 39.00 | 39.50 | 42.00  | 39.00 | 40.50 | 44.00          | 39.00 | 41.50 | 41.50           | 43.50 | 42.50 |
|                | PBZ Drenching (120 ppm)   | 40.00              | 40.00 | 40.00 | 40.00              | 44.50 | 42.25 | 45.00  | 41.25 | 43.13 | 40.50          | 38.00 | 39.25 | 41.00           | 39.00 | 40.00 |
|                | Mean                      | 40.00              | 40.00 | 40.00 | 42.75              | 41.50 | 42.13 | 44.00  | 40.34 | 42.17 | 42.88          | 39.19 | 41.03 | 41.00           | 41.88 | 41.44 |
|                | SEM                       | 0.471              |       |       | 1.751              |       |       | 2.178  |       |       | 1.342          |       |       | 1.018           |       |       |
|                | CD at 5 %                 |                    |       |       |                    |       |       |        |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                |       |       | 1.784              |       |       | 2.219  |       |       | 1.367          |       |       | 1.037           |       |       |
|                | Treatment (T)             | N/A                |       |       | N/A                |       |       | N/A    |       |       | N/A            |       |       | 1.467           |       |       |
|                | Varieties (V)             | 0.48               |       |       | N/A                |       |       | 2.219  |       |       | 1.367          |       |       | N/A             |       |       |
|                | W x T =                   | N/A                |       |       | N/A                |       |       | N/A    |       |       | N/A            |       |       | N/A             |       |       |
|                | W x V =                   | N/A                |       |       | N/A                |       |       | N/A    |       |       | N/A            |       |       | 1.467           |       |       |
|                | B x V =                   | N/A                |       |       | N/A                |       |       | N/A    |       |       | 2.734          |       |       | N/A             |       |       |
|                | W x B x V=                | N/A                |       |       | N/A                |       |       | N/A    |       |       | N/A            |       |       | 2.934           |       |       |

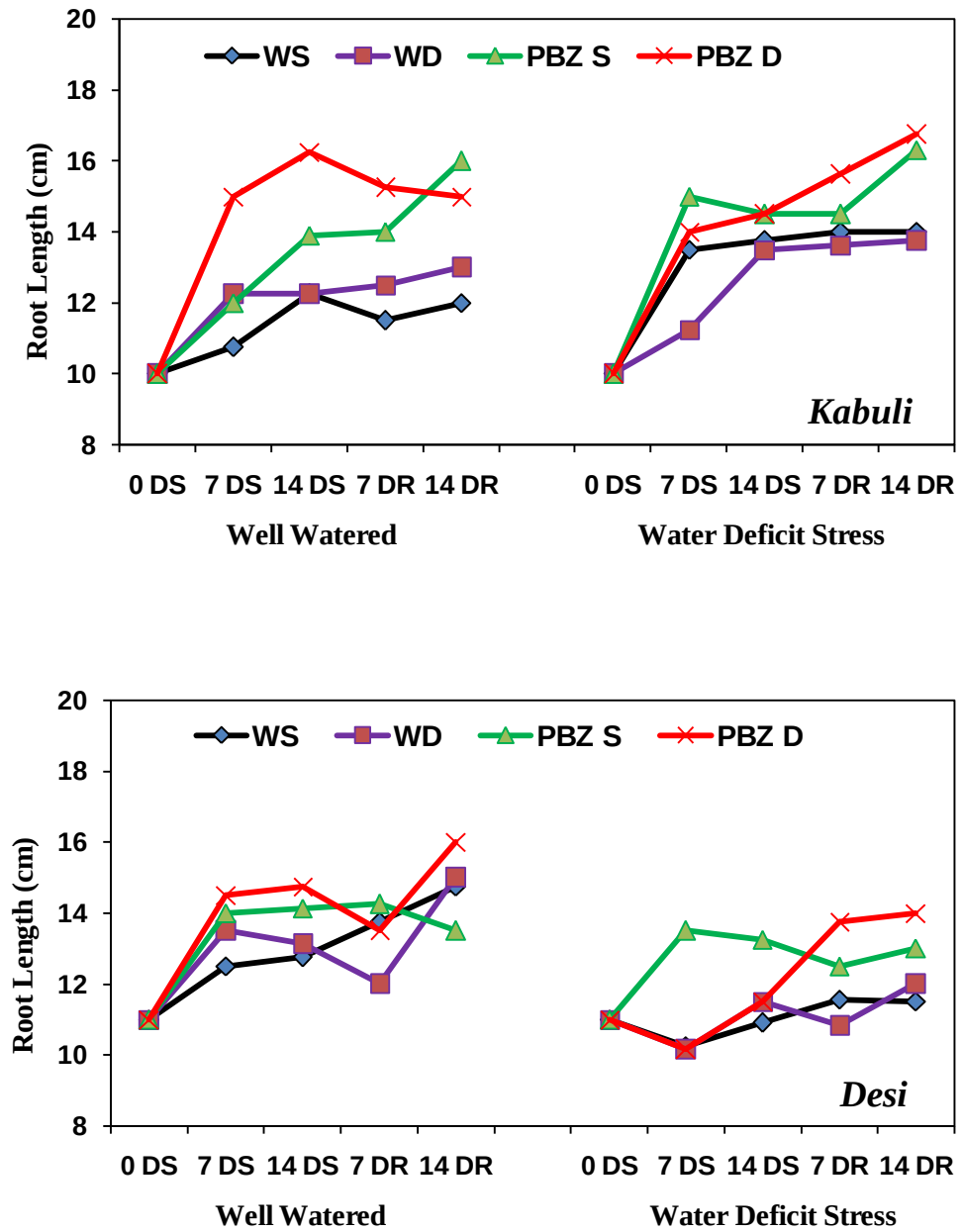


Fig. 4.3.1.2.3.2. Effect of PBZ on root length (cm) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.2 Effect of PBZ on root length in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Root length(cm)    |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|--------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli             | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 10.00              | 11.00 | 10.50 | 10.75              | 12.50 | 11.63 | 12.25               | 12.75 | 12.50 | 11.50          | 13.75 | 12.63 | 12.00           | 14.75 | 13.38 |
|                | Water Drenching           | 10.00              | 11.00 | 10.50 | 12.25              | 13.50 | 12.88 | 12.25               | 13.13 | 12.69 | 12.50          | 12.00 | 12.25 | 13.00           | 15.00 | 14.00 |
|                | PBZ Foliar spray (60 ppm) | 10.00              | 11.00 | 10.50 | 12.00              | 14.00 | 13.00 | 13.90               | 14.13 | 14.01 | 14.00          | 14.25 | 14.13 | 16.00           | 13.50 | 14.75 |
|                | PBZ Drenching (120 ppm)   | 10.00              | 11.00 | 10.50 | 15.00              | 14.50 | 14.75 | 16.25               | 14.75 | 15.50 | 15.25          | 13.50 | 14.38 | 15.00           | 16.00 | 15.50 |
|                | Mean                      | 10.00              | 11.00 | 10.50 | 12.50              | 13.63 | 13.06 | 13.66               | 13.69 | 13.68 | 13.31          | 13.38 | 13.34 | 14.00           | 14.81 | 14.41 |
| Water stressed | Water Foliar spray        | 10.00              | 11.00 | 10.50 | 13.50              | 10.25 | 11.88 | 13.75               | 10.91 | 12.33 | 14.00          | 11.56 | 12.78 | 14.00           | 11.50 | 12.75 |
|                | Water Drenching           | 10.00              | 11.00 | 10.50 | 11.25              | 10.15 | 10.70 | 13.50               | 11.50 | 12.50 | 13.63          | 10.83 | 12.23 | 13.75           | 12.00 | 12.88 |
|                | PBZ Foliar spray (60 ppm) | 10.00              | 11.00 | 10.50 | 15.00              | 13.50 | 14.25 | 14.50               | 13.25 | 13.88 | 14.50          | 12.50 | 13.50 | 16.30           | 13.00 | 14.65 |
|                | PBZ Drenching (120 ppm)   | 10.00              | 11.00 | 10.50 | 14.00              | 10.15 | 12.08 | 14.50               | 11.50 | 13.00 | 15.63          | 13.75 | 14.69 | 16.75           | 14.00 | 15.38 |
|                | Mean                      | 10.00              | 11.00 | 10.50 | 13.44              | 11.01 | 12.23 | 14.06               | 11.79 | 12.93 | 14.44          | 12.16 | 13.30 | 15.20           | 12.63 | 13.91 |
|                | SEM                       | 0.471              |       |       | 0.776              |       |       | 0.947               |       |       | 1.384          |       |       | 1.135           |       |       |
|                | CD at 5 %                 |                    |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                |       |       | 0.79               |       |       | N/A                 |       |       | N/A            |       |       | N/A             |       |       |
|                | Treatment (T)             | N/A                |       |       | 1.117              |       |       | 1.365               |       |       | N/A            |       |       | 1.635           |       |       |
|                | Varieties (V)             | 0.48               |       |       | N/A                |       |       | 0.965               |       |       | N/A            |       |       | N/A             |       |       |
|                | W x T =                   | N/A                |       |       | 1.58               |       |       | N/A                 |       |       | N/A            |       |       | N/A             |       |       |
|                | W x V =                   | N/A                |       |       | 1.117              |       |       | N/A                 |       |       | N/A            |       |       | 1.635           |       |       |
|                | B x V =                   | N/A                |       |       | N/A                |       |       | N/A                 |       |       | N/A            |       |       | N/A             |       |       |
|                | W x Tx V=                 | 1.358              |       |       | N/A                |       |       | N/A                 |       |       | N/A            |       |       | N/A             |       |       |

#### **4.3.1.2.3.3. Leaf number**

In general, both foliar and drenching application of PBZ in both the varieties under both well watered and water deficit conditions enhanced the leaf number per plant. Moreover, during recovery after water stress termination plants treated with PBZ had the higher leaf number (Fig. 4.3.1.2.3.3. and Table 4.3.1.2.3.3.).

#### **4.3.1.2.3.4. Leaf area**

Under well watered condition, all the treatments had higher leaf area than imposed water deficit condition. Leaf area gradually decreased with increasing the period of imposed water stress at reproductive stage in both *desi* and *kabuli* varieties (Fig. 4.3.1.2.3.4. and Table 4.3.1.2.3.4.). Foliar and drenching applications of PBZ under water deficit condition maintained significantly higher leaf area over their respective controls. Plants treated with PBZ drenching beared the highest leaf area under water deficit condition in both the varieties (Fig. 4.3.1.2.3.4. and Table 4.3.1.2.3.4.).

#### **4.3.1.2.3.5. Shoot dry weight**

Under water deficit conditions, both *kabuli* and *desi* varieties showed the reduction in their shoot dry weight as compared to well watered conditions. Foliar and drenching application of PBZ enhanced the shoot dry weight under well watered and water deficit conditions in both *kabuli* and *desi* varieties (Fig. 4.3.1.2.3.5. and Table 4.3.1.2.3.5.).

#### **4.3.1.2.3.6. Root dry weight**

Plants treated with foliar and drenching applications of PBZ showed the higher root dry weight in both *kabuli* and *desi* varieties under both well watered and water deficit conditions. In general, plants treated with PBZ drenching application had higher root dry weight than foliar application of PBZ under both well watered and water deficit conditions (Fig. 4.3.1.2.3.6. and Table 4.3.1.2.3.6.).

#### **4.3.1.2.3.7. Leaf dry weight**

Imposed water deficit condition resulted gradual reduction on leaf dry weight maximum reduction was obtained on 14<sup>th</sup> day. Plants treated with foliar and drenching application of PBZ had the higher leaf dry weight under both well watered and water deficit

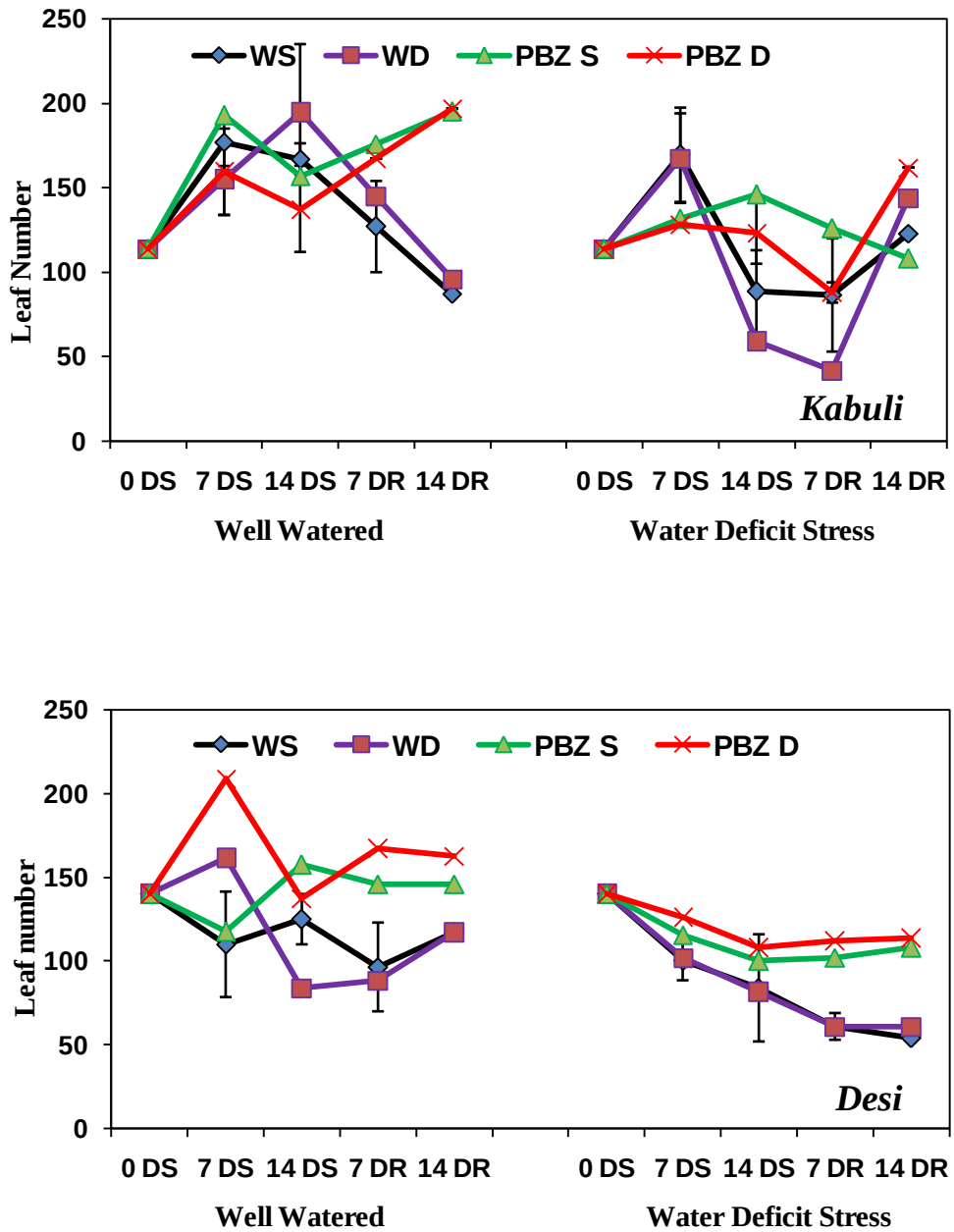


Fig. 4.3.1.2.3.3. Effect of PBZ on leaf number in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.3. Effect of PBZ on leaf number in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Leaf number        |        |        |                    |        |        |                     |        |        |                |        |        |                 |        |        |
|----------------|---------------------------|--------------------|--------|--------|--------------------|--------|--------|---------------------|--------|--------|----------------|--------|--------|-----------------|--------|--------|
|                |                           | 0 day water stress |        |        | 7 day water stress |        |        | 14 day water stress |        |        | 7 day recovery |        |        | 14 day recovery |        |        |
|                |                           | Kabuli             | Desi   | Mean   | Kabuli             | Desi   | Mean   | Kabuli              | Desi   | Mean   | Kabuli         | Desi   | Mean   | Kabuli          | Desi   | Mean   |
| Well watered   | Water Foliar spray        | 114.00             | 140.00 | 127.00 | 177.00             | 110.00 | 143.50 | 167.00              | 125.00 | 146.00 | 127.00         | 96.50  | 111.75 | 87.00           | 117.00 | 102.00 |
|                | Water Drenching           | 114.00             | 140.00 | 127.00 | 155.00             | 161.50 | 158.25 | 194.50              | 84.00  | 139.25 | 145.25         | 88.50  | 116.88 | 96.00           | 117.00 | 106.50 |
|                | PBZ Foliar spray (60 ppm) | 114.00             | 140.00 | 127.00 | 193.00             | 117.50 | 155.25 | 157.00              | 157.50 | 157.25 | 176.00         | 146.00 | 161.00 | 195.00          | 146.00 | 170.50 |
|                | PBZ Drenching (120 ppm)   | 114.00             | 140.00 | 127.00 | 159.50             | 209.00 | 184.25 | 137.50              | 137.50 | 137.50 | 167.25         | 167.00 | 167.13 | 197.00          | 163.00 | 180.00 |
|                | Mean                      | 114.00             | 140.00 | 127.00 | 171.13             | 149.50 | 160.31 | 164.00              | 126.00 | 145.00 | 153.88         | 124.50 | 139.19 | 143.75          | 135.75 | 139.75 |
| Water stressed | Water Foliar spray        | 114.00             | 140.00 | 127.00 | 169.50             | 100.00 | 134.75 | 88.50               | 84.00  | 86.25  | 86.50          | 61.00  | 73.75  | 123.00          | 54.00  | 88.50  |
|                | Water Drenching           | 114.00             | 140.00 | 127.00 | 167.50             | 102.00 | 134.75 | 59.00               | 81.50  | 70.25  | 42.00          | 61.00  | 51.50  | 144.00          | 61.00  | 102.50 |
|                | PBZ Foliar spray (60 ppm) | 114.00             | 140.00 | 127.00 | 131.50             | 115.50 | 123.50 | 146.00              | 100.00 | 123.00 | 126.00         | 102.00 | 114.00 | 108.00          | 108.00 | 108.00 |
|                | PBZ Drenching (120 ppm)   | 114.00             | 140.00 | 127.00 | 128.50             | 126.00 | 127.25 | 123.50              | 108.00 | 115.75 | 88.00          | 112.00 | 100.00 | 162.00          | 113.50 | 137.75 |
|                | Mean                      | 114.00             | 140.00 | 127.00 | 149.25             | 110.88 | 130.06 | 104.25              | 93.38  | 98.81  | 85.63          | 84.00  | 84.81  | 134.25          | 84.13  | 109.19 |
|                | SEM                       | 1.448              |        |        | 15.703             |        |        | 22.653              |        |        | 13.74          |        |        | 9.302           |        |        |
|                | CD at 5 %                 |                    |        |        |                    |        |        |                     |        |        |                |        |        |                 |        |        |
|                | Water status (W)          | N/A                |        |        | 15.998             |        |        | 23.078              |        |        | 13.997         |        |        | 9.476           |        |        |
|                | Treatment (T)             | N/A                |        |        | N/A                |        |        | N/A                 |        |        | 19.795         |        |        | 13.401          |        |        |
|                | Varieties (V)             | 1.475              |        |        | 15.998             |        |        | N/A                 |        |        | 13.997         |        |        | 9.476           |        |        |
|                | W x T =                   | 2.951              |        |        | N/A                |        |        | N/A                 |        |        | N/A            |        |        | 18.952          |        |        |
|                | W x V =                   | N/A                |        |        | N/A                |        |        | 32.637              |        |        | 19.795         |        |        | N/A             |        |        |
|                | Tx V =                    | 2.951              |        |        | 31.995             |        |        | N/A                 |        |        | N/A            |        |        | 18.952          |        |        |
|                | W x Tx V=                 | N/A                |        |        | 45.248             |        |        | N/A                 |        |        | 39.59          |        |        | 26.802          |        |        |

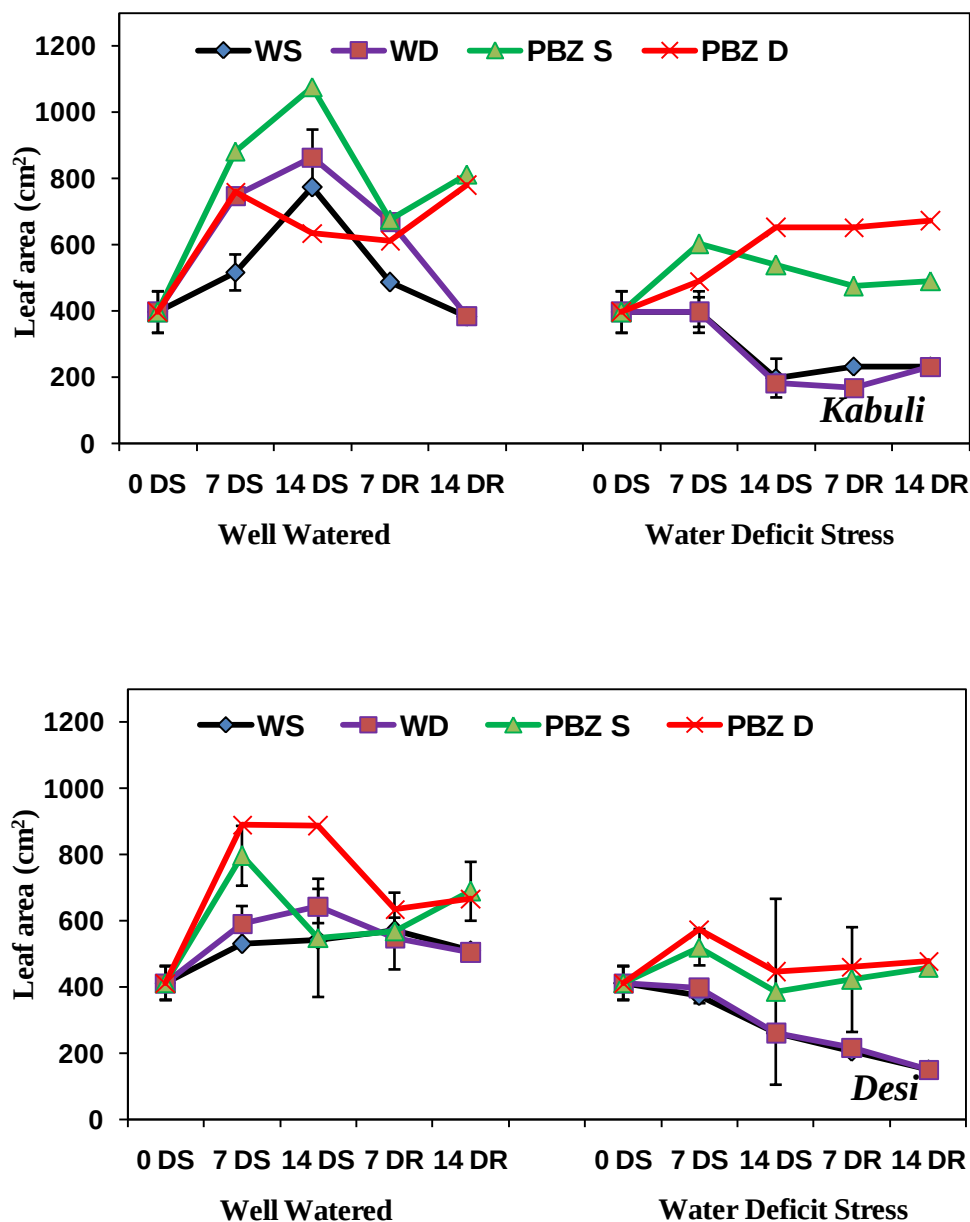


Fig. 4.3.1.2.3.4. Effect of PBZ on leaf area in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.4. Effect of PBZ on leaf area in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Leaf area (cm <sup>2</sup> ) |        |        |                    |        |        |                     |        |        |                |        |        |                 |        |        |
|----------------|---------------------------|------------------------------|--------|--------|--------------------|--------|--------|---------------------|--------|--------|----------------|--------|--------|-----------------|--------|--------|
|                |                           | 0 day water stress           |        |        | 7 day water stress |        |        | 14 day water stress |        |        | 7 day recovery |        |        | 14 day recovery |        |        |
|                |                           | Kabuli                       | Desi   | Mean   | Kabuli             | Desi   | Mean   | Kabuli              | Desi   | Mean   | Kabuli         | Desi   | Mean   | Kabuli          | Desi   | Mean   |
| Well watered   | Water Foliar spray        | 396.78                       | 412.00 | 404.39 | 516.56             | 529.12 | 522.84 | 773.91              | 543.18 | 658.55 | 487.00         | 569.89 | 528.45 | 383.00          | 511.00 | 447.00 |
|                | Water Drenching           | 396.78                       | 412.00 | 404.39 | 748.73             | 592.84 | 670.78 | 865.16              | 644.59 | 754.87 | 670.22         | 548.37 | 609.30 | 387.00          | 503.00 | 445.00 |
|                | PBZ Foliar spray (60 ppm) | 396.78                       | 412.00 | 404.39 | 882.04             | 796.32 | 839.18 | 1077.48             | 548.37 | 812.93 | 676.00         | 569.00 | 622.50 | 813.00          | 689.00 | 751.00 |
|                | PBZ Drenching (120 ppm)   | 396.78                       | 412.00 | 404.39 | 761.08             | 890.68 | 825.88 | 636.39              | 886.73 | 761.56 | 611.70         | 636.01 | 623.86 | 780.00          | 667.00 | 723.50 |
|                | Mean                      | 396.78                       | 412.00 | 404.39 | 727.10             | 702.24 | 714.67 | 838.23              | 655.72 | 746.98 | 611.23         | 580.82 | 596.02 | 590.75          | 592.50 | 591.63 |
| Water stressed | Water Foliar spray        | 396.78                       | 412.00 | 404.39 | 396.78             | 373.01 | 384.89 | 197.34              | 260.51 | 228.93 | 232.44         | 205.00 | 218.72 | 232.44          | 150.77 | 191.61 |
|                | Water Drenching           | 396.78                       | 412.00 | 404.39 | 396.78             | 395.67 | 396.22 | 183.07              | 260.51 | 221.79 | 169.00         | 218.00 | 193.50 | 232.44          | 150.77 | 191.61 |
|                | PBZ Foliar spray (60 ppm) | 396.78                       | 412.00 | 404.39 | 602.19             | 520.13 | 561.16 | 538.32              | 385.52 | 461.92 | 474.45         | 422.33 | 448.39 | 491.26          | 459.14 | 475.20 |
|                | PBZ Drenching (120 ppm)   | 396.78                       | 412.00 | 404.39 | 491.26             | 574.90 | 533.08 | 651.59              | 446.23 | 548.91 | 653.33         | 462.22 | 557.78 | 671.89          | 478.22 | 575.06 |
|                | Mean                      | 396.78                       | 412.00 | 404.39 | 471.75             | 465.93 | 468.84 | 392.58              | 338.19 | 365.39 | 382.31         | 326.89 | 354.60 | 407.01          | 309.73 | 358.37 |
|                | SEM                       | 33.067                       |        |        | 92.827             |        |        | 93.47               |        |        | 51.495         |        |        | 50.997          |        |        |
|                | CD at 5 %                 |                              |        |        |                    |        |        |                     |        |        |                |        |        |                 |        |        |
|                | Water status (W)          | N/A                          |        |        | 94.568             |        |        | 95.223              |        |        | 52.461         |        |        | 51.953          |        |        |
|                | Treatment (T)             | N/A                          |        |        | 133.739            |        |        | 134.665             |        |        | 74.191         |        |        | 73.473          |        |        |
|                | Varieties (V)             | 33.687                       |        |        | N/A                |        |        | 95.223              |        |        | N/A            |        |        | N/A             |        |        |
|                | W x T =                   | 67.375                       |        |        | N/A                |        |        | N/A                 |        |        | 104.921        |        |        | N/A             |        |        |
|                | W x V =                   | 47.641                       |        |        | N/A                |        |        | N/A                 |        |        | N/A            |        |        | N/A             |        |        |
|                | Tx V =                    | 67.375                       |        |        | N/A                |        |        | N/A                 |        |        | 104.921        |        |        | N/A             |        |        |
|                | W x Tx V=                 | N/A                          |        |        | N/A                |        |        | 269.33              |        |        | 148.381        |        |        | 146.946         |        |        |

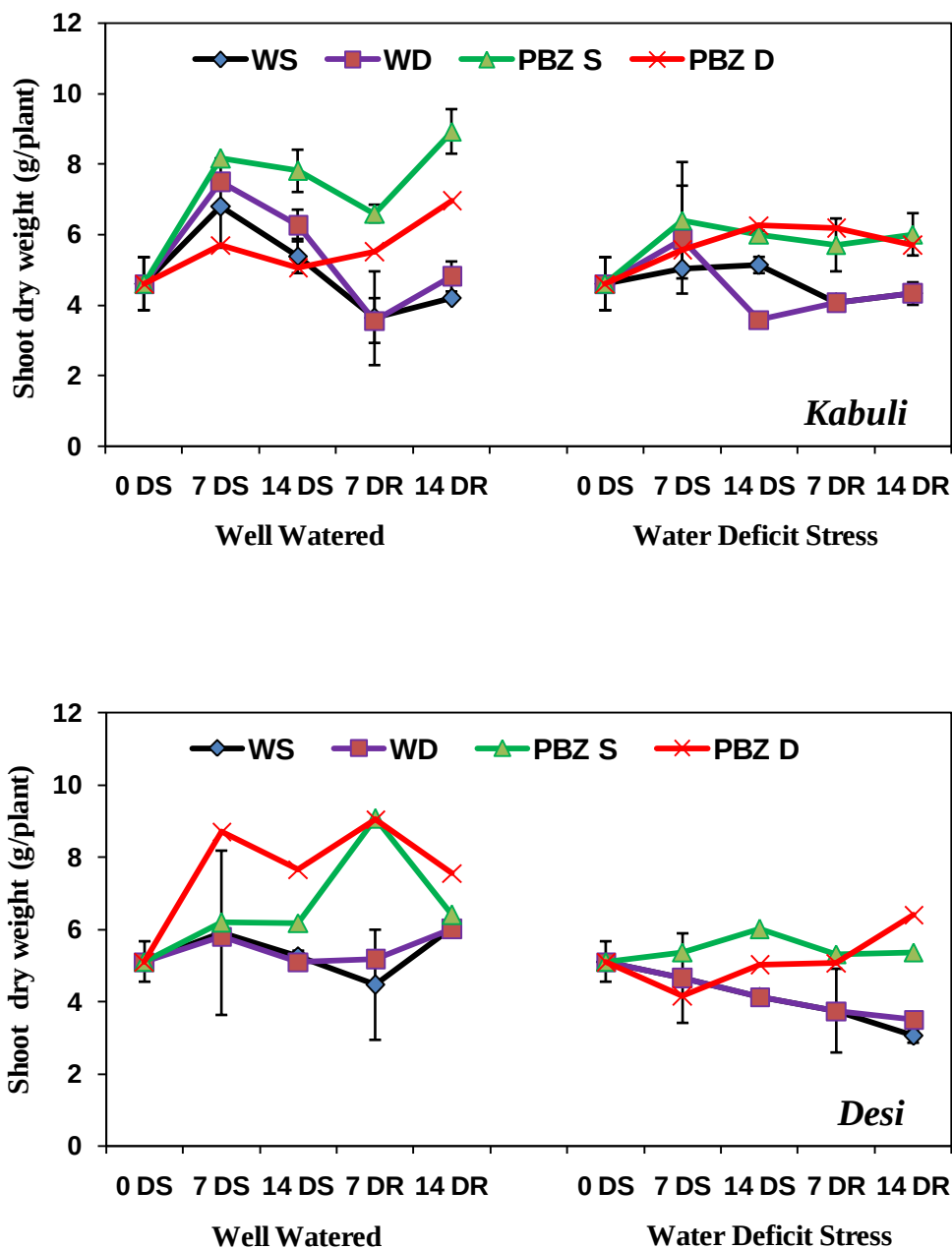


Fig. 4.3.1.2.3.5. Effect of PBZ on shoot dry weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.5. Effect of PBZ on shoot dry weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Shoot dry weight (g) |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|----------------|---------------------------|----------------------|------|------|--------------------|------|------|---------------------|------|------|----------------|------|------|-----------------|------|------|
|                |                           | 0 day water stress   |      |      | 7 day water stress |      |      | 14 day water stress |      |      | 7 day recovery |      |      | 14 day recovery |      |      |
|                |                           | Kabuli               | Desi | Mean | Kabuli             | Desi | Mean | Kabuli              | Desi | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi | Mean |
| Well watered   | Water Foliar spray        | 4.60                 | 5.10 | 4.85 | 6.79               | 5.90 | 6.34 | 5.39                | 5.25 | 5.32 | 3.62           | 4.46 | 4.04 | 4.19            | 6.03 | 5.11 |
|                | Water Drenching           | 4.60                 | 5.10 | 4.85 | 7.48               | 5.80 | 6.64 | 6.25                | 5.10 | 5.68 | 3.56           | 5.17 | 4.36 | 4.81            | 6.00 | 5.40 |
|                | PBZ Foliar spray (60 ppm) | 4.60                 | 5.10 | 4.85 | 8.15               | 6.18 | 7.16 | 7.80                | 6.16 | 6.98 | 6.59           | 9.06 | 7.83 | 8.92            | 6.39 | 7.65 |
|                | PBZ Drenching (120 ppm)   | 4.60                 | 5.10 | 4.85 | 5.71               | 8.71 | 7.21 | 5.05                | 7.66 | 6.36 | 5.50           | 9.05 | 7.27 | 6.95            | 7.55 | 7.25 |
|                | Mean                      | 4.60                 | 5.10 | 4.85 | 7.03               | 6.64 | 6.84 | 6.12                | 6.04 | 6.08 | 4.82           | 6.93 | 5.87 | 6.21            | 6.49 | 6.35 |
| Water stressed | Water Foliar spray        | 4.60                 | 5.10 | 4.85 | 5.02               | 4.64 | 4.83 | 5.13                | 4.12 | 4.63 | 4.06           | 3.74 | 3.90 | 4.32            | 3.04 | 3.68 |
|                | Water Drenching           | 4.60                 | 5.10 | 4.85 | 5.85               | 4.64 | 5.25 | 3.58                | 4.12 | 3.85 | 4.06           | 3.74 | 3.90 | 4.32            | 3.50 | 3.91 |
|                | PBZ Foliar spray (60 ppm) | 4.60                 | 5.10 | 4.85 | 6.40               | 5.36 | 5.88 | 6.00                | 6.00 | 6.00 | 5.70           | 5.30 | 5.50 | 6.00            | 5.36 | 5.68 |
|                | PBZ Drenching (120 ppm)   | 4.60                 | 5.10 | 4.85 | 5.56               | 4.14 | 4.85 | 6.27                | 5.00 | 5.64 | 6.17           | 5.06 | 5.62 | 5.69            | 6.41 | 6.05 |
|                | Mean                      | 4.60                 | 5.10 | 4.85 | 5.71               | 4.70 | 5.20 | 5.25                | 4.81 | 5.03 | 5.00           | 4.46 | 4.73 | 5.08            | 4.58 | 4.83 |
|                | SEM                       | 0.245                |      |      | 0.862              |      |      | 0.661               |      |      | 0.834          |      |      | 1.056           |      |      |
|                | CD at 5 %                 |                      |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|                | Water status (W)          | N/A                  |      |      | N/A                |      |      | 0.674               |      |      | 0.731          |      |      | 1.076           |      |      |
|                | Treatment (T)             | N/A                  |      |      | N/A                |      |      | 0.953               |      |      | 0.983          |      |      | N/A             |      |      |
|                | Varieties (V)             | 0.25                 |      |      | N/A                |      |      | N/A                 |      |      | N/A            |      |      | 1.522           |      |      |
|                | W x T =                   | N/A                  |      |      | N/A                |      |      | N/A                 |      |      | N/A            |      |      | 1.076           |      |      |
|                | W x V =                   | N/A                  |      |      | N/A                |      |      | N/A                 |      |      | N/A            |      |      | N/A             |      |      |
|                | Tx V =                    | N/A                  |      |      | N/A                |      |      | N/A                 |      |      | N/A            |      |      | 2.153           |      |      |
|                | W x Tx V=                 | N/A                  |      |      | N/A                |      |      | 1.905               |      |      | 1.581          |      |      | N/A             |      |      |

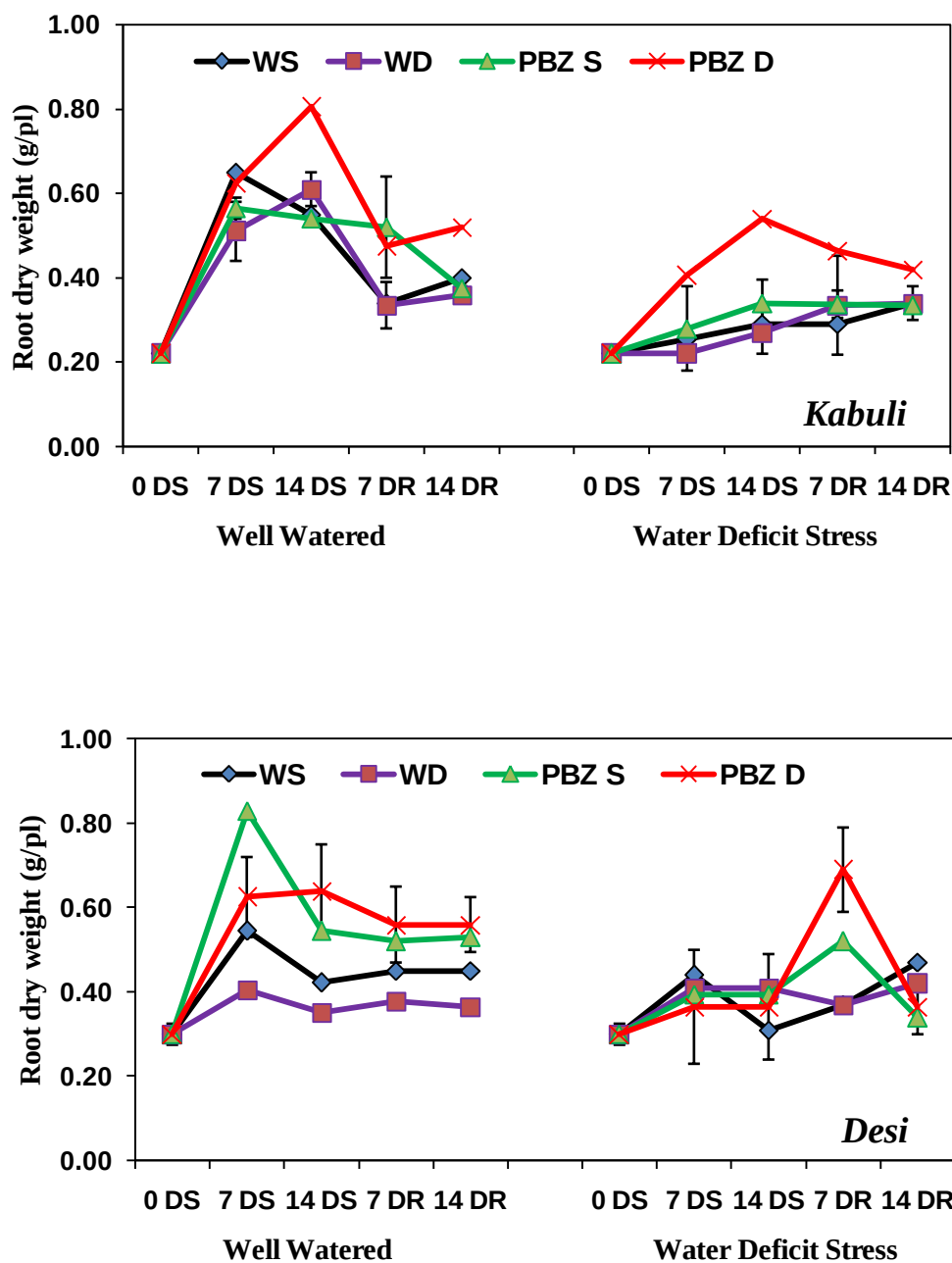


Fig. 4.3.1.2.3.6. Effect of PBZ on root dry weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.6. Effect of PBZ on root dry weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Root dry weight (g) |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|----------------|---------------------------|---------------------|------|------|--------------------|------|------|---------------------|------|------|----------------|------|------|-----------------|------|------|
|                |                           | 0 day water stress  |      |      | 7 day water stress |      |      | 14 day water stress |      |      | 7 day recovery |      |      | 14 day recovery |      |      |
|                |                           | Kabuli              | Desi | Mean | Kabuli             | Desi | Mean | Kabuli              | Desi | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi | Mean |
| Well watered   | Water Foliar spray        | 0.22                | 0.30 | 0.26 | 0.65               | 0.55 | 0.60 | 0.55                | 0.42 | 0.49 | 0.34           | 0.45 | 0.40 | 0.40            | 0.45 | 0.43 |
|                | Water Drenching           | 0.22                | 0.30 | 0.26 | 0.51               | 0.41 | 0.46 | 0.61                | 0.35 | 0.48 | 0.34           | 0.38 | 0.36 | 0.36            | 0.37 | 0.36 |
|                | PBZ Foliar spray (60 ppm) | 0.22                | 0.30 | 0.26 | 0.57               | 0.83 | 0.70 | 0.54                | 0.55 | 0.54 | 0.52           | 0.52 | 0.52 | 0.38            | 0.53 | 0.45 |
|                | PBZ Drenching (120 ppm)   | 0.22                | 0.30 | 0.26 | 0.63               | 0.63 | 0.63 | 0.81                | 0.64 | 0.72 | 0.48           | 0.56 | 0.52 | 0.52            | 0.56 | 0.54 |
|                | Mean                      | 0.22                | 0.30 | 0.26 | 0.59               | 0.60 | 0.59 | 0.63                | 0.49 | 0.56 | 0.42           | 0.48 | 0.45 | 0.41            | 0.48 | 0.45 |
| Water stressed | Water Foliar spray        | 0.22                | 0.30 | 0.26 | 0.26               | 0.44 | 0.35 | 0.29                | 0.31 | 0.30 | 0.29           | 0.37 | 0.33 | 0.34            | 0.47 | 0.41 |
|                | Water Drenching           | 0.22                | 0.30 | 0.26 | 0.22               | 0.41 | 0.32 | 0.27                | 0.41 | 0.34 | 0.34           | 0.37 | 0.35 | 0.34            | 0.42 | 0.38 |
|                | PBZ Foliar spray (60 ppm) | 0.22                | 0.30 | 0.26 | 0.28               | 0.39 | 0.34 | 0.34                | 0.39 | 0.37 | 0.34           | 0.52 | 0.43 | 0.34            | 0.34 | 0.34 |
|                | PBZ Drenching (120 ppm)   | 0.22                | 0.30 | 0.26 | 0.41               | 0.37 | 0.39 | 0.54                | 0.37 | 0.45 | 0.47           | 0.69 | 0.58 | 0.42            | 0.37 | 0.39 |
|                | Mean                      | 0.22                | 0.30 | 0.26 | 0.29               | 0.40 | 0.35 | 0.36                | 0.37 | 0.36 | 0.36           | 0.49 | 0.42 | 0.36            | 0.40 | 0.38 |
|                | SEM                       | 0.015               |      |      | 0.099              |      |      | 0.042               |      |      | 0.055          |      |      | 0.044           |      |      |
|                | CD at 5 %                 |                     |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|                | Water status (W)          | N/A                 |      |      | 0.1                |      |      | 0.043               |      |      | N/A            |      |      | N/A             |      |      |
|                | Treatment (T)             | N/A                 |      |      | N/A                |      |      | 0.061               |      |      | 0.079          |      |      | N/A             |      |      |
|                | Varieties (V)             | 0.016               |      |      | N/A                |      |      | 0.043               |      |      | 0.056          |      |      | N/A             |      |      |
|                | W x T =                   | N/A                 |      |      | N/A                |      |      | N/A                 |      |      | 0.056          |      |      | N/A             |      |      |
|                | W x V =                   | N/A                 |      |      | N/A                |      |      | 0.061               |      |      | N/A            |      |      | N/A             |      |      |
|                | Tx V =                    | N/A                 |      |      | N/A                |      |      | 0.086               |      |      | N/A            |      |      | N/A             |      |      |
|                | W x Tx V=                 | 0.045               |      |      | N/A                |      |      | 0.122               |      |      | N/A            |      |      | N/A             |      |      |

conditions in both varieties. In general efficacy of PBZ drenching in terms of leaf dry weight was recorded better than foliar application of PBZ (Fig. 4.3.1.2.3.7. and Table 4.3.1.2.3.7.).

#### **4.3.1.2.3.8. Specific leaf weight**

Foliar and drenching applications of PBZ in general, reduced the specific leaf weight under water deficit condition in both *kabuli* and *desi* varieties. However foliar application of PBZ in *desi* variety enhanced the SLW under well watered conditions (Fig. 4.3.1.2.3.8. and Table 4.3.1.2.3.8.).

#### **4.3.1.2.3.9. Specific leaf area**

On the other hand, foliar and drenching application of PBZ reduced the SLA in both *kabuli* and *desi* varieties. In PBZ treated *kabuli* plants SLA was obtained higher under well watered condition while in *desi* chickpea it was higher under water deficit condition (Fig. 4.3.1.2.3.9. and Table 4.3.1.2.3.9.).

#### **4.3.1.2.3.10. Leaf area ratio**

Under water deficit conditions, in both the varieties, reduction in LAR was reported. Foliar and drenching applications of PBZ maintained the higher LAR under imposed water deficit conditions. LAR performance of plant treated with PBZ drenching application was found better than plants treated with PBZ foliar application. However, under well watered condition in both the varieties foliar and drenching application of PBZ didn't show the significant alteration in LAR in both *desi* and *kabuli* varieties (Fig. 4.3.1.2.3.10. and Table 4.3.1.2.3.10.).

#### **4.3.1.2.4. Chloroplast ultrastructure**

Under imposed water deficit condition, swelling and destruction of thylakoid, appearance of lipid droplets, disappearance of starch inclusions, changes in the lamellar structure, number of grana with loose type of thylakoid, large increase in osmophilic granules and reduction of amount of starch in the chloroplast were noted in chloroplast of both *kabuli* and *desi* varieties. However, water deficit stress with foliar and drenching applications of PBZ maintained integrity of the chloroplast structure and grana were recorded with compact type of thylakoid in both varieties. Water stressed with foliar and drenching application of PBZ maintained the chloroplast structure similar to the control (Fig.4.3.1.2.4.). Moreover, PBZ

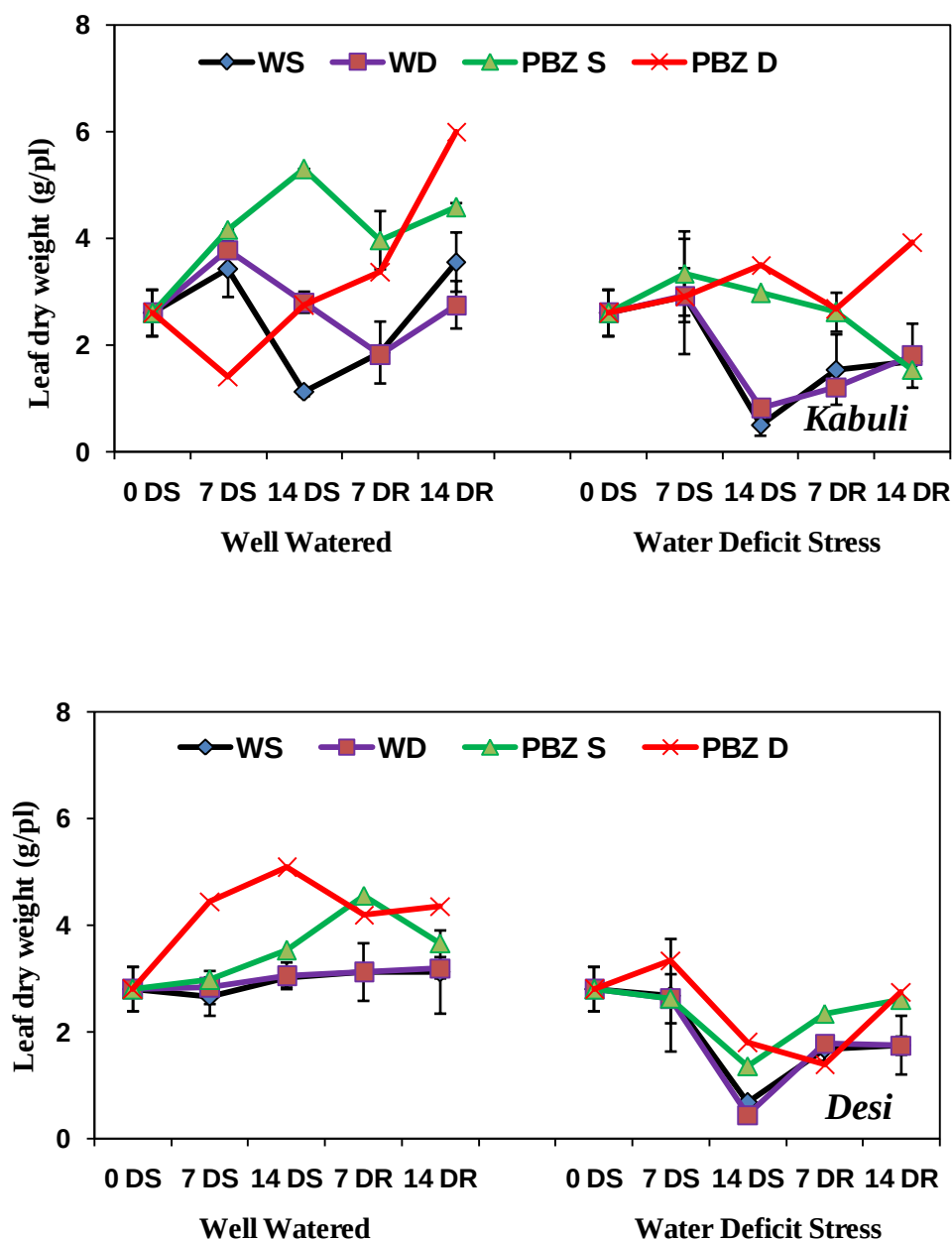


Fig. 4.3.1.2.3.7. Effect of PBZ on leaf dry weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.7. Effect of PBZ on leaf dry weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Leaf dry weight(g) |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|----------------|---------------------------|--------------------|------|------|--------------------|------|------|---------------------|------|------|----------------|------|------|-----------------|------|------|
|                |                           | 0 day water stress |      |      | 7 day water stress |      |      | 14 day water stress |      |      | 7 day recovery |      |      | 14 day recovery |      |      |
|                |                           | Kabuli             | Desi | Mean | Kabuli             | Desi | Mean | Kabuli              | Desi | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi | Mean |
| Well watered   | Water Foliar spray        | 2.60               | 2.80 | 2.70 | 3.42               | 2.66 | 3.04 | 1.13                | 3.02 | 2.07 | 1.86           | 3.12 | 2.49 | 3.56            | 3.12 | 3.34 |
|                | Water Drenching           | 2.60               | 2.80 | 2.70 | 3.79               | 2.83 | 3.31 | 2.80                | 3.05 | 2.93 | 1.82           | 3.12 | 2.47 | 2.76            | 3.20 | 2.98 |
|                | PBZ Foliar spray (60 ppm) | 2.60               | 2.80 | 2.70 | 4.16               | 2.98 | 3.57 | 5.30                | 3.53 | 4.42 | 3.97           | 4.56 | 4.26 | 4.58            | 3.66 | 4.12 |
|                | PBZ Drenching (120 ppm)   | 2.60               | 2.80 | 2.70 | 1.42               | 4.45 | 2.93 | 2.74                | 5.08 | 3.91 | 3.38           | 4.20 | 3.79 | 6.00            | 4.36 | 5.18 |
|                | Mean                      | 2.60               | 2.80 | 2.70 | 3.20               | 3.23 | 3.21 | 2.99                | 3.67 | 3.33 | 2.75           | 3.75 | 3.25 | 4.22            | 3.59 | 3.90 |
| Water stressed | Water Foliar spray        | 2.60               | 2.80 | 2.70 | 2.91               | 2.69 | 2.80 | 0.50                | 0.67 | 0.59 | 1.54           | 1.68 | 1.61 | 1.70            | 1.74 | 1.72 |
|                | Water Drenching           | 2.60               | 2.80 | 2.70 | 2.94               | 2.62 | 2.78 | 0.82                | 0.45 | 0.63 | 1.22           | 1.79 | 1.51 | 1.80            | 1.75 | 1.78 |
|                | PBZ Foliar spray (60 ppm) | 2.60               | 2.80 | 2.70 | 3.34               | 2.63 | 2.98 | 2.98                | 1.36 | 2.17 | 2.62           | 2.34 | 2.48 | 1.54            | 2.60 | 2.07 |
|                | PBZ Drenching (120 ppm)   | 2.60               | 2.80 | 2.70 | 2.91               | 3.34 | 3.13 | 3.50                | 1.80 | 2.65 | 2.67           | 1.40 | 2.03 | 3.93            | 2.76 | 3.34 |
|                | Mean                      | 2.60               | 2.80 | 2.70 | 3.02               | 2.82 | 2.92 | 1.95                | 1.07 | 1.51 | 2.01           | 1.80 | 1.91 | 2.24            | 2.21 | 2.23 |
|                | SEM                       | 0.021              |      |      | 0.399              |      |      | 0.15                |      |      | 0.307          |      |      | 0.306           |      |      |
|                | CD at 5 %                 |                    |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|                | Water status (W)          | N/A                |      |      | N/A                |      |      | 0.152               |      |      | 0.312          |      |      | 0.312           |      |      |
|                | Treatment (T)             | N/A                |      |      | N/A                |      |      | 0.216               |      |      | 0.442          |      |      | 0.441           |      |      |
|                | Varieties (V)             | 0.022              |      |      | N/A                |      |      | N/A                 |      |      | 0.312          |      |      | N/A             |      |      |
|                | W x T =                   | 0.043              |      |      | N/A                |      |      | 0.305               |      |      | N/A            |      |      | N/A             |      |      |
|                | W x V =                   | 0.03               |      |      | N/A                |      |      | 0.216               |      |      | 0.442          |      |      | 0.441           |      |      |
|                | Tx V =                    | 0.043              |      |      | 0.814              |      |      | 0.305               |      |      | 0.625          |      |      | 0.623           |      |      |
|                | W x Tx V=                 | N/A                |      |      | 1.151              |      |      | 0.431               |      |      | N/A            |      |      | 0.881           |      |      |

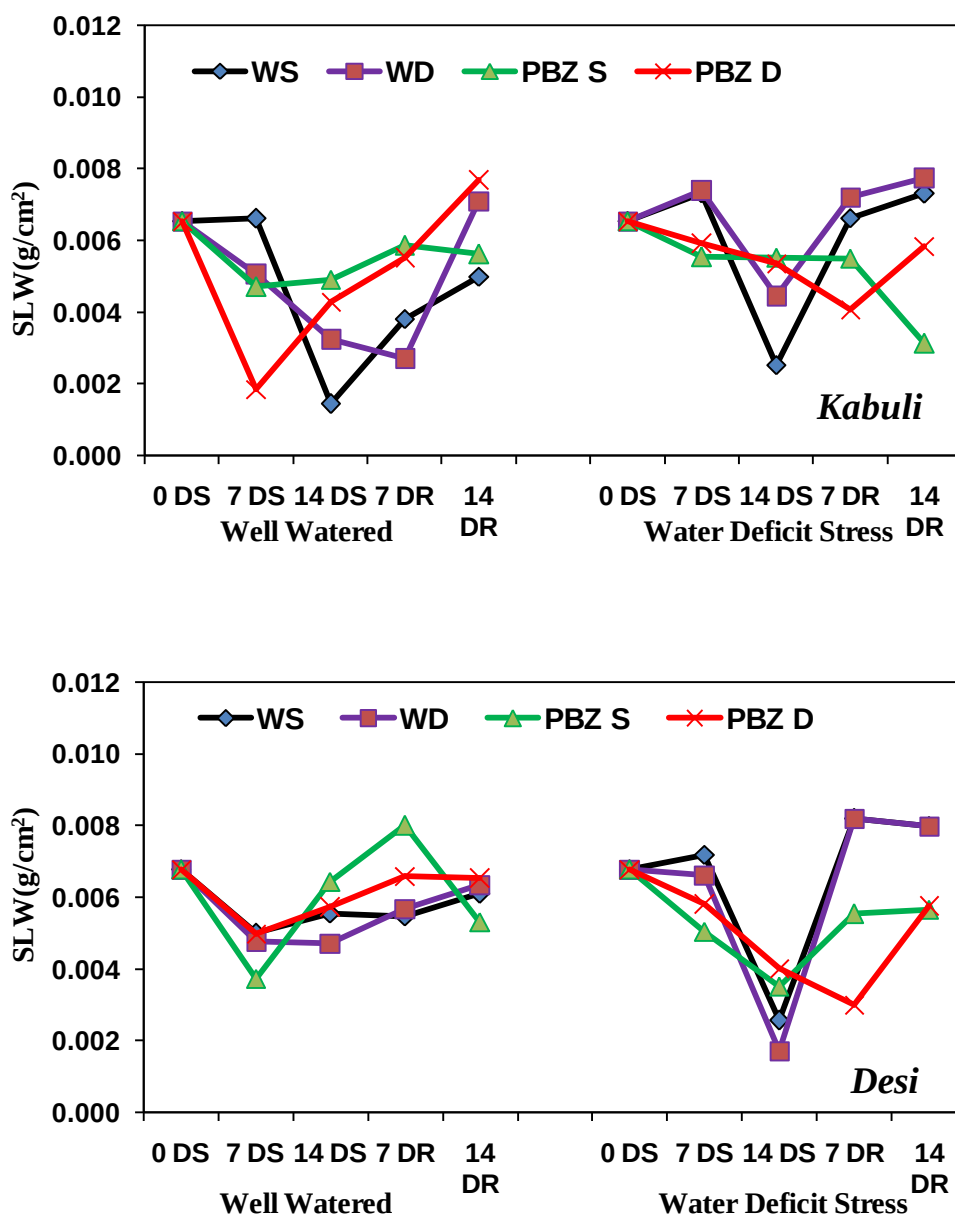


Fig. 4.3.1.2.3.8. Effect of PBZ on specific leaf weight (SLW) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.8. Effect of PBZ on specific leaf weight in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | SLW(g/cm <sup>2</sup> ) |              |              |                    |              |              |                     |              |              |                |              |              |                 |              |              |
|----------------|---------------------------|-------------------------|--------------|--------------|--------------------|--------------|--------------|---------------------|--------------|--------------|----------------|--------------|--------------|-----------------|--------------|--------------|
|                |                           | 0 day water stress      |              |              | 7 day water stress |              |              | 14 day water stress |              |              | 7 day recovery |              |              | 14 day recovery |              |              |
|                |                           | Kabuli                  | Desi         | Mean         | Kabuli             | Desi         | Mean         | Kabuli              | Desi         | Mean         | Kabuli         | Desi         | Mean         | Kabuli          | Desi         | Mean         |
| Well watered   | Water Foliar spray        | 0.007                   | 0.007        | <b>0.007</b> | 0.007              | 0.005        | <b>0.006</b> | 0.001               | 0.006        | <b>0.004</b> | 0.004          | 0.005        | <b>0.005</b> | 0.005           | 0.006        | <b>0.006</b> |
|                | Water Drenching           | 0.007                   | 0.007        | <b>0.007</b> | 0.005              | 0.005        | <b>0.005</b> | 0.003               | 0.005        | <b>0.004</b> | 0.003          | 0.006        | <b>0.004</b> | 0.007           | 0.006        | <b>0.007</b> |
|                | PBZ Foliar spray (60 ppm) | 0.007                   | 0.007        | <b>0.007</b> | 0.005              | 0.004        | <b>0.004</b> | 0.005               | 0.006        | <b>0.006</b> | 0.006          | 0.008        | <b>0.007</b> | 0.006           | 0.005        | <b>0.005</b> |
|                | PBZ Drenching (120 ppm)   | 0.007                   | 0.007        | <b>0.007</b> | 0.002              | 0.005        | <b>0.003</b> | 0.004               | 0.006        | <b>0.005</b> | 0.006          | 0.007        | <b>0.006</b> | 0.008           | 0.007        | <b>0.007</b> |
|                | Mean                      | <b>0.007</b>            | <b>0.007</b> | <b>0.007</b> | <b>0.005</b>       | <b>0.005</b> | <b>0.005</b> | <b>0.003</b>        | <b>0.006</b> | <b>0.005</b> | <b>0.004</b>   | <b>0.006</b> | <b>0.005</b> | <b>0.006</b>    | <b>0.006</b> | <b>0.006</b> |
| Water stressed | Water Foliar spray        | 0.007                   | 0.007        | <b>0.007</b> | 0.007              | 0.007        | <b>0.007</b> | 0.003               | 0.003        | <b>0.003</b> | 0.007          | 0.008        | <b>0.007</b> | 0.007           | 0.008        | <b>0.008</b> |
|                | Water Drenching           | 0.007                   | 0.007        | <b>0.007</b> | 0.007              | 0.007        | <b>0.007</b> | 0.004               | 0.002        | <b>0.003</b> | 0.007          | 0.008        | <b>0.008</b> | 0.008           | 0.008        | <b>0.008</b> |
|                | PBZ Foliar spray (60 ppm) | 0.007                   | 0.007        | <b>0.007</b> | 0.006              | 0.005        | <b>0.005</b> | 0.006               | 0.004        | <b>0.005</b> | 0.006          | 0.006        | <b>0.006</b> | 0.003           | 0.006        | <b>0.004</b> |
|                | PBZ Drenching (120 ppm)   | 0.007                   | 0.007        | <b>0.007</b> | 0.006              | 0.006        | <b>0.006</b> | 0.005               | 0.004        | <b>0.005</b> | 0.004          | 0.003        | <b>0.004</b> | 0.006           | 0.006        | <b>0.006</b> |
|                | Mean                      | <b>0.007</b>            | <b>0.007</b> | <b>0.007</b> | <b>0.007</b>       | <b>0.006</b> | <b>0.006</b> | <b>0.004</b>        | <b>0.003</b> | <b>0.004</b> | <b>0.006</b>   | <b>0.006</b> | <b>0.006</b> | <b>0.006</b>    | <b>0.007</b> | <b>0.006</b> |
|                | SEM                       | 0                       |              |              | 0.001              |              |              | 8.583               |              |              | 0.001          |              |              | 0.001           |              |              |
|                | CD at 5 %                 |                         |              |              |                    |              |              |                     |              |              |                |              |              |                 |              |              |
|                | Water status (W)          | N/A                     |              |              | 0.001              |              |              | N/A                 |              |              | N/A            |              |              | N/A             |              |              |
|                | Treatment (T)             | N/A                     |              |              | 0.001              |              |              | N/A                 |              |              | 0.002          |              |              | 0.001           |              |              |
|                | Varieties (V)             | N/A                     |              |              | N/A                |              |              | N/A                 |              |              | 0.001          |              |              | 0.001           |              |              |
|                | W x T =                   | 0                       |              |              | N/A                |              |              | N/A                 |              |              | N/A            |              |              | 0.002           |              |              |
|                | W x V =                   | N/A                     |              |              | N/A                |              |              | N/A                 |              |              | N/A            |              |              | N/A             |              |              |
|                | T x V =                   | N/A                     |              |              | N/A                |              |              | N/A                 |              |              | N/A            |              |              | N/A             |              |              |
|                | W x T x V =               | 0                       |              |              | N/A                |              |              | N/A                 |              |              | N/A            |              |              | N/A             |              |              |

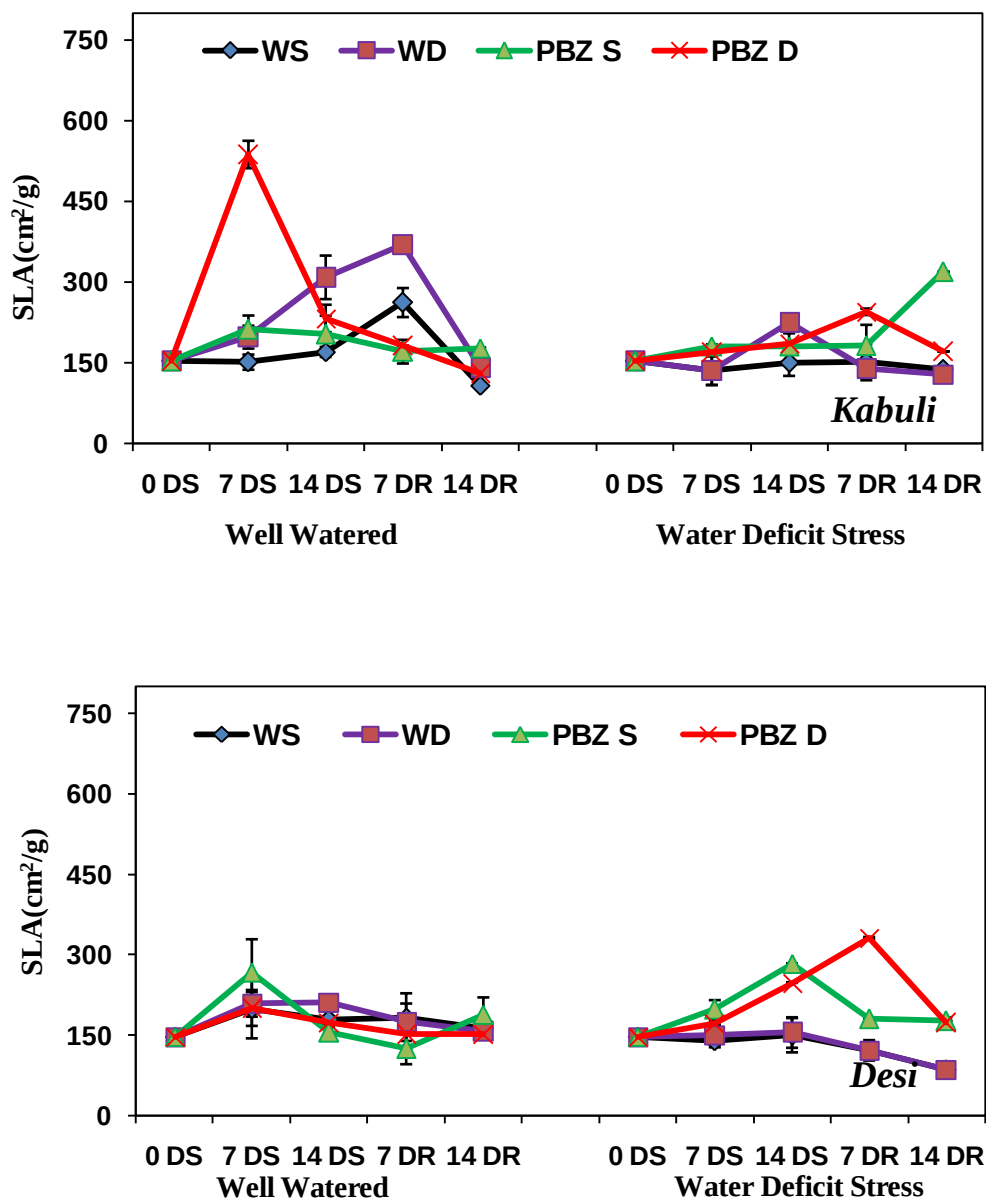


Fig. 4.3.1.2.3.9. Effect of PBZ on specific leaf area (SLA) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.3.9. Effect of PBZ on specific leaf area in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | SLA(cm <sup>2</sup> /g) |               |               |                    |               |               |                     |               |               |                |               |               |                 |               |               |
|----------------|---------------------------|-------------------------|---------------|---------------|--------------------|---------------|---------------|---------------------|---------------|---------------|----------------|---------------|---------------|-----------------|---------------|---------------|
|                |                           | 0 day water stress      |               |               | 7 day water stress |               |               | 14 day water stress |               |               | 7 day recovery |               |               | 14 day recovery |               |               |
|                |                           | Kabuli                  | Desi          | Mean          | Kabuli             | Desi          | Mean          | Kabuli              | Desi          | Mean          | Kabuli         | Desi          | Mean          | Kabuli          | Desi          | Mean          |
| Well watered   | Water Foliar spray        | 152.61                  | 147.14        | <b>149.87</b> | 151.04             | 198.92        | <b>174.98</b> | 170.00              | 179.86        | <b>174.93</b> | 261.83         | 182.66        | <b>222.24</b> | 107.74          | 163.78        | <b>135.76</b> |
|                | Water Drenching           | 152.61                  | 147.14        | <b>149.87</b> | 197.55             | 209.48        | <b>203.52</b> | 308.71              | 211.34        | <b>260.02</b> | 369.27         | 175.76        | <b>272.51</b> | 140.47          | 157.19        | <b>148.83</b> |
|                | PBZ Foliar spray (60 ppm) | 152.61                  | 147.14        | <b>149.87</b> | 212.03             | 267.67        | <b>239.85</b> | 203.30              | 155.35        | <b>179.32</b> | 170.49         | 124.92        | <b>147.70</b> | 177.51          | 188.25        | <b>182.88</b> |
|                | PBZ Drenching (120 ppm)   | 152.61                  | 147.14        | <b>149.87</b> | 537.11             | 200.15        | <b>368.63</b> | 232.26              | 174.55        | <b>203.41</b> | 181.24         | 151.43        | <b>166.34</b> | 130.00          | 152.98        | <b>141.49</b> |
|                | Mean                      | <b>152.61</b>           | <b>147.14</b> | <b>149.87</b> | <b>274.43</b>      | <b>219.05</b> | <b>246.74</b> | <b>228.57</b>       | <b>180.28</b> | <b>204.42</b> | <b>245.71</b>  | <b>158.69</b> | <b>202.20</b> | <b>138.93</b>   | <b>165.55</b> | <b>152.24</b> |
| Water stressed | Water Foliar spray        | 152.61                  | 147.14        | <b>149.87</b> | 136.35             | 138.92        | <b>137.64</b> | 150.00              | 150.00        | <b>150.00</b> | 150.94         | 122.02        | <b>136.48</b> | 136.73          | 86.65         | <b>111.69</b> |
|                | Water Drenching           | 152.61                  | 147.14        | <b>149.87</b> | 135.19             | 151.02        | <b>143.10</b> | 224.63              | 155.00        | <b>189.81</b> | 138.52         | 121.79        | <b>130.16</b> | 129.13          | 86.15         | <b>107.64</b> |
|                | PBZ Foliar spray (60 ppm) | 152.61                  | 147.14        | <b>149.87</b> | 180.30             | 198.14        | <b>189.22</b> | 180.80              | 283.47        | <b>232.13</b> | 181.43         | 180.48        | <b>180.96</b> | 319.00          | 176.59        | <b>247.79</b> |
|                | PBZ Drenching (120 ppm)   | 152.61                  | 147.14        | <b>149.87</b> | 168.82             | 172.12        | <b>170.47</b> | 186.17              | 247.90        | <b>217.03</b> | 244.69         | 331.34        | <b>288.02</b> | 170.96          | 173.58        | <b>172.27</b> |
|                | Mean                      | <b>152.61</b>           | <b>147.14</b> | <b>149.87</b> | <b>155.16</b>      | <b>165.05</b> | <b>160.11</b> | <b>185.40</b>       | <b>209.09</b> | <b>197.25</b> | <b>178.90</b>  | <b>188.91</b> | <b>183.90</b> | <b>188.96</b>   | <b>130.74</b> | <b>159.85</b> |
|                | SEM                       | 12.511                  |               |               | 226.252            |               |               | 59.758              |               |               | 65.817         |               |               | 17.535          |               |               |
|                | CD at 5 %                 |                         |               |               |                    |               |               |                     |               |               |                |               |               |                 |               |               |
|                | Water status (W)          | N/A                     |               |               | N/A                |               |               | 60.878              |               |               | N/A            |               |               | N/A             |               |               |
|                | Treatment (T)             | N/A                     |               |               | N/A                |               |               | 86.094              |               |               | 94.825         |               |               | 25.263          |               |               |
|                | Varieties (V)             | 12.746                  |               |               | N/A                |               |               | N/A                 |               |               | N/A            |               |               | 17.864          |               |               |
|                | W x T =                   | 25.491                  |               |               | N/A                |               |               | N/A                 |               |               | 134.103        |               |               | 35.728          |               |               |
|                | W x V =                   | 18.025                  |               |               | N/A                |               |               | 86.094              |               |               | N/A            |               |               | 25.263          |               |               |
|                | Tx V =                    | 25.491                  |               |               | N/A                |               |               | 121.756             |               |               | N/A            |               |               | 35.728          |               |               |
|                | W x Tx V=                 | N/A                     |               |               | N/A                |               |               | N/A                 |               |               | N/A            |               |               | 50.527          |               |               |

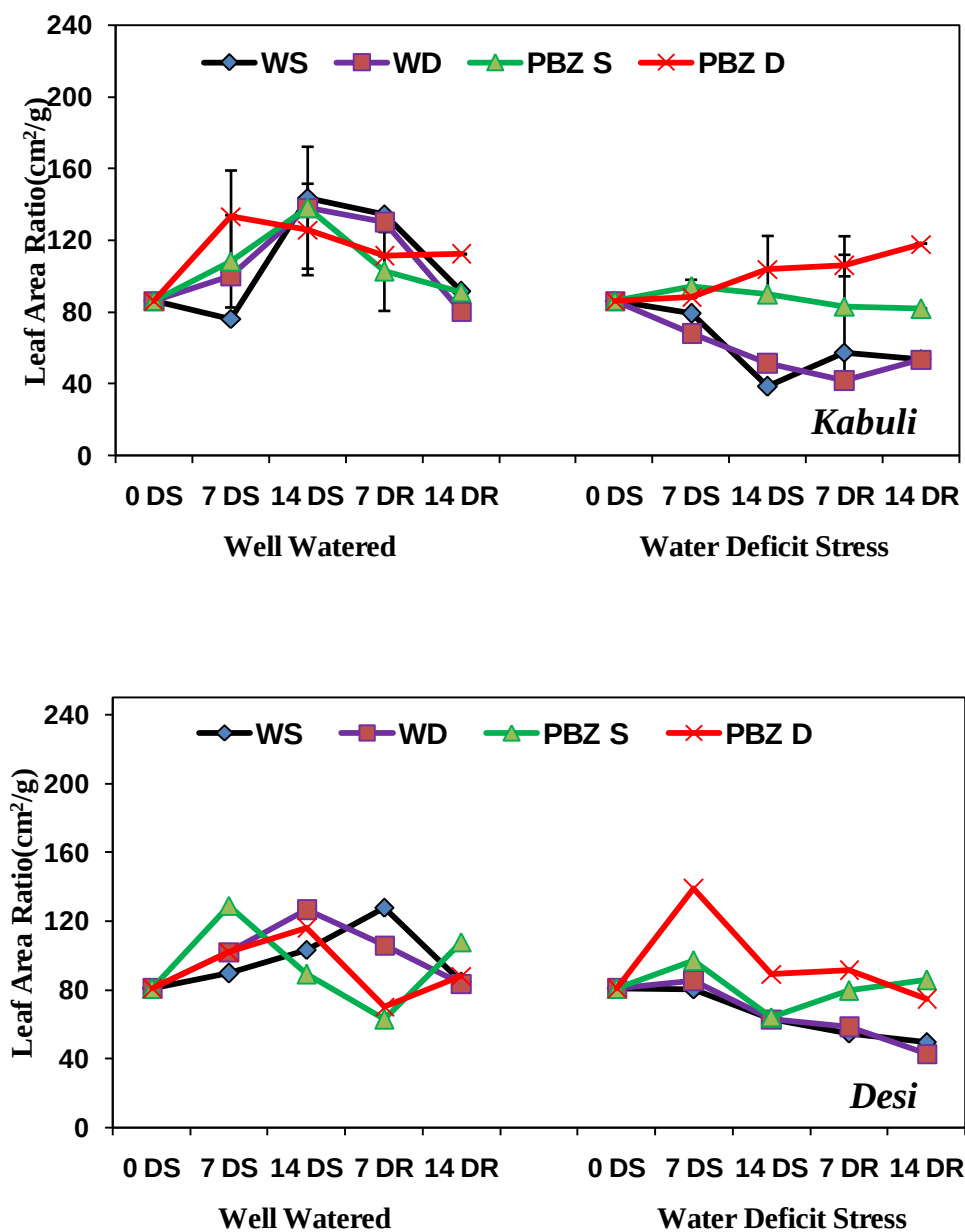


Fig. 4.3.1.2.3.10. Effect of PBZ on leaf area ratio (LAR) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

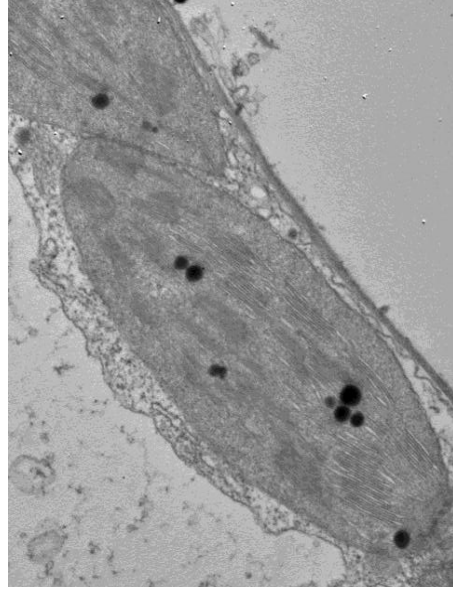
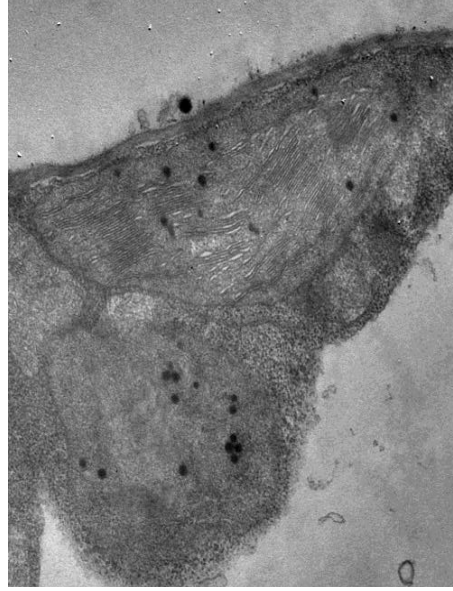
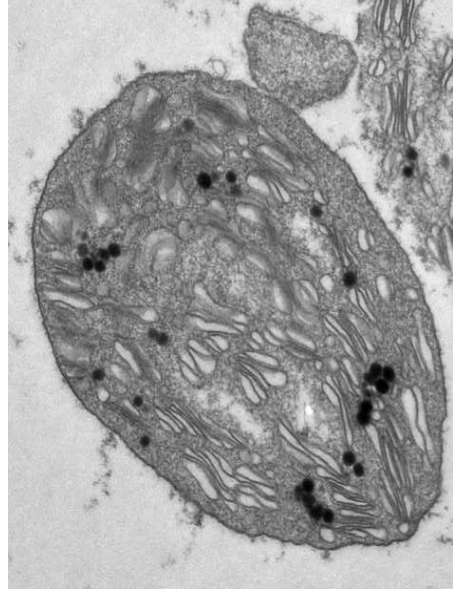
**Table 4.3.1.2.3.10.Effect of PBZ on leaf area ratio in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | LAR(cm <sup>2</sup> /g) |       |       |                    |        |        |                     |        |        |                |        |        |                 |        |        |
|----------------|---------------------------|-------------------------|-------|-------|--------------------|--------|--------|---------------------|--------|--------|----------------|--------|--------|-----------------|--------|--------|
|                |                           | 0 day water stress      |       |       | 7 day water stress |        |        | 14 day water stress |        |        | 7 day recovery |        |        | 14 day recovery |        |        |
|                |                           | Kabuli                  | Desi  | Mean  | Kabuli             | Desi   | Mean   | Kabuli              | Desi   | Mean   | Kabuli         | Desi   | Mean   | Kabuli          | Desi   | Mean   |
| Well watered   | Water Foliar spray        | 86.26                   | 80.78 | 83.52 | 76.13              | 89.76  | 82.94  | 143.72              | 103.46 | 123.59 | 134.53         | 127.92 | 131.23 | 91.41           | 84.81  | 88.11  |
|                | Water Drenching           | 86.26                   | 80.78 | 83.52 | 100.10             | 102.21 | 101.16 | 138.42              | 126.39 | 132.41 | 130.00         | 106.17 | 118.09 | 80.54           | 83.83  | 82.19  |
|                | PBZ Foliar spray (60 ppm) | 86.26                   | 80.78 | 83.52 | 108.23             | 128.96 | 118.59 | 138.14              | 89.02  | 113.58 | 102.58         | 62.80  | 82.69  | 91.16           | 107.82 | 99.49  |
|                | PBZ Drenching (120 ppm)   | 86.26                   | 80.78 | 83.52 | 133.41             | 102.32 | 117.86 | 126.02              | 115.76 | 120.89 | 111.22         | 70.32  | 90.77  | 112.31          | 88.34  | 100.33 |
|                | Mean                      | 86.26                   | 80.78 | 83.52 | 104.47             | 105.81 | 105.14 | 136.57              | 108.66 | 122.62 | 119.58         | 91.80  | 105.69 | 93.85           | 91.20  | 92.53  |
| Water stressed | Water Foliar spray        | 86.26                   | 80.78 | 83.52 | 79.04              | 80.39  | 79.71  | 38.47               | 63.20  | 50.83  | 57.32          | 54.81  | 56.07  | 53.81           | 49.68  | 51.74  |
|                | Water Drenching           | 86.26                   | 80.78 | 83.52 | 67.83              | 85.27  | 76.55  | 51.14               | 63.20  | 57.17  | 41.68          | 58.29  | 49.98  | 53.81           | 43.08  | 48.44  |
|                | PBZ Foliar spray (60 ppm) | 86.26                   | 80.78 | 83.52 | 94.09              | 97.01  | 95.55  | 89.72               | 64.25  | 76.99  | 83.24          | 79.68  | 81.46  | 81.88           | 85.64  | 83.76  |
|                | PBZ Drenching (120 ppm)   | 86.26                   | 80.78 | 83.52 | 88.43              | 138.86 | 113.65 | 103.92              | 89.25  | 96.58  | 105.89         | 91.29  | 98.59  | 118.08          | 74.66  | 96.37  |
|                | Mean                      | 86.26                   | 80.78 | 83.52 | 82.35              | 100.38 | 91.37  | 70.81               | 69.97  | 70.39  | 72.03          | 71.02  | 71.53  | 76.89           | 63.26  | 70.08  |
|                | SEM                       | 6.335                   |       |       | 19.741             |        |        | 16.03               |        |        | 9.533          |        |        | 34.937          |        |        |
|                | CD at 5 %                 |                         |       |       |                    |        |        |                     |        |        |                |        |        |                 |        |        |
|                | Water status (W)          | 6.454                   |       |       | N/A                |        |        | 16.33               |        |        | 9.711          |        |        | N/A             |        |        |
|                | Treatment (T)             | 9.128                   |       |       | N/A                |        |        | N/A                 |        |        | 13.734         |        |        | 50.334          |        |        |
|                | Varieties (V)             | N/A                     |       |       | N/A                |        |        | N/A                 |        |        | N/A            |        |        | N/A             |        |        |
|                | W x T =                   | N/A                     |       |       | N/A                |        |        | N/A                 |        |        | 19.423         |        |        | N/A             |        |        |
|                | W x V =                   | N/A                     |       |       | N/A                |        |        | N/A                 |        |        | N/A            |        |        | N/A             |        |        |
|                | Tx V =                    | N/A                     |       |       | N/A                |        |        | 32.661              |        |        | 19.423         |        |        | N/A             |        |        |
|                | W x Tx V=                 | 18.255                  |       |       | 56.882             |        |        | N/A                 |        |        | 27.468         |        |        | N/A             |        |        |

Water stress (WS)

WS + PBZ Foliar Spray

WS + PBZ Drenching



**Desi (Pusa 362)**

**Kabuli (Pusa 1108)**

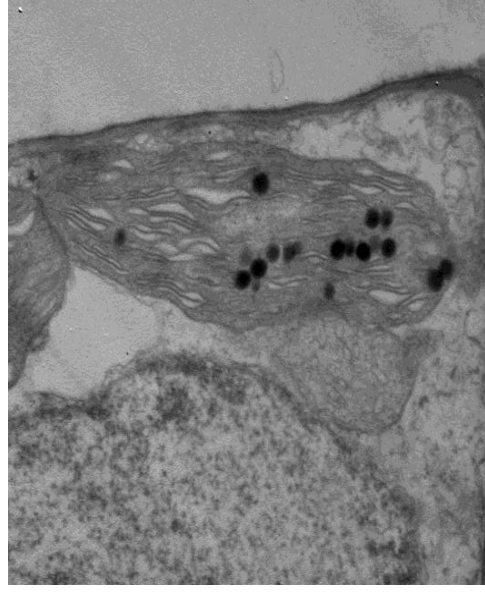
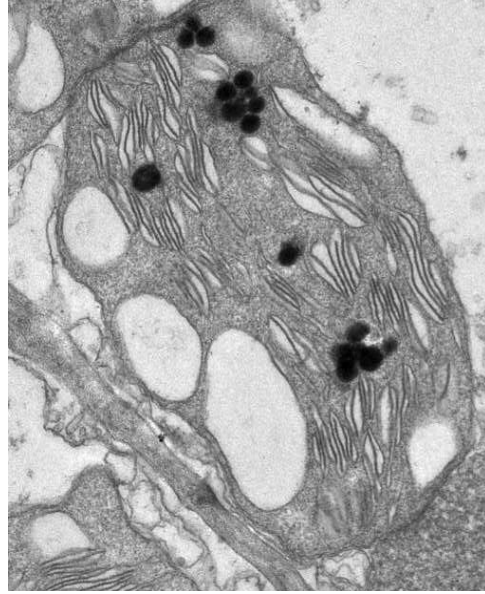


Fig. 4.3.1.2.4 Effect of PBZ on chloroplast ultra structure in chickpea genotypes under imposed water deficit stressed conditions.

treatment had large and high number of starch grains and low number of osmophilic granules in chloroplast in *desi* chickpea variety (Fig. 4.3.1.2.4.).

#### **4.3.1.2.5. Photosynthesis and its associated parameters**

##### **4.3.1.2.5.1. Photosynthesis rate**

Rate of photosynthesis was reported to decrease with the advancement of water stress duration and maximum reduction was obtained on 14<sup>th</sup> day of imposed water deficit stress in both the varieties. Foliar and drenching application of PBZ maintained the higher rate of photosynthesis under water deficit condition and also showed the fast recovery after water stress termination. However, the foliar and drenching application of PBZ under well watered condition were unable to show significant increase in rate of photosynthesis. Drenching application of the PBZ showed the better efficacy in terms of photosynthesis rate (Fig.4.3.1.2.5.1.and Table 4.3.1.2.5.1.).

##### **4.3.1.2.5.2. Stomatal conductance**

Foliar and drenching applications, of PBZ reduced the stomatal conductance under well watered condition in general particularly in *desi* variety. Stomatal conductance gradually decreased with advancement in water deficit period and recovered after water stress termination. Faster recovery was obtained with the foliar and drenching application of PBZ particularly in *kabuli* variety (Fig.4.3.1.2.5.2.and Table 4.3.1.2.5.2.).

##### **4.3.1.2.5.3. Transpiration rate**

Imposed water deficit condition in both *desi* and *kabuli* varieties drastically reduced the rate of transpiration. There was no significant alteration in transpiration rate with the foliar and drenching application of PBZ under well watered condition. However, under water deficit condition, both foliar and drenching application significantly reduced the rate of transpiration in both *desi* and *kabuli* varieties (Fig.4.3.1.2.5.3.and Table 4.3.1.2.5.3.).

##### **4.3.1.2.5.4. Leaf water use efficiency**

In both *desi* and *kabuli* varieties foliar and drenching applications had the non-significant change under well watered condition. However, during water deficit condition and after water deficit stress termination highly significant enhancement in leaf water use efficiency was

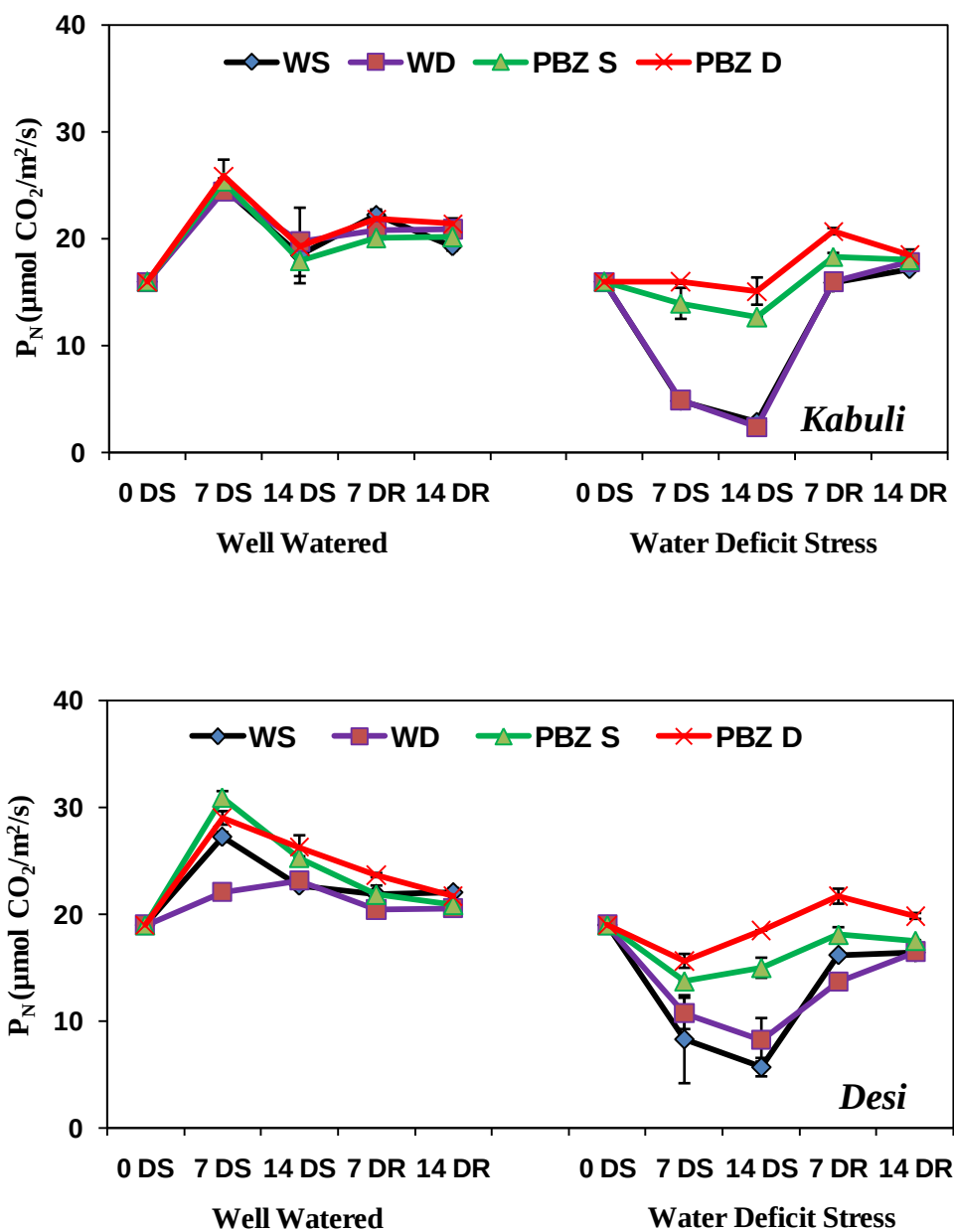


Fig. 4.3.1.2.5.1. Effect of PBZ on photosynthetic rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.5.1. Effect of PBZ on photosynthetic rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Photosynthetic rate (μmol CO <sub>2</sub> /m <sup>2</sup> /s) |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|---------------------------------------------------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress                                            |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                                                        | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 16.00                                                         | 19.00 | 17.50 | 24.77              | 27.25 | 26.01 | 18.43               | 22.68 | 20.56 | 22.27          | 21.89 | 22.08 | 19.30           | 22.05 | 20.67 |
|                | Water Drenching           | 16.00                                                         | 19.00 | 17.50 | 24.47              | 22.10 | 23.28 | 19.70               | 23.15 | 21.43 | 20.83          | 20.44 | 20.64 | 20.85           | 20.59 | 20.72 |
|                | PBZ Foliar spray (60 ppm) | 16.00                                                         | 19.00 | 17.50 | 25.35              | 30.93 | 28.14 | 17.92               | 25.30 | 21.61 | 20.07          | 21.85 | 20.96 | 20.15           | 20.90 | 20.52 |
|                | PBZ Drenching (120 ppm)   | 16.00                                                         | 19.00 | 17.50 | 25.90              | 29.03 | 27.47 | 19.32               | 26.30 | 22.81 | 21.87          | 23.68 | 22.77 | 21.45           | 21.73 | 21.59 |
|                | Mean                      | 16.00                                                         | 19.00 | 17.50 | 25.12              | 27.33 | 26.22 | 18.84               | 24.36 | 21.60 | 21.26          | 21.96 | 21.61 | 20.44           | 21.32 | 20.88 |
| Water stressed | Water Foliar spray        | 16.00                                                         | 19.00 | 17.50 | 4.84               | 8.32  | 6.58  | 2.88                | 5.71  | 4.30  | 15.84          | 16.20 | 16.02 | 17.15           | 16.43 | 16.79 |
|                | Water Drenching           | 16.00                                                         | 19.00 | 17.50 | 4.88               | 10.73 | 7.81  | 2.42                | 8.26  | 5.34  | 15.94          | 13.69 | 14.81 | 17.89           | 16.50 | 17.19 |
|                | PBZ Foliar spray (60 ppm) | 16.00                                                         | 19.00 | 17.50 | 13.95              | 13.75 | 13.85 | 12.65               | 15.01 | 13.83 | 18.34          | 18.10 | 18.22 | 18.02           | 17.54 | 17.78 |
|                | PBZ Drenching (120 ppm)   | 16.00                                                         | 19.00 | 17.50 | 15.93              | 15.65 | 15.79 | 15.10               | 18.51 | 16.81 | 20.71          | 21.70 | 21.20 | 18.44           | 19.86 | 19.15 |
|                | Mean                      | 16.00                                                         | 19.00 | 17.50 | 9.90               | 12.11 | 11.01 | 8.26                | 11.87 | 10.07 | 17.71          | 17.42 | 17.56 | 17.88           | 17.58 | 17.73 |
|                | SEM                       | 0.289                                                         |       |       | 0.722              |       |       | 0.712               |       |       | 0.377          |       |       | 0.231           |       |       |
|                | CD at 5 %                 |                                                               |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                                                           |       |       | 0.736              |       |       | 0.725               |       |       | 0.384          |       |       | 0.236           |       |       |
|                | Treatment (T)             | N/A                                                           |       |       | 1.041              |       |       | 1.025               |       |       | 0.543          |       |       | 0.333           |       |       |
|                | Varieties (V)             | 0.294                                                         |       |       | 0.736              |       |       | 0.725               |       |       | N/A            |       |       | 0.236           |       |       |
|                | W x T =                   | N/A                                                           |       |       | 1.472              |       |       | 1.45                |       |       | 0.768          |       |       | 0.471           |       |       |
|                | W x V =                   | N/A                                                           |       |       | N/A                |       |       | 1.025               |       |       | 0.543          |       |       | 0.333           |       |       |
|                | Tx V =                    | N/A                                                           |       |       | N/A                |       |       | N/A                 |       |       | 0.768          |       |       | 0.471           |       |       |
|                | W x Tx V=                 | N/A                                                           |       |       | 2.081              |       |       | 2.051               |       |       | N/A            |       |       | 0.666           |       |       |

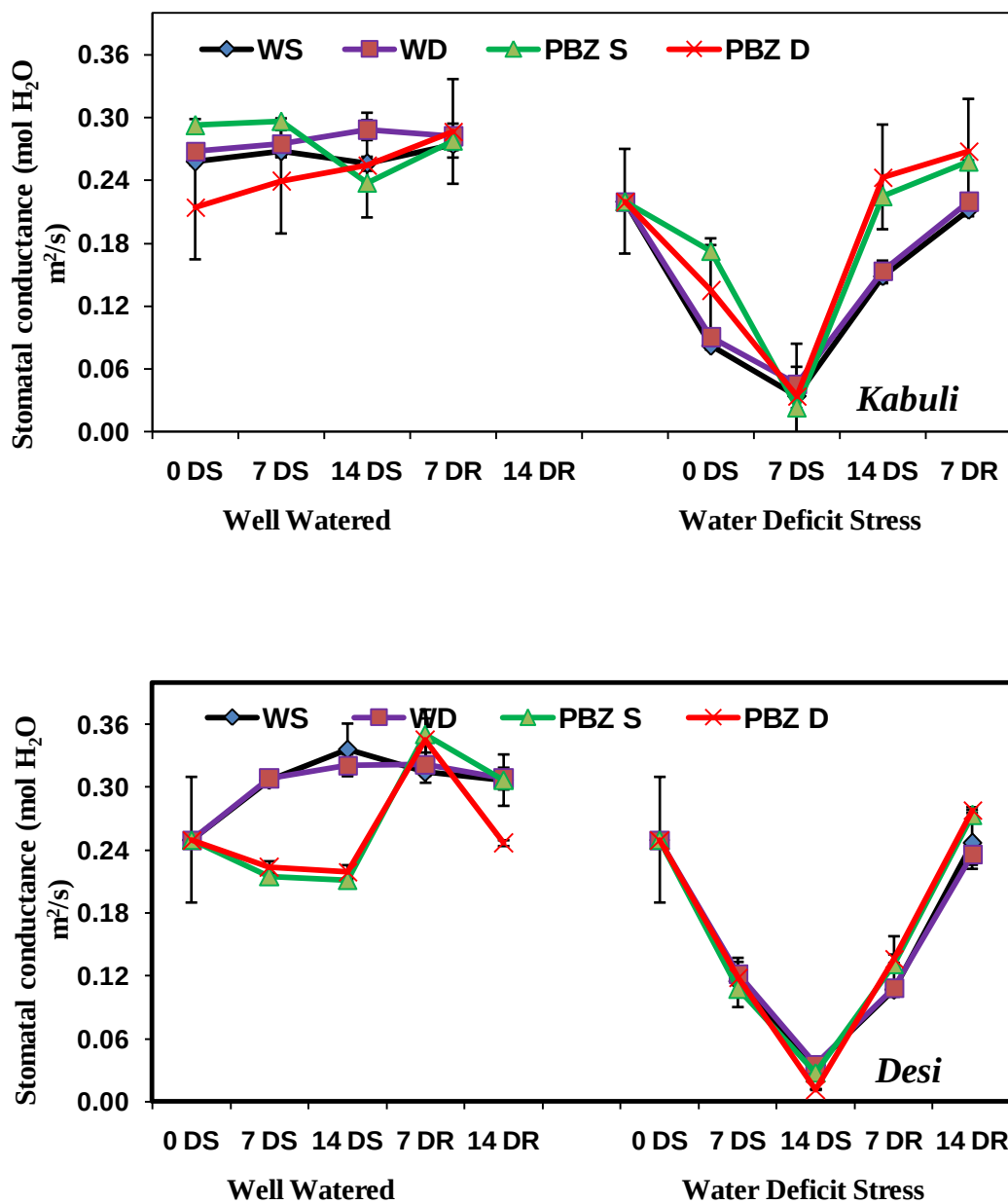


Fig. 4.3.1.2.5.2. Effect of PBZ on stomatal conductance rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.5.2. Effect of PBZ on stomatal conductance rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Stomatal Conductance (mol H <sub>2</sub> O m <sup>2</sup> /s) |             |             |                    |             |             |                     |             |             |                |             |             |                 |             |             |
|----------------|---------------------------|---------------------------------------------------------------|-------------|-------------|--------------------|-------------|-------------|---------------------|-------------|-------------|----------------|-------------|-------------|-----------------|-------------|-------------|
|                |                           | 0 day water stress                                            |             |             | 7 day water stress |             |             | 14 day water stress |             |             | 7 day recovery |             |             | 14 day recovery |             |             |
|                |                           | Kabuli                                                        | Desi        | Mean        | Kabuli             | Desi        | Mean        | Kabuli              | Desi        | Mean        | Kabuli         | Desi        | Mean        | Kabuli          | Desi        | Mean        |
| Well watered   | Water Foliar spray        | 0.22                                                          | 0.25        | <b>0.24</b> | 0.26               | 0.31        | <b>0.28</b> | 0.27                | 0.34        | <b>0.30</b> | 0.26           | 0.31        | <b>0.29</b> | 0.27            | 0.31        | <b>0.29</b> |
|                | Water Drenching           | 0.22                                                          | 0.25        | <b>0.24</b> | 0.27               | 0.31        | <b>0.29</b> | 0.28                | 0.32        | <b>0.30</b> | 0.29           | 0.32        | <b>0.31</b> | 0.28            | 0.31        | <b>0.30</b> |
|                | PBZ Foliar spray (60 ppm) | 0.22                                                          | 0.25        | <b>0.24</b> | 0.29               | 0.22        | <b>0.25</b> | 0.30                | 0.21        | <b>0.25</b> | 0.24           | 0.35        | <b>0.29</b> | 0.28            | 0.31        | <b>0.29</b> |
|                | PBZ Drenching (120 ppm)   | 0.22                                                          | 0.25        | <b>0.24</b> | 0.21               | 0.22        | <b>0.22</b> | 0.24                | 0.22        | <b>0.23</b> | 0.25           | 0.34        | <b>0.30</b> | 0.29            | 0.25        | <b>0.27</b> |
|                | Mean                      | <b>0.22</b>                                                   | <b>0.25</b> | <b>0.24</b> | <b>0.26</b>        | <b>0.26</b> | <b>0.26</b> | <b>0.27</b>         | <b>0.27</b> | <b>0.27</b> | <b>0.26</b>    | <b>0.33</b> | <b>0.30</b> | <b>0.28</b>     | <b>0.29</b> | <b>0.29</b> |
| Water stressed | Water Foliar spray        | 0.22                                                          | 0.25        | <b>0.24</b> | 0.08               | 0.11        | <b>0.10</b> | 0.03                | 0.03        | <b>0.03</b> | 0.15           | 0.11        | <b>0.13</b> | 0.21            | 0.25        | <b>0.23</b> |
|                | Water Drenching           | 0.22                                                          | 0.25        | <b>0.24</b> | 0.09               | 0.12        | <b>0.11</b> | 0.05                | 0.04        | <b>0.04</b> | 0.15           | 0.11        | <b>0.13</b> | 0.22            | 0.24        | <b>0.23</b> |
|                | PBZ Foliar spray (60 ppm) | 0.22                                                          | 0.25        | <b>0.24</b> | 0.17               | 0.11        | <b>0.14</b> | 0.02                | 0.03        | <b>0.03</b> | 0.22           | 0.13        | <b>0.18</b> | 0.26            | 0.27        | <b>0.27</b> |
|                | PBZ Drenching (120 ppm)   | 0.22                                                          | 0.25        | <b>0.24</b> | 0.13               | 0.12        | <b>0.13</b> | 0.03                | 0.01        | <b>0.02</b> | 0.24           | 0.14        | <b>0.19</b> | 0.27            | 0.28        | <b>0.27</b> |
|                | Mean                      | <b>0.22</b>                                                   | <b>0.25</b> | <b>0.24</b> | <b>0.12</b>        | <b>0.12</b> | <b>0.12</b> | <b>0.03</b>         | <b>0.03</b> | <b>0.03</b> | <b>0.19</b>    | <b>0.12</b> | <b>0.16</b> | <b>0.24</b>     | <b>0.26</b> | <b>0.25</b> |
|                | SEM                       | 0.006                                                         |             |             | 35.301             |             |             | 0.008               |             |             | 0.007          |             |             | 0.005           |             |             |
|                | CD at 5 %                 |                                                               |             |             |                    |             |             |                     |             |             |                |             |             |                 |             |             |
|                | Water status (W)          | N/A                                                           |             |             | 0.008              |             |             | 0.009               |             |             | 0.008          |             |             | 0.005           |             |             |
|                | Treatment (T)             | N/A                                                           |             |             | 0.011              |             |             | 0.012               |             |             | 0.011          |             |             | 0.007           |             |             |
|                | Varieties (V)             | 0.006                                                         |             |             | N/A                |             |             | N/A                 |             |             | N/A            |             |             | 0.005           |             |             |
|                | W x T =                   | N/A                                                           |             |             | 0.016              |             |             | 0.017               |             |             | 0.015          |             |             | 0.01            |             |             |
|                | W x V =                   | N/A                                                           |             |             | N/A                |             |             | N/A                 |             |             | 0.011          |             |             | 0.007           |             |             |
|                | Tx V =                    | N/A                                                           |             |             | 0.016              |             |             | 0.017               |             |             | N/A            |             |             | 0.01            |             |             |
|                | W x Tx V=                 | 0.017                                                         |             |             | N/A                |             |             | 0.024               |             |             | 0.021          |             |             | N/A             |             |             |

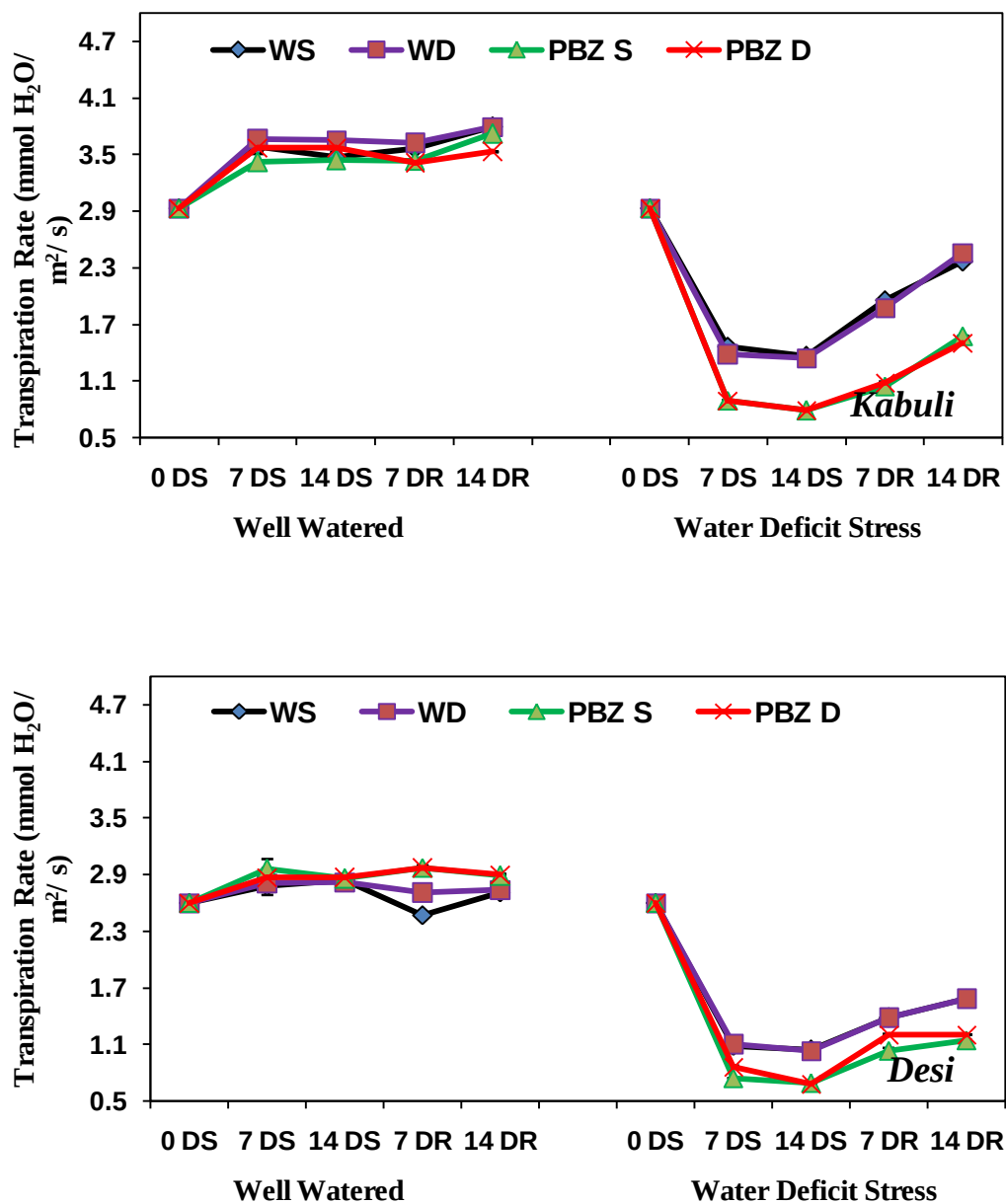


Fig. 4.3.1.2.5.3. Effect of PBZ on transpiration rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.5.3. Effect of PBZ on transpiration rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Transpiration Rate (mmol H <sub>2</sub> O/ m <sup>2</sup> / s) |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|----------------|---------------------------|----------------------------------------------------------------|------|------|--------------------|------|------|---------------------|------|------|----------------|------|------|-----------------|------|------|
|                |                           | 0 day water stress                                             |      |      | 7 day water stress |      |      | 14 day water stress |      |      | 7 day recovery |      |      | 14 day recovery |      |      |
|                |                           | Kabuli                                                         | Desi | Mean | Kabuli             | Desi | Mean | Kabuli              | Desi | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi | Mean |
| Well watered   | Water Foliar spray        | 2.93                                                           | 2.60 | 2.77 | 3.59               | 2.77 | 3.18 | 3.47                | 2.84 | 3.15 | 3.57           | 2.47 | 3.02 | 3.79            | 2.71 | 3.25 |
|                | Water Drenching           | 2.93                                                           | 2.60 | 2.77 | 3.66               | 2.81 | 3.24 | 3.66                | 2.82 | 3.24 | 3.62           | 2.71 | 3.16 | 3.79            | 2.74 | 3.27 |
|                | PBZ Foliar spray (60 ppm) | 2.93                                                           | 2.60 | 2.77 | 3.42               | 2.96 | 3.19 | 3.44                | 2.86 | 3.15 | 3.43           | 2.97 | 3.20 | 3.72            | 2.89 | 3.30 |
|                | PBZ Drenching (120 ppm)   | 2.93                                                           | 2.60 | 2.77 | 3.58               | 2.87 | 3.23 | 3.57                | 2.87 | 3.22 | 3.41           | 2.97 | 3.19 | 3.53            | 2.90 | 3.22 |
|                | Mean                      | 2.93                                                           | 2.60 | 2.77 | 3.56               | 2.85 | 3.21 | 3.53                | 2.85 | 3.19 | 3.51           | 2.78 | 3.14 | 3.71            | 2.81 | 3.26 |
| Water stressed | Water Foliar spray        | 2.93                                                           | 2.60 | 2.77 | 1.47               | 1.08 | 1.28 | 1.96                | 1.04 | 1.50 | 1.96           | 1.38 | 1.67 | 2.36            | 1.59 | 1.98 |
|                | Water Drenching           | 2.93                                                           | 2.60 | 2.77 | 1.38               | 1.10 | 1.24 | 1.88                | 1.03 | 1.45 | 1.88           | 1.38 | 1.63 | 2.46            | 1.58 | 2.02 |
|                | PBZ Foliar spray (60 ppm) | 2.93                                                           | 2.60 | 2.77 | 0.89               | 0.74 | 0.82 | 1.04                | 0.70 | 0.87 | 1.04           | 1.04 | 1.04 | 1.57            | 1.14 | 1.36 |
|                | PBZ Drenching (120 ppm)   | 2.93                                                           | 2.60 | 2.77 | 0.89               | 0.86 | 0.88 | 1.08                | 0.68 | 0.88 | 1.08           | 1.21 | 1.14 | 1.50            | 1.20 | 1.35 |
|                | Mean                      | 2.93                                                           | 2.60 | 2.77 | 1.16               | 0.95 | 1.05 | 1.49                | 0.86 | 1.18 | 1.49           | 1.25 | 1.37 | 1.98            | 1.38 | 1.68 |
|                | SEM                       | 0.025                                                          |      |      | 0.035              |      |      | 0.043               |      |      | 0.039          |      |      | 0.027           |      |      |
|                | CD at 5 %                 |                                                                |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|                | Water status (W)          | N/A                                                            |      |      | 0.036              |      |      | 0.044               |      |      | 0.039          |      |      | 0.027           |      |      |
|                | Treatment (T)             | N/A                                                            |      |      | 0.051              |      |      | 0.062               |      |      | 0.056          |      |      | 0.038           |      |      |
|                | Varieties (V)             | 0.026                                                          |      |      | 0.036              |      |      | 0.044               |      |      | 0.039          |      |      | 0.027           |      |      |
|                | W x T =                   | 0.051                                                          |      |      | 0.072              |      |      | 0.088               |      |      | 0.079          |      |      | 0.054           |      |      |
|                | W x V =                   | 0.036                                                          |      |      | 0.051              |      |      | 0.062               |      |      | 0.056          |      |      | 0.038           |      |      |
|                | Tx V =                    | 0.051                                                          |      |      | 0.072              |      |      | 0.088               |      |      | 0.079          |      |      | 0.054           |      |      |
|                | W x Tx V=                 | N/A                                                            |      |      | 0.101              |      |      | N/A                 |      |      | N/A            |      |      | 0.077           |      |      |

estimated in both the varieties. Maximum leaf water use efficiency was recorded with the drenching application of PBZ (Fig.4.3.1.2.5.4.and Table 4.3.1.2.5.4.).

#### **4.3.1.2.5.5. Fv/Fm ratio**

Under water deficit condition, Fv/Fm ratio drastically reduced and recovered gradually after water deficit termination. Foliar and drenching applications of PBZ enhanced the quantum yield of both *kabuli* and *desi* varieties under well watered condition and maintained high Fv/Fm ratio under water deficit condition (Fig.4.3.1.2.5.5.).

#### **4.3.1.2.6. Photosynthetic pigments**

##### **4.3.1.2.6.1. Chlorophyll a content**

Plants of both *kabuli* and *desi* varieties, treated with foliar and drenching application enhanced the chlorophyll a content over their respective controls under both well watered and water deficit conditions. Maximum enhancement was recorded with the drenching applications of PBZ in both the varieties under both the conditions (Fig.4.3.1.2.6.1.and Table 4.3.1.2.6.1.).

##### **4.3.1.2.6.2. Chlorophyll b content**

Chlorophyll b content was also recorded higher with the foliar and drenching application of PBZ in both *kabuli* and *desi* varieties under well watered and water stress conditions. Plants treated with drenching application of PBZ had the highest value of chlorophyll b content in both varieties under normal and water stress conditions (Fig.4.3.1.2.6.2.and Table 4.3.1.2.6.2.).

##### **4.3.1.2.6.3. Total chlorophyll content**

Similarly, total chlorophyll content was also estimated higher with the foliar and drenching application of PBZ in both *kabuli* and *desi* varieties under both well watered and water stress conditions. Highest value of total chlorophyll content was obtained in plants treated with drenching application of PBZ in both varieties under both well watered and water deficit stress conditions (Fig.4.3.1.2.6.3.and Table 4.3.1.2.6.3.).

##### **4.3.1.2.6.4. Total carotenoid content**

In both *desi* and *kabuli* chickpea varieties, plants treated with foliar and drenching applications of PBZ had the higher content of total carotenoids under well watered and water

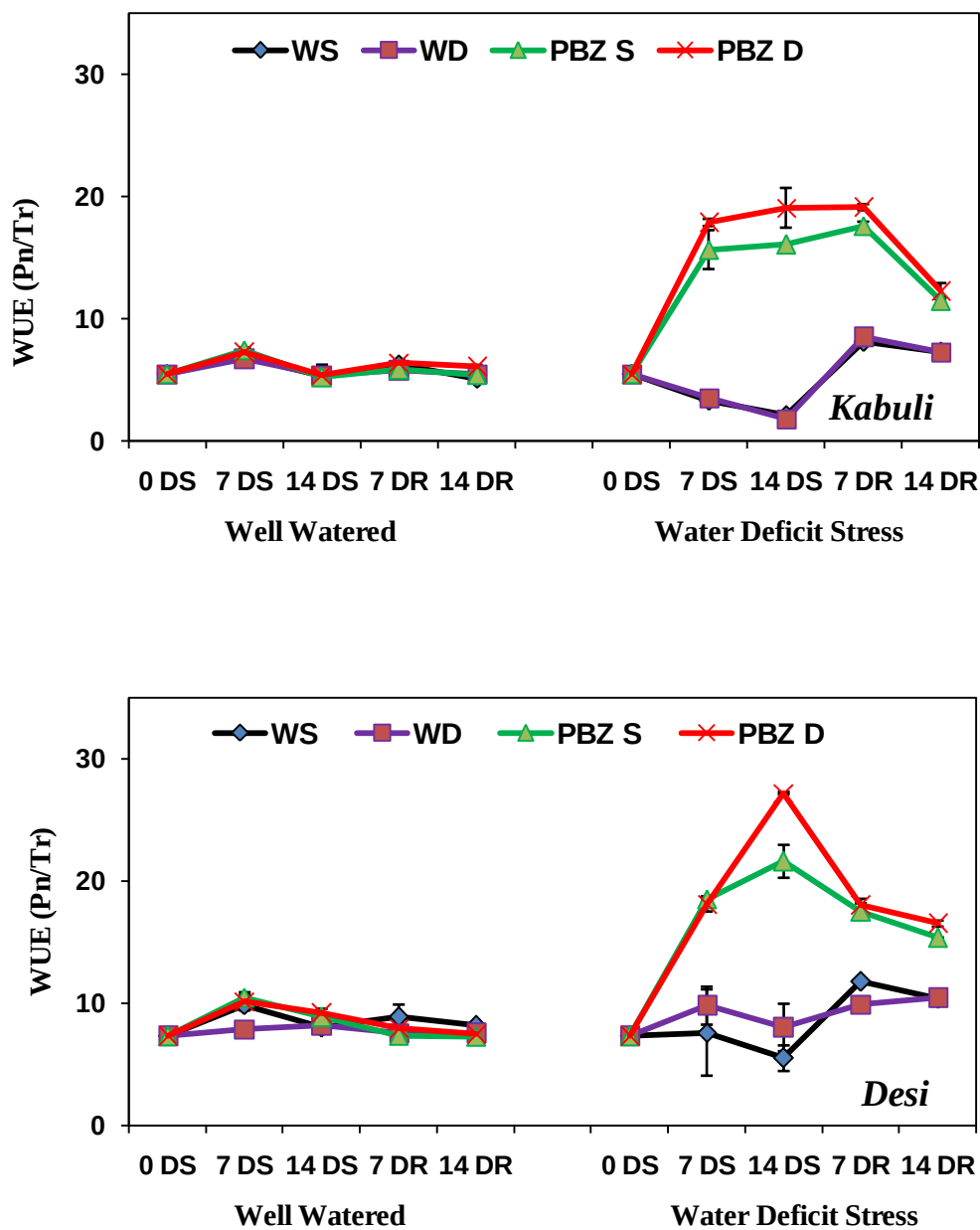


Fig. 4.3.1.2.5.4. Effect of PBZ on leaf water use efficiency (WUE) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.5.4. Effect of PBZ on leaf water use efficiency in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Water Use Efficiency (PN/Transpiration rate) |      |      |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|----------------------------------------------|------|------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress                           |      |      | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                                       | Desi | Mean | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 5.46                                         | 7.31 | 6.38 | 6.91               | 9.82  | 8.37  | 5.32                | 7.98  | 6.65  | 6.24           | 8.86  | 7.55  | 5.09            | 8.14  | 6.61  |
|                | Water Drenching           | 5.46                                         | 7.31 | 6.38 | 6.68               | 7.87  | 7.27  | 5.39                | 8.20  | 6.79  | 5.76           | 7.55  | 6.65  | 5.50            | 7.51  | 6.51  |
|                | PBZ Foliar spray (60 ppm) | 5.46                                         | 7.31 | 6.38 | 7.42               | 10.46 | 8.94  | 5.21                | 8.85  | 7.03  | 5.84           | 7.35  | 6.60  | 5.42            | 7.24  | 6.33  |
|                | PBZ Drenching (120 ppm)   | 5.46                                         | 7.31 | 6.38 | 7.24               | 10.13 | 8.69  | 5.40                | 9.17  | 7.29  | 6.41           | 7.97  | 7.19  | 6.08            | 7.48  | 6.78  |
|                | Mean                      | 5.46                                         | 7.31 | 6.38 | 7.06               | 9.57  | 8.32  | 5.33                | 8.55  | 6.94  | 6.06           | 7.93  | 7.00  | 5.52            | 7.59  | 6.56  |
| Water stressed | Water Foliar spray        | 5.46                                         | 7.31 | 6.38 | 3.30               | 7.58  | 5.44  | 2.12                | 5.48  | 3.80  | 8.09           | 11.73 | 9.91  | 7.25            | 10.35 | 8.80  |
|                | Water Drenching           | 5.46                                         | 7.31 | 6.38 | 3.54               | 9.78  | 6.66  | 1.80                | 8.00  | 4.90  | 8.50           | 9.90  | 9.20  | 7.27            | 10.42 | 8.85  |
|                | PBZ Foliar spray (60 ppm) | 5.46                                         | 7.31 | 6.38 | 15.65              | 18.52 | 17.09 | 16.05               | 21.59 | 18.82 | 17.60          | 17.48 | 17.54 | 11.45           | 15.34 | 13.40 |
|                | PBZ Drenching (120 ppm)   | 5.46                                         | 7.31 | 6.38 | 17.87              | 18.09 | 17.98 | 19.07               | 27.17 | 23.12 | 19.12          | 17.98 | 18.55 | 12.26           | 16.49 | 14.37 |
|                | Mean                      | 5.46                                         | 7.31 | 6.38 | 10.09              | 13.49 | 11.79 | 9.76                | 15.56 | 12.66 | 13.33          | 14.27 | 13.80 | 9.56            | 13.15 | 11.35 |
|                | SEM                       | 0.018                                        |      |      | 0.627              |       |       | 0.49                |       |       | 0.246          |       |       | 0.134           |       |       |
|                | CD at 5 %                 |                                              |      |      |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                                          |      |      | 0.639              |       |       | 0.499               |       |       | 0.251          |       |       | 0.137           |       |       |
|                | Treatment (T)             | N/A                                          |      |      | 0.903              |       |       | 0.706               |       |       | 0.355          |       |       | 0.194           |       |       |
|                | Varieties (V)             | 0.018                                        |      |      | 0.639              |       |       | 0.499               |       |       | 0.251          |       |       | 0.137           |       |       |
|                | W x T =                   | N/A                                          |      |      | 1.277              |       |       | 0.999               |       |       | 0.502          |       |       | 0.274           |       |       |
|                | W x V =                   | N/A                                          |      |      | N/A                |       |       | 0.706               |       |       | 0.355          |       |       | 0.194           |       |       |
|                | Tx V =                    | N/A                                          |      |      | N/A                |       |       | 0.999               |       |       | 0.502          |       |       | 0.274           |       |       |
|                | W x Tx V=                 | 0.051                                        |      |      | 1.806              |       |       | N/A                 |       |       | 0.709          |       |       | 0.387           |       |       |

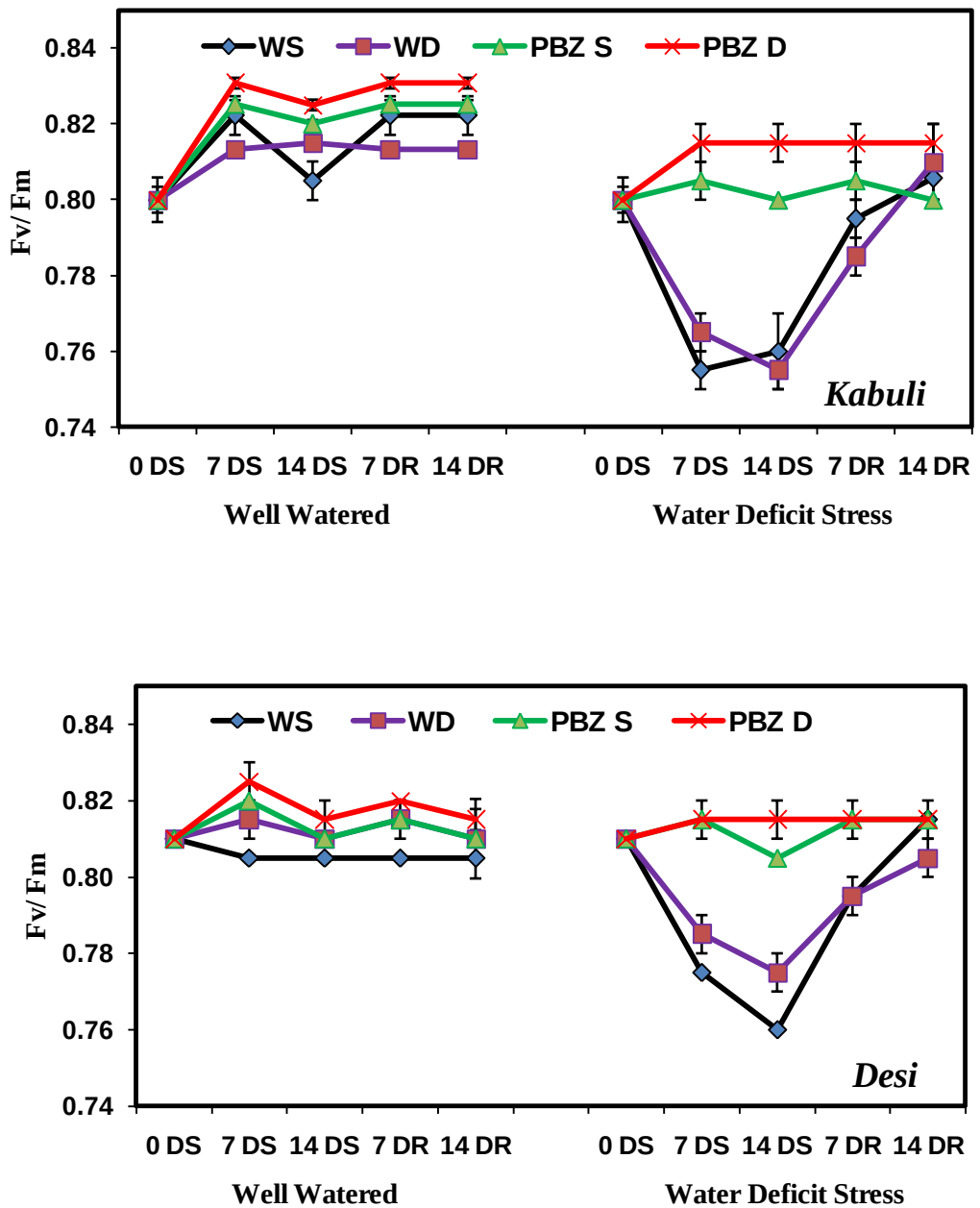


Fig. 4.3.1.2.5.5. Effect of PBZ on  $F_v/F_m$  in chickpea genotypes under well watered and imposed water deficit stressed conditions.

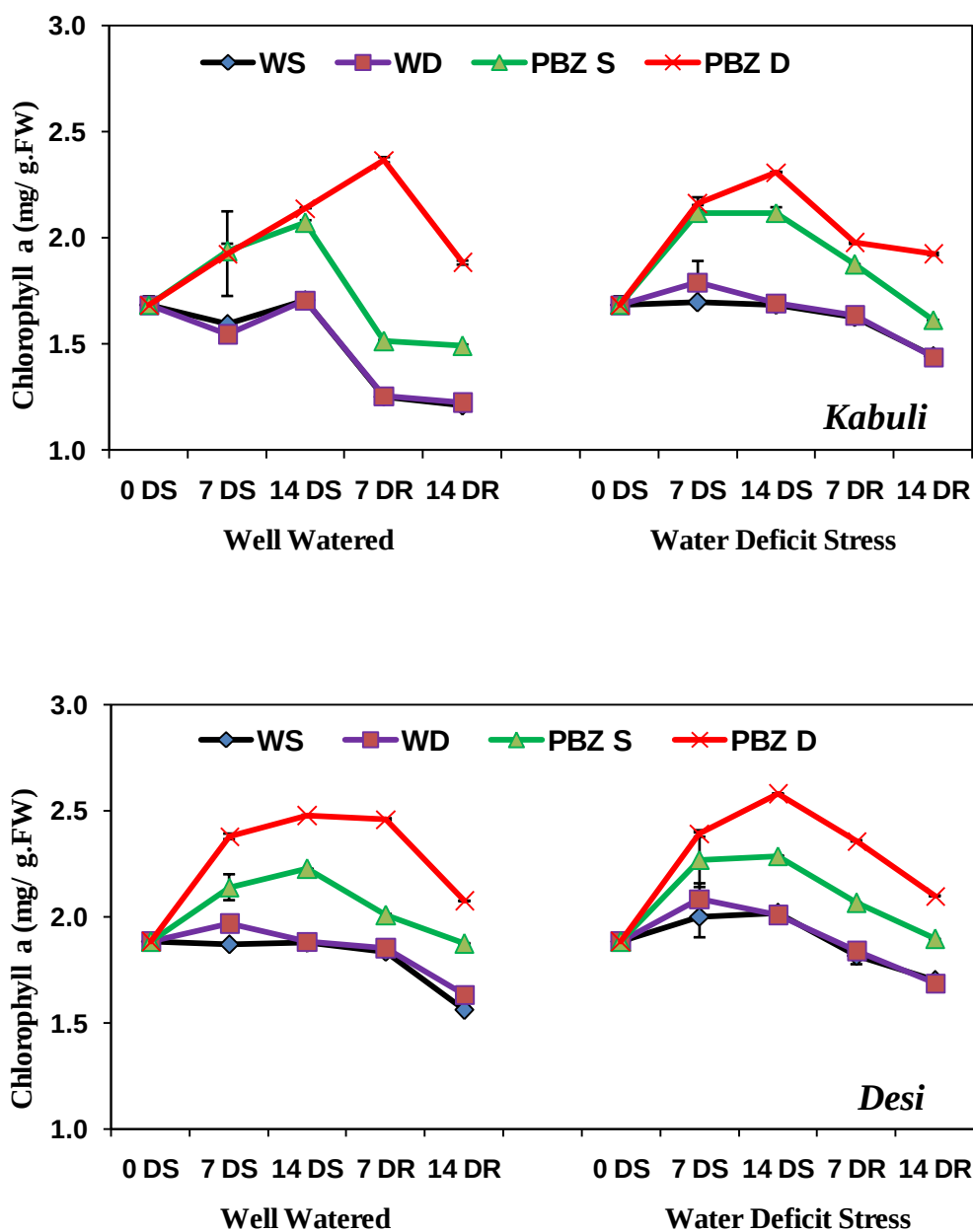


Fig. 4.3.1.2.6.1. Effect of PBZ on chlorophyll a in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.6.1. Effect of PBZ on chlorophyll a in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Chlorophyll a (mg/ g.FW) |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|----------------|---------------------------|--------------------------|------|------|--------------------|------|------|---------------------|------|------|----------------|------|------|-----------------|------|------|
|                |                           | 0 day water stress       |      |      | 7 day water stress |      |      | 14 day water stress |      |      | 7 day recovery |      |      | 14 day recovery |      |      |
|                |                           | Kabuli                   | Desi | Mean | Kabuli             | Desi | Mean | Kabuli              | Desi | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi | Mean |
| Well watered   | Water Foliar spray        | 1.69                     | 1.88 | 1.78 | 1.60               | 1.87 | 1.73 | 1.71                | 1.88 | 1.79 | 1.25           | 1.83 | 1.54 | 1.21            | 1.56 | 1.39 |
|                | Water Drenching           | 1.69                     | 1.88 | 1.78 | 1.55               | 1.97 | 1.76 | 1.71                | 1.89 | 1.80 | 1.26           | 1.85 | 1.55 | 1.22            | 1.63 | 1.43 |
|                | PBZ Foliar spray (60 ppm) | 1.69                     | 1.88 | 1.78 | 1.94               | 2.14 | 2.04 | 2.07                | 2.23 | 2.15 | 1.51           | 2.01 | 1.76 | 1.49            | 1.87 | 1.68 |
|                | PBZ Drenching (120 ppm)   | 1.69                     | 1.88 | 1.78 | 1.93               | 2.38 | 2.15 | 2.14                | 2.48 | 2.31 | 2.37           | 2.46 | 2.41 | 1.88            | 2.08 | 1.98 |
|                | Mean                      | 1.69                     | 1.88 | 1.78 | 1.75               | 2.09 | 1.92 | 1.91                | 2.12 | 2.01 | 1.60           | 2.04 | 1.82 | 1.45            | 1.79 | 1.62 |
| Water stressed | Water Foliar spray        | 1.69                     | 1.88 | 1.78 | 1.70               | 2.00 | 1.85 | 1.68                | 2.02 | 1.85 | 1.63           | 1.82 | 1.72 | 1.44            | 1.70 | 1.57 |
|                | Water Drenching           | 1.69                     | 1.88 | 1.78 | 1.79               | 2.08 | 1.94 | 1.69                | 2.01 | 1.85 | 1.63           | 1.84 | 1.74 | 1.44            | 1.69 | 1.56 |
|                | PBZ Foliar spray (60 ppm) | 1.69                     | 1.88 | 1.78 | 2.12               | 2.27 | 2.19 | 2.12                | 2.29 | 2.20 | 1.88           | 2.07 | 1.97 | 1.61            | 1.90 | 1.75 |
|                | PBZ Drenching (120 ppm)   | 1.69                     | 1.88 | 1.78 | 2.16               | 2.39 | 2.28 | 2.31                | 2.58 | 2.45 | 1.98           | 2.36 | 2.17 | 1.92            | 2.10 | 2.01 |
|                | Mean                      | 1.69                     | 1.88 | 1.78 | 1.94               | 2.19 | 2.06 | 1.95                | 2.22 | 2.09 | 1.78           | 2.02 | 1.90 | 1.61            | 1.85 | 1.73 |
|                | SEM                       | 0.014                    |      |      | 0.043              |      |      | 0.006               |      |      | 0.007          |      |      | 0.003           |      |      |
|                | CD at 5 %                 |                          |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|                | Water status (W)          | N/A                      |      |      | 0.044              |      |      | 0.007               |      |      | N/A            |      |      | 0.003           |      |      |
|                | Treatment (T)             | N/A                      |      |      | 0.062              |      |      | 0.009               |      |      | 0.01           |      |      | 0.004           |      |      |
|                | Varieties (V)             | 0.014                    |      |      | 0.044              |      |      | 0.007               |      |      | 0.007          |      |      | 0.003           |      |      |
|                | W x T =                   | 0.028                    |      |      | N/A                |      |      | 0.013               |      |      | 0.014          |      |      | 0.006           |      |      |
|                | W x V =                   | N/A                      |      |      | N/A                |      |      | 0.009               |      |      | 0.01           |      |      | 0.004           |      |      |
|                | Tx V =                    | N/A                      |      |      | 0.088              |      |      | 0.013               |      |      | 0.014          |      |      | 0.006           |      |      |
|                | W x Tx V=                 | N/A                      |      |      | N/A                |      |      | 0.018               |      |      | 0.02           |      |      | 0.008           |      |      |

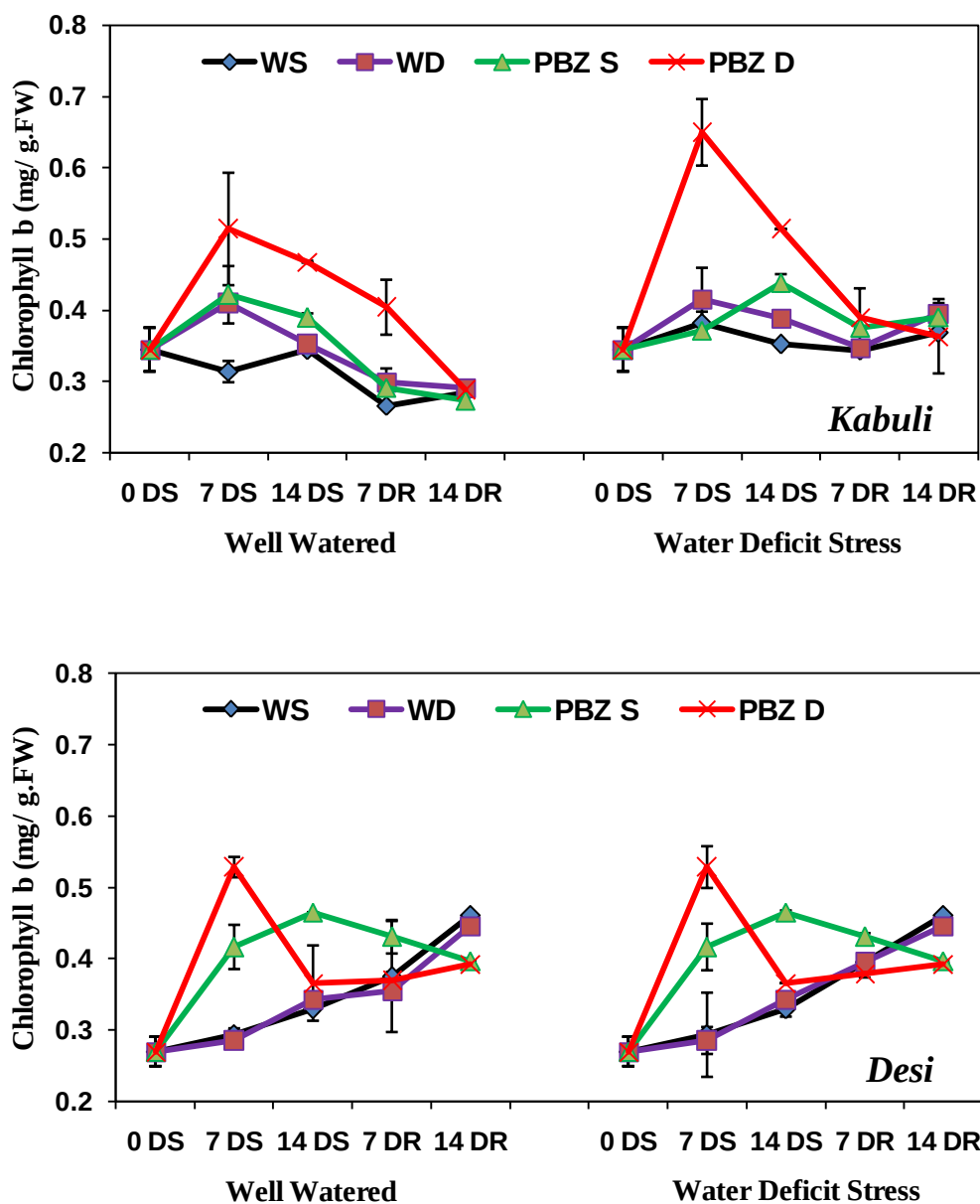


Fig. 4.3.1.2.6.2. Effect of PBZ on chlorophyll b in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.6.2. Effect of PBZ on chlorophyll b in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status              | PBZ Treatments            | Chlorophyll b (mg/ g.FW) |      |       |                    |      |       |                     |      |       |                |      |       |                 |      |      |
|---------------------------|---------------------------|--------------------------|------|-------|--------------------|------|-------|---------------------|------|-------|----------------|------|-------|-----------------|------|------|
|                           |                           | 0 day water stress       |      |       | 7 day water stress |      |       | 14 day water stress |      |       | 7 day recovery |      |       | 14 day recovery |      |      |
|                           |                           | Kabuli                   | Desi | Mean  | Kabuli             | Desi | Mean  | Kabuli              | Desi | Mean  | Kabuli         | Desi | Mean  | Kabuli          | Desi | Mean |
| Well watered              | Water Foliar spray        | 0.34                     | 0.27 | 0.31  | 0.31               | 0.29 | 0.30  | 0.34                | 0.33 | 0.34  | 0.27           | 0.37 | 0.32  | 0.28            | 0.46 | 0.37 |
|                           | Water Drenching           | 0.34                     | 0.27 | 0.31  | 0.41               | 0.28 | 0.35  | 0.35                | 0.34 | 0.35  | 0.30           | 0.35 | 0.33  | 0.29            | 0.45 | 0.37 |
|                           | PBZ Foliar spray (60 ppm) | 0.34                     | 0.27 | 0.31  | 0.42               | 0.42 | 0.42  | 0.39                | 0.46 | 0.43  | 0.29           | 0.43 | 0.36  | 0.27            | 0.40 | 0.33 |
|                           | PBZ Drenching (120 ppm)   | 0.34                     | 0.27 | 0.31  | 0.51               | 0.53 | 0.52  | 0.47                | 0.37 | 0.42  | 0.40           | 0.37 | 0.39  | 0.29            | 0.39 | 0.34 |
|                           | Mean                      | 0.34                     | 0.27 | 0.31  | 0.41               | 0.38 | 0.40  | 0.39                | 0.38 | 0.38  | 0.31           | 0.38 | 0.35  | 0.28            | 0.42 | 0.35 |
|                           | Water stressed            | Water Foliar spray       | 0.34 | 0.27  | 0.31               | 0.38 | 0.29  | 0.34                | 0.35 | 0.33  | 0.34           | 0.34 | 0.39  | 0.37            | 0.37 | 0.46 |
| Water Drenching           |                           | 0.34                     | 0.27 | 0.31  | 0.42               | 0.28 | 0.35  | 0.39                | 0.34 | 0.37  | 0.35           | 0.39 | 0.37  | 0.40            | 0.45 | 0.42 |
| PBZ Foliar spray (60 ppm) |                           | 0.34                     | 0.27 | 0.31  | 0.37               | 0.42 | 0.39  | 0.44                | 0.46 | 0.45  | 0.38           | 0.43 | 0.40  | 0.39            | 0.40 | 0.39 |
| PBZ Drenching (120 ppm)   |                           | 0.34                     | 0.27 | 0.31  | 0.65               | 0.53 | 0.59  | 0.51                | 0.37 | 0.44  | 0.39           | 0.38 | 0.38  | 0.36            | 0.39 | 0.38 |
| Mean                      |                           | 0.34                     | 0.27 | 0.31  | 0.45               | 0.38 | 0.42  | 0.42                | 0.38 | 0.40  | 0.36           | 0.40 | 0.38  | 0.38            | 0.42 | 0.40 |
| SEM                       |                           | 0.024                    |      |       | 0.02               |      |       | 0.008               |      |       | 0.015          |      |       | 0.008           |      |      |
| CD at 5 %                 |                           |                          |      |       |                    |      |       |                     |      |       |                |      |       |                 |      |      |
| Water status (W)          | N/A                       |                          |      | 0.021 |                    |      | 0.008 |                     |      | 0.015 |                |      | 0.008 |                 |      |      |
| Treatment (T)             | N/A                       |                          |      | 0.029 |                    |      | 0.012 |                     |      | 0.022 |                |      | 0.012 |                 |      |      |
| Varieties (V)             | 0.024                     |                          |      | 0.021 |                    |      | 0.008 |                     |      | 0.015 |                |      | 0.008 |                 |      |      |
| W x T =                   | 0.048                     |                          |      | 0.041 |                    |      | 0.016 |                     |      | 0.031 |                |      | 0.016 |                 |      |      |
| W x V =                   | 0.034                     |                          |      | 0.029 |                    |      | 0.012 |                     |      | N/A   |                |      | 0.012 |                 |      |      |
| Tx V =                    | 0.048                     |                          |      | 0.041 |                    |      | 0.016 |                     |      | 0.031 |                |      | 0.016 |                 |      |      |
| W x Tx V=                 | N/A                       |                          |      | 0.058 |                    |      | 0.023 |                     |      | 0.043 |                |      | 0.023 |                 |      |      |

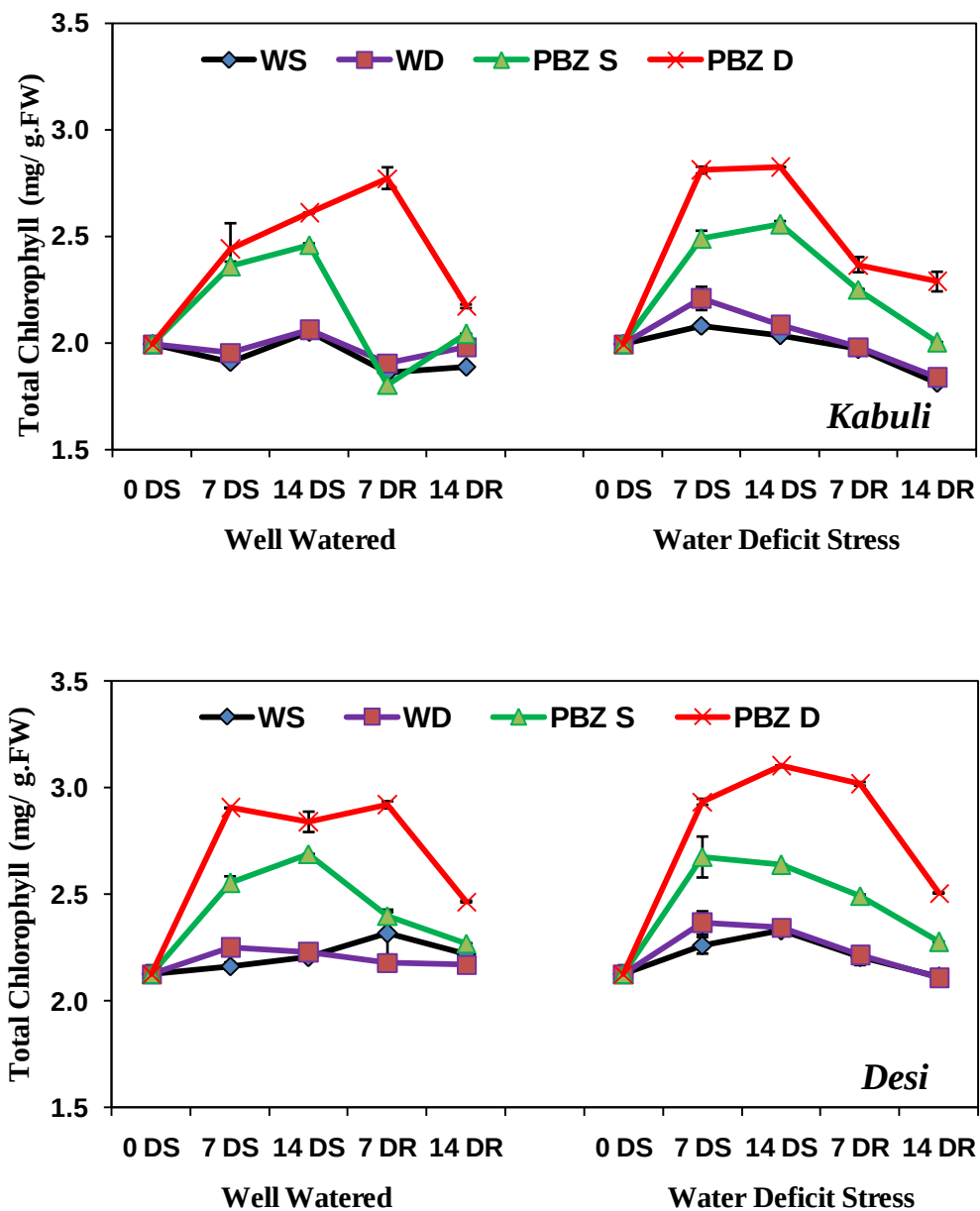


Fig. 4.3.1.2.6.3. Effect of PBZ on total chlorophyll in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.6.3. Effect of PBZ on total chlorophyll in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Total Chlorophyll (mg/ g.FW) |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|----------------|---------------------------|------------------------------|------|------|--------------------|------|------|---------------------|------|------|----------------|------|------|-----------------|------|------|
|                |                           | 0 day water stress           |      |      | 7 day water stress |      |      | 14 day water stress |      |      | 7 day recovery |      |      | 14 day recovery |      |      |
|                |                           | Kabuli                       | Desi | Mean | Kabuli             | Desi | Mean | Kabuli              | Desi | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi | Mean |
| Well watered   | Water Foliar spray        | 2.00                         | 2.13 | 2.06 | 1.91               | 2.16 | 2.04 | 2.05                | 2.21 | 2.13 | 1.86           | 2.32 | 2.09 | 1.89            | 2.22 | 2.05 |
|                | Water Drenching           | 2.00                         | 2.13 | 2.06 | 1.95               | 2.25 | 2.10 | 2.06                | 2.23 | 2.14 | 1.91           | 2.18 | 2.04 | 1.98            | 2.17 | 2.08 |
|                | PBZ Foliar spray (60 ppm) | 2.00                         | 2.13 | 2.06 | 2.36               | 2.56 | 2.46 | 2.46                | 2.69 | 2.57 | 1.80           | 2.40 | 2.10 | 2.04            | 2.27 | 2.16 |
|                | PBZ Drenching (120 ppm)   | 2.00                         | 2.13 | 2.06 | 2.44               | 2.91 | 2.67 | 2.61                | 2.84 | 2.72 | 2.77           | 2.92 | 2.85 | 2.17            | 2.47 | 2.32 |
|                | Mean                      | 2.00                         | 2.13 | 2.06 | 2.17               | 2.47 | 2.32 | 2.29                | 2.49 | 2.39 | 2.08           | 2.46 | 2.27 | 2.02            | 2.28 | 2.15 |
| Water stressed | Water Foliar spray        | 2.00                         | 2.13 | 2.06 | 2.08               | 2.26 | 2.17 | 2.04                | 2.33 | 2.18 | 1.97           | 2.21 | 2.09 | 1.81            | 2.11 | 1.96 |
|                | Water Drenching           | 2.00                         | 2.13 | 2.06 | 2.21               | 2.37 | 2.29 | 2.08                | 2.35 | 2.21 | 1.98           | 2.21 | 2.10 | 1.84            | 2.11 | 1.97 |
|                | PBZ Foliar spray (60 ppm) | 2.00                         | 2.13 | 2.06 | 2.49               | 2.68 | 2.58 | 2.56                | 2.64 | 2.60 | 2.25           | 2.49 | 2.37 | 2.00            | 2.28 | 2.14 |
|                | PBZ Drenching (120 ppm)   | 2.00                         | 2.13 | 2.06 | 2.81               | 2.94 | 2.87 | 2.82                | 3.10 | 2.96 | 2.37           | 3.02 | 2.69 | 2.29            | 2.51 | 2.40 |
|                | Mean                      | 2.00                         | 2.13 | 2.06 | 2.40               | 2.56 | 2.48 | 2.37                | 2.61 | 2.49 | 2.14           | 2.48 | 2.31 | 1.98            | 2.25 | 2.12 |
|                | SEM                       | 0.005                        |      |      | 0.028              |      |      | 0.008               |      |      | 0.019          |      |      | 0.007           |      |      |
|                | CD at 5 %                 |                              |      |      |                    |      |      |                     |      |      |                |      |      |                 |      |      |
|                | Water status (W)          | 0.005                        |      |      | 0.028              |      |      | 0.008               |      |      | 0.02           |      |      | 0.008           |      |      |
|                | Treatment (T)             | 0.007                        |      |      | 0.04               |      |      | 0.012               |      |      | 0.028          |      |      | 0.011           |      |      |
|                | Varieties (V)             | 0.005                        |      |      | 0.028              |      |      | 0.008               |      |      | 0.02           |      |      | 0.008           |      |      |
|                | W x T =                   | N/A                          |      |      | N/A                |      |      | 0.016               |      |      | 0.039          |      |      | 0.015           |      |      |
|                | W x V =                   | N/A                          |      |      | 0.04               |      |      | 0.012               |      |      | N/A            |      |      | N/A             |      |      |
|                | Tx V =                    | N/A                          |      |      | N/A                |      |      | 0.016               |      |      | 0.039          |      |      | 0.015           |      |      |
|                | W x Tx V=                 | 0.014                        |      |      | 0.079              |      |      | 0.023               |      |      | 0.055          |      |      | 0.021           |      |      |

deficit stress conditions. Furthermore, plants treated with drenching application of PBZ had the highest content of total carotenoids in both the varieties (Fig.4.3.1.2.6.4.and Table 4.3.1.2.6.4.).

#### **4.3.1.2.6.5. Photosynthetic pigment profile**

With the help of thin layer chromatography photosynthetic pigments were separated and their profiles were scrutinized under imposed water deficit condition on 14<sup>th</sup> day at reproductive stage in both *desi* and *kabuli* varieties. Plants treated with foliar and drenching application of PBZ showed denser photosynthetic pigment band under imposed water stress condition in both *desi* and *kabuli* varieties. Density of the carotenoids bands in PBZ treated plant was observed much sharper than that of untreated one. The photosynthetic pigments profile of PBZ treated plants under imposed water stress condition exhibited the distinct differences in terms of appearance of all the carotenoids particularly belonging to zeaxanthin cycle than their respective controls. The sharpness of these bands in both *desi* and *kabuli* varieties was much more apparent in PBZ foliar and drenching treated plants (Fig.4.3.1.2.6.5.).

#### **4.3.1.2.7. Yield and its attributes**

##### **4.3.1.2.7.1. Plant height**

Under water deficit conditions plant height of both the varieties were significantly reduced. As compared to *desi* type, severe reduction in plant height was observed in *kabuli* type. Foliar and drenching application of PBZ increased the plant height under well watered condition. However under water deficit condition there was non-significant change in plant height (Table 4.3.1.2.7. (a)).

##### **4.3.1.2.7.2. Root length**

On an average, there was significant reduction in root length of both chickpea varieties under water deficit condition. However, foliar and drenching PBZ applications under both well watered and water stress conditions enhanced the root length of both varieties over their respective control (Table 4.3.1.2.7. (a)).

##### **4.3.1.2.7.3. Number of main branches**

There was negligible effect of water stress on number of main branches of both chickpea varieties. However, under water stress conditions PBZ treatments improved the number of branches (Table 4.3.1.2.7. (a)).

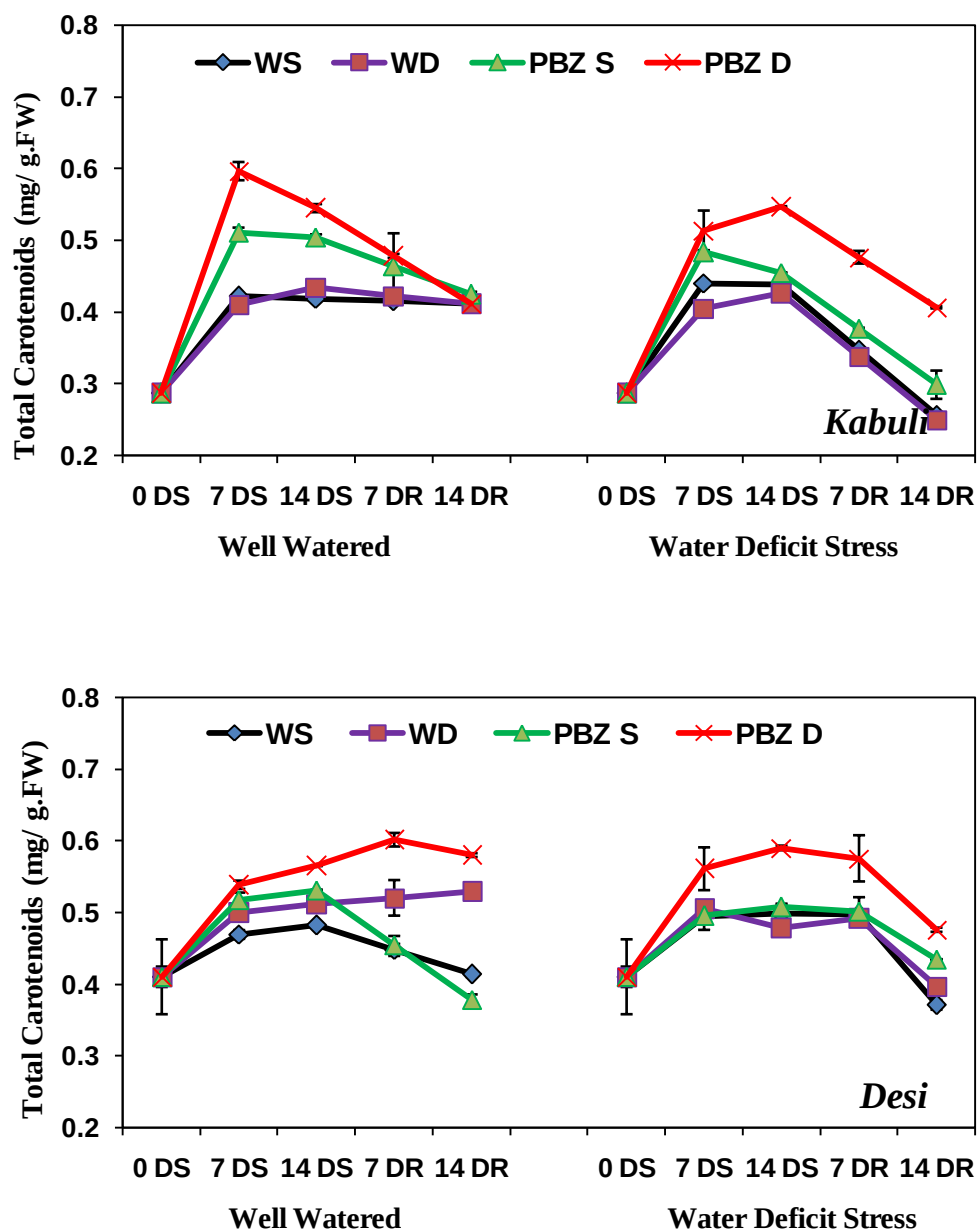
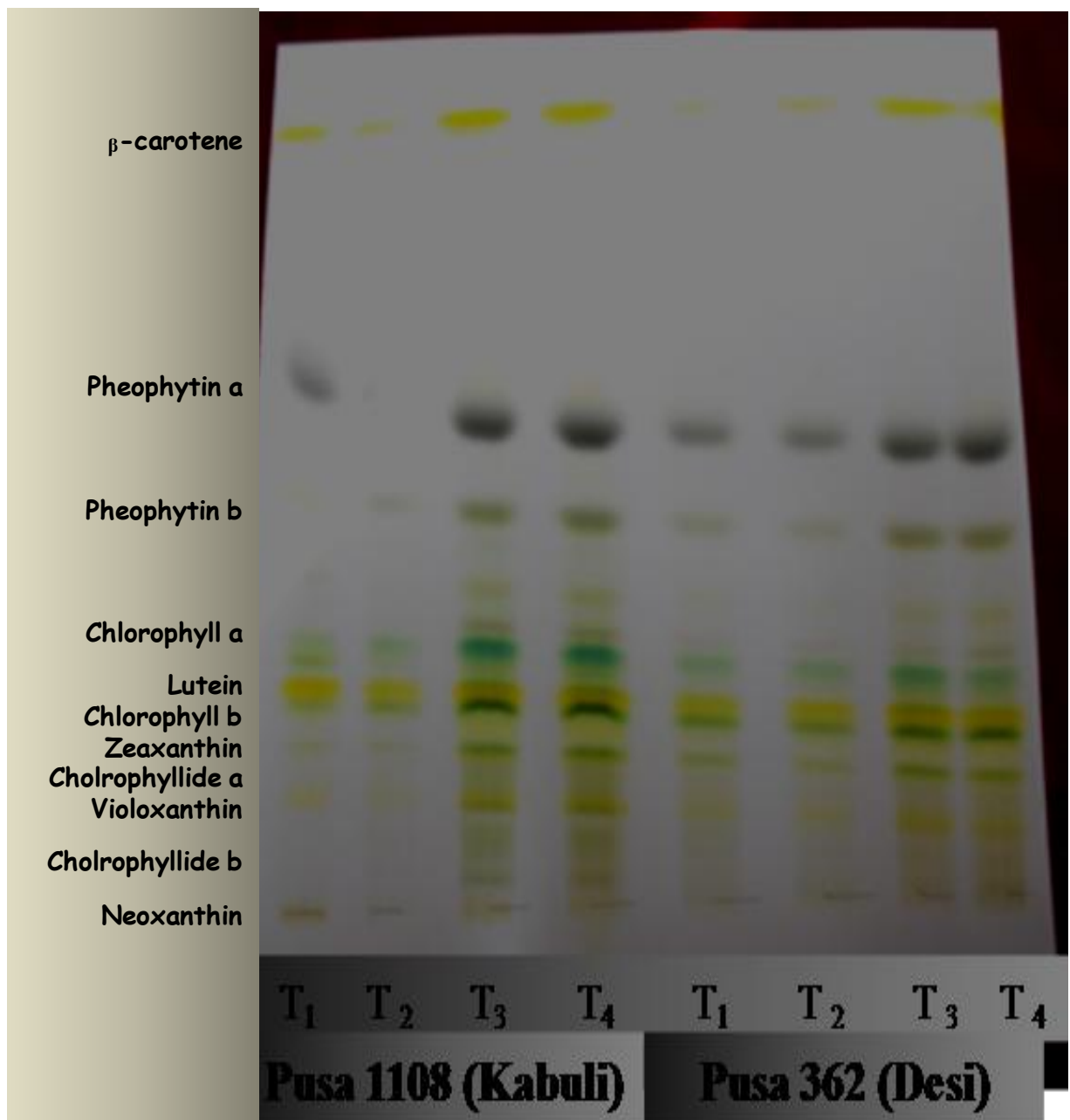


Fig. 4.3.1.2.6.4. Effect of PBZ on total carotenoids in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 4.3.1.2.6.4. Effect of PBZ on total carotenoids in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status              | PBZ Treatments            | Total Carotenoids (mg/ g.FW) |       |      |                    |       |      |                     |       |      |                |      |      |                 |       |      |
|---------------------------|---------------------------|------------------------------|-------|------|--------------------|-------|------|---------------------|-------|------|----------------|------|------|-----------------|-------|------|
|                           |                           | 0 day water stress           |       |      | 7 day water stress |       |      | 14 day water stress |       |      | 7 day recovery |      |      | 14 day recovery |       |      |
|                           |                           | Kabuli                       | Desi  | Mean | Kabuli             | Desi  | Mean | Kabuli              | Desi  | Mean | Kabuli         | Desi | Mean | Kabuli          | Desi  | Mean |
| Well watered              | Water Foliar spray        | 0.29                         | 0.41  | 0.35 | 0.42               | 0.47  | 0.45 | 0.42                | 0.48  | 0.45 | 0.42           | 0.45 | 0.43 | 0.41            | 0.41  | 0.41 |
|                           | Water Drenching           | 0.29                         | 0.41  | 0.35 | 0.41               | 0.50  | 0.45 | 0.43                | 0.51  | 0.47 | 0.42           | 0.52 | 0.47 | 0.41            | 0.53  | 0.47 |
|                           | PBZ Foliar spray (60 ppm) | 0.29                         | 0.41  | 0.35 | 0.51               | 0.52  | 0.51 | 0.50                | 0.53  | 0.52 | 0.46           | 0.45 | 0.46 | 0.42            | 0.38  | 0.40 |
|                           | PBZ Drenching (120 ppm)   | 0.29                         | 0.41  | 0.35 | 0.60               | 0.54  | 0.57 | 0.54                | 0.57  | 0.56 | 0.48           | 0.60 | 0.54 | 0.41            | 0.58  | 0.50 |
|                           | Mean                      | 0.29                         | 0.41  | 0.35 | 0.48               | 0.51  | 0.50 | 0.48                | 0.52  | 0.50 | 0.45           | 0.51 | 0.48 | 0.41            | 0.48  | 0.45 |
|                           | Water stressed            | Water Foliar spray           | 0.29  | 0.41 | 0.35               | 0.44  | 0.50 | 0.47                | 0.44  | 0.50 | 0.47           | 0.35 | 0.50 | 0.42            | 0.26  | 0.37 |
| Water Drenching           |                           | 0.29                         | 0.41  | 0.35 | 0.41               | 0.50  | 0.45 | 0.43                | 0.48  | 0.45 | 0.34           | 0.49 | 0.41 | 0.25            | 0.40  | 0.32 |
| PBZ Foliar spray (60 ppm) |                           | 0.29                         | 0.41  | 0.35 | 0.48               | 0.50  | 0.49 | 0.45                | 0.51  | 0.48 | 0.38           | 0.50 | 0.44 | 0.30            | 0.43  | 0.37 |
| PBZ Drenching (120 ppm)   |                           | 0.29                         | 0.41  | 0.35 | 0.51               | 0.56  | 0.54 | 0.55                | 0.59  | 0.57 | 0.48           | 0.58 | 0.53 | 0.41            | 0.48  | 0.44 |
| Mean                      |                           | 0.29                         | 0.41  | 0.35 | 0.46               | 0.51  | 0.49 | 0.47                | 0.52  | 0.49 | 0.38           | 0.52 | 0.45 | 0.30            | 0.42  | 0.36 |
|                           |                           | SEM                          | 0.696 |      |                    | 0.007 |      |                     | 0.003 |      |                | 0.01 |      |                 | 0.004 |      |
|                           | CD at 5 %                 |                              |       |      |                    |       |      |                     |       |      |                |      |      |                 |       |      |
|                           | Water status (W)          | N/A                          |       |      | 0.008              |       |      | 0.003               |       |      | 0.01           |      |      | 0.004           |       |      |
|                           | Treatment (T)             | N/A                          |       |      | 0.011              |       |      | 0.004               |       |      | 0.014          |      |      | 0.005           |       |      |
|                           | Varieties (V)             | 0.709                        |       |      | 0.008              |       |      | 0.003               |       |      | 0.01           |      |      | 0.004           |       |      |
|                           | W x T =                   | 1.418                        |       |      | 0.015              |       |      | 0.005               |       |      | 0.02           |      |      | 0.007           |       |      |
|                           | W x V =                   | 1.003                        |       |      | 0.011              |       |      | N/A                 |       |      | 0.014          |      |      | 0.005           |       |      |
|                           | Tx V =                    | 1.418                        |       |      | 0.015              |       |      | 0.005               |       |      | 0.02           |      |      | 0.007           |       |      |
|                           | W x Tx V=                 | N/A                          |       |      | 0.022              |       |      | 0.008               |       |      | 0.028          |      |      | 0.01            |       |      |



T<sub>1</sub> = Water spray ;                      T<sub>2</sub> = Water drenching;  
T<sub>3</sub> = PBZ Foliar spray;                T<sub>4</sub> = PBZ drenching

Fig. 4.3.1.2.6.5. Effect of PBZ on photosynthetic pigments profile of *kabuli* and *desi* chickpea genotypes under imposed water deficit stress conditions.

#### **4.3.1.2.7.4. Number of secondary branches**

Similarly, there was non-significant effect of water deficit on number of secondary branches. However, PBZ application reduced the number of secondary branches particularly in *kabuli* type under normal conditions (Table 4.3.1.2.7. (a).).

#### **4.3.1.2.7.5. Number of pods per plant**

In both *kabuli* and *desi* chickpea varieties, significant reduction was noted in number of pods per plant under water deficit condition. As compared to *desi* chickpea reduction in number of pods per plant under water deficit was recorded higher in *kabuli* one. Further foliar and drenching application enhanced the number of pods per plant under both well watered and water deficit condition and the highest number of pods per plant were recorded with the drenching application of PBZ in both the varieties (Table 4.3.1.2.7. (a).).

#### **4.3.1.2.7.6. Shoot dry weight**

Under water deficit conditions, shoot dry weight of both the varieties were significantly reduced. As compared to *desi* type, severe reduction in shoot dry weight was observed in *kabuli* type. Foliar and drenching application of PBZ increased the shoot dry weight under well watered condition. However, under water deficit condition there was significant enhancement in shoot dry weight was noted only in *kabuli* type (Table 4.3.1.2.7. (b) ).

#### **4.3.1.2.7.7. Leaf dry weight**

Under water deficit conditions, significant reduction was noted in both *desi* and *kabuli* varieties. In addition to this, foliar and drenching application improved the leaf dry weight (Table 4.3.1.2.7. (b).).

#### **4.3.1.2.7.8. Pod dry weight**

Under water deficit conditions, both *desi* and *kabuli* chickpea showed the highly significant reduction in pod weight per plant. *Kabuli* chickpea showed higher reduction in pod weight under imposed water deficit than *desi* one. Both PBZ treatments (Foliar and drenching) significantly enhanced the pod weight per plant under both well watered and water deficit conditions. Maximum enhancement in pod weight was found with the drenching application of PBZ in both chickpea varieties (Table 4.3.1.2.7. (b).).

**Table 4.3.1.2.7.a. Effect of PBZ on post harvest records in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Post Harvest Records |       |       |                  |       |       |               |      |      |                    |      |      |                      |       |       |
|----------------|---------------------------|----------------------|-------|-------|------------------|-------|-------|---------------|------|------|--------------------|------|------|----------------------|-------|-------|
|                |                           | Plant height (cm)    |       |       | Root length (cm) |       |       | Main branches |      |      | Secondary branches |      |      | No. of pod per plant |       |       |
|                |                           | Kabuli               | Desi  | Mean  | Kabuli           | Desi  | Mean  | Kabuli        | Desi | Mean | Kabuli             | Desi | Mean | Kabuli               | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 44.20                | 44.50 | 44.35 | 9.75             | 12.83 | 11.29 | 2.00          | 3.50 | 2.75 | 3.50               | 4.50 | 4.00 | 26.20                | 21.17 | 23.68 |
|                | Water Drenching           | 49.90                | 50.00 | 49.95 | 12.50            | 12.00 | 12.25 | 2.33          | 3.50 | 2.92 | 2.83               | 4.50 | 3.67 | 26.20                | 21.17 | 23.68 |
|                | PBZ Foliar spray (60 ppm) | 56.48                | 49.00 | 52.74 | 12.40            | 14.60 | 13.50 | 2.00          | 3.17 | 2.58 | 3.67               | 4.33 | 4.00 | 28.50                | 25.83 | 27.17 |
|                | PBZ Drenching (120 ppm)   | 59.88                | 40.33 | 50.11 | 15.00            | 16.75 | 15.88 | 2.00          | 3.33 | 2.67 | 2.67               | 4.33 | 3.50 | 34.33                | 28.17 | 31.25 |
|                | Mean                      | 52.62                | 45.96 | 49.29 | 12.41            | 14.05 | 13.23 | 2.08          | 3.38 | 2.73 | 3.17               | 4.42 | 3.79 | 28.81                | 24.08 | 26.45 |
| Water stressed | Water Foliar spray        | 39.83                | 40.83 | 40.33 | 9.09             | 8.07  | 8.58  | 2.17          | 3.00 | 2.58 | 3.00               | 4.33 | 3.67 | 6.60                 | 15.80 | 11.20 |
|                | Water Drenching           | 43.17                | 42.50 | 42.83 | 8.17             | 10.71 | 9.44  | 2.67          | 3.60 | 3.13 | 2.67               | 4.33 | 3.50 | 9.40                 | 14.50 | 11.95 |
|                | PBZ Foliar spray (60 ppm) | 42.67                | 44.00 | 43.33 | 12.85            | 10.83 | 11.84 | 2.83          | 3.00 | 2.92 | 2.60               | 4.40 | 3.50 | 13.83                | 19.00 | 16.42 |
|                | PBZ Drenching (120 ppm)   | 39.50                | 38.33 | 38.92 | 16.67            | 10.83 | 13.75 | 2.83          | 2.83 | 2.83 | 3.17               | 4.40 | 3.78 | 22.67                | 17.00 | 19.83 |
|                | Mean                      | 41.29                | 41.42 | 41.35 | 11.69            | 10.11 | 10.90 | 2.63          | 3.11 | 2.87 | 2.86               | 4.37 | 3.61 | 13.13                | 16.58 | 14.85 |
|                | SEM                       | 1.915                |       |       | 1.523            |       |       | 0.257         |      |      | 0.53               |      |      | 2.013                |       |       |
|                | CD at 5 %                 |                      |       |       |                  |       |       |               |      |      |                    |      |      |                      |       |       |
|                | Water status (W)          | 1.951                |       |       | 1.551            |       |       | N/A           |      |      | N/A                |      |      | 2.003                |       |       |
|                | Treatment (T)             | 2.759                |       |       | 2.194            |       |       | 0.37          |      |      | 0.764              |      |      | 2.833                |       |       |
|                | Varieties (V)             | N/A                  |       |       | N/A              |       |       | N/A           |      |      | N/A                |      |      | N/A                  |       |       |
|                | W x T =                   | 3.901                |       |       | N/A              |       |       | N/A           |      |      | N/A                |      |      | N/A                  |       |       |
|                | W x V =                   | N/A                  |       |       | N/A              |       |       | N/A           |      |      | N/A                |      |      | 2.833                |       |       |
|                | Tx V =                    | N/A                  |       |       | N/A              |       |       | N/A           |      |      | N/A                |      |      | 4.007                |       |       |
| W x Tx V=      | N/A                       |                      |       | N/A   |                  |       | N/A   |               |      | N/A  |                    |      | N/A  |                      |       |       |

**Kabuli**



**Desi**



**Fig. 4.3.1.2.7.a: Effect of PBZ applications on podding of chickpea varieties under well watered condition**

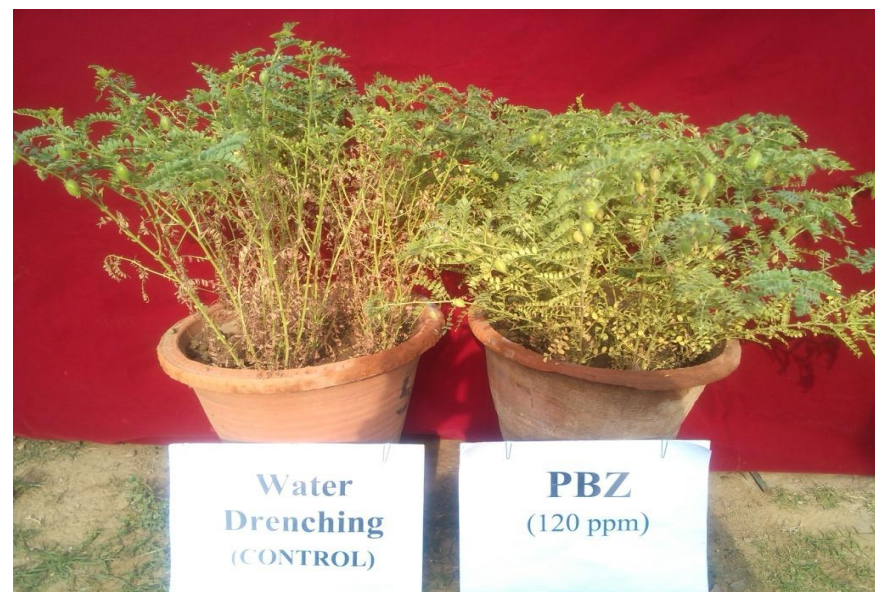


**Fig. 4.3.1.2.7.b: Effect of PBZ applications on podding of chickpea varieties under water deficit stress condition**

#### **4.3.1.2.7.9. Root dry weight**

On an average, under water deficit conditions, there was a significant reduction in root dry weight of both chickpea varieties. *Kabuli* variety had higher reduction in root dry weight than *desi* variety. Foliar and drenching application enhanced the root dry weight as compared to their respective controls under both well watered and water stress conditions (Table 4.3.1.2.7. (b)).

#### **4.3.1.2.7.10. Total dry matter (TDM)**

Similarly, under water deficit condition both *kabuli* and *desi* varieties showed highly significant reduction in total dry matter. Severe reduction in TDM was noted in *kabuli* type under water stress than *desi* one. Foliar and drenching applications enhanced total dry matter under both well watered and water stress conditions (Table 4.3.1.2.7. (b)).

#### **4.3.1.2.7.11. Ten pod weight**

On an average 10 pod weight was significantly reduced under water stress conditions. Foliar and drenching application enhanced the 10 pod weight of both *desi* and *kabuli* varieties under water deficit conditions (Table 4.3.1.2.7. (c)).

#### **4.3.1.2.7.12. Ten pod seed weight**

Similarly, under water deficit condition reduction in 10 pod seed weight was analysed in both *kabuli* and *desi* varieties. Further both PBZ treatments enhanced the 10 pod seed weight of both varieties under water deficit condition (Table 4.3.1.2.7. (c)).

#### **4.3.1.2.7.13. Total seed weight**

On an average both *kabuli* and *desi* varieties showed the significant reduction in total seed weight under water deficit conditions. Comparatively higher reduction in seed weight was noted in *kabuli* type than *desi*. Furthermore foliar and drenching application of PBZ significantly enhanced the seed weight over their respective controls under both well watered and water stress conditions. Maximum enhancement in seed weight was recorded with the drenching application of PBZ under both well watered and water stress conditions (Table 4.3.1.2.7. (c)).

**Table 4.3.1.2.7.b. Effect of PBZ on post harvest records in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Post Harvest Records |      |      |                     |      |      |                          |      |      |                 |      |      |         |      |      |
|----------------|---------------------------|----------------------|------|------|---------------------|------|------|--------------------------|------|------|-----------------|------|------|---------|------|------|
|                |                           | Shoot dry weight (g) |      |      | Leaf dry weight (g) |      |      | Pod weight per plant (g) |      |      | Root dry weight |      |      | TDM (g) |      |      |
|                |                           | Kabuli               | Desi | Mean | Kabuli              | Desi | Mean | Kabuli                   | Desi | Mean | Kabuli          | Desi | Mean | Kabuli  | Desi | Mean |
| Well watered   | Water Foliar spray        | 4.87                 | 4.38 | 4.63 | 0.86                | 0.75 | 0.80 | 8.38                     | 5.65 | 7.01 | 0.49            | 0.42 | 0.46 | 5.80    | 4.95 | 5.37 |
|                | Water Drenching           | 5.07                 | 4.50 | 4.78 | 1.05                | 0.98 | 1.01 | 8.81                     | 5.65 | 7.23 | 0.63            | 0.42 | 0.53 | 6.78    | 5.21 | 6.00 |
|                | PBZ Foliar spray (60 ppm) | 6.39                 | 4.75 | 5.57 | 1.24                | 1.40 | 1.32 | 9.29                     | 6.27 | 7.78 | 0.86            | 0.46 | 0.66 | 8.20    | 6.28 | 7.24 |
|                | PBZ Drenching (120 ppm)   | 6.41                 | 4.81 | 5.61 | 1.40                | 1.22 | 1.31 | 8.83                     | 8.24 | 8.54 | 0.90            | 0.51 | 0.70 | 8.77    | 6.36 | 7.56 |
|                | Mean                      | 5.69                 | 4.61 | 5.15 | 1.14                | 1.09 | 1.11 | 8.83                     | 6.45 | 7.64 | 0.72            | 0.45 | 0.59 | 7.39    | 5.70 | 6.54 |
| Water stressed | Water Foliar spray        | 2.87                 | 3.19 | 3.03 | 0.66                | 0.89 | 0.78 | 1.40                     | 3.00 | 2.20 | 0.26            | 0.29 | 0.27 | 3.13    | 3.80 | 3.47 |
|                | Water Drenching           | 2.78                 | 3.76 | 3.27 | 0.69                | .70  | 0.97 | 2.44                     | 2.76 | 2.60 | 0.26            | 0.29 | 0.27 | 3.54    | 3.80 | 3.67 |
|                | PBZ Foliar spray (60 ppm) | 2.59                 | 3.85 | 3.22 | 0.63                | 1.14 | 0.88 | 3.62                     | 3.86 | 3.74 | 0.35            | 0.43 | 0.39 | 4.27    | 4.82 | 4.55 |
|                | PBZ Drenching (120 ppm)   | 3.63                 | 3.54 | 3.59 | 1.01                | 0.88 | 0.95 | 6.69                     | 4.19 | 5.44 | 0.40            | 0.42 | 0.41 | 4.87    | 4.11 | 4.49 |
|                | Mean                      | 2.97                 | 3.58 | 3.28 | 0.75                | .902 | 0.89 | 3.54                     | 3.45 | 3.49 | 0.31            | 0.36 | 0.34 | 3.95    | 4.13 | 4.04 |
|                | SEM                       | 0.369                |      |      | 0.223               |      |      | 0.973                    |      |      | 0.143           |      |      | 0.584   |      |      |
|                | CD at 5 %                 |                      |      |      |                     |      |      |                          |      |      |                 |      |      |         |      |      |
|                | Water status (W)          | 0.532                |      |      | 0.227               |      |      | 0.991                    |      |      | 0.146           |      |      | 0.595   |      |      |
|                | Treatment (T)             | 0.752                |      |      | N/A                 |      |      | 1.401                    |      |      | 0.206           |      |      | 0.841   |      |      |
|                | Varieties (V)             | 0.532                |      |      | N/A                 |      |      | 0.991                    |      |      | N/A             |      |      | N/A     |      |      |
|                | W x T =                   | N/A                  |      |      | N/A                 |      |      | N/A                      |      |      | N/A             |      |      | N/A     |      |      |
|                | W x V =                   | N/A                  |      |      | N/A                 |      |      | N/A                      |      |      | N/A             |      |      | N/A     |      |      |
|                | Tx V =                    | N/A                  |      |      | 0.455               |      |      | N/A                      |      |      | N/A             |      |      | N/A     |      |      |
|                | W x Tx V=                 | N/A                  |      |      | N/A                 |      |      | N/A                      |      |      | N/A             |      |      | N/A     |      |      |

**Table 4.3.1.2.7.c. Effect of PBZ on post harvest records in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Post Harvest Records |      |      |                        |      |      |                       |      |      |                     |       |       |
|----------------|---------------------------|----------------------|------|------|------------------------|------|------|-----------------------|------|------|---------------------|-------|-------|
|                |                           | 10 pod weight(g)     |      |      | 10 pod seed weight (g) |      |      | Total seed weight (g) |      |      | 100 seed weight (g) |       |       |
|                |                           | Kabuli               | Desi | Mean | Kabuli                 | Desi | Mean | Kabuli                | Desi | Mean | Kabuli              | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 3.20                 | 2.67 | 2.93 | 2.16                   | 1.69 | 1.92 | 5.62                  | 3.70 | 4.66 | 21.47               | 17.47 | 19.47 |
|                | Water Drenching           | 3.36                 | 2.67 | 3.02 | 3.51                   | 1.69 | 2.60 | 5.62                  | 3.70 | 4.66 | 21.47               | 17.47 | 19.47 |
|                | PBZ Foliar spray (60 ppm) | 3.26                 | 2.43 | 2.84 | 2.44                   | 1.73 | 2.09 | 6.77                  | 4.47 | 5.62 | 23.75               | 17.28 | 20.52 |
|                | PBZ Drenching (120 ppm)   | 2.57                 | 2.93 | 2.75 | 2.24                   | 1.88 | 2.06 | 7.63                  | 5.39 | 6.51 | 22.23               | 19.12 | 20.68 |
|                | Mean                      | 3.10                 | 2.67 | 2.89 | 2.59                   | 1.75 | 2.17 | 6.41                  | 4.31 | 5.36 | 22.23               | 17.84 | 20.03 |
| Water stressed | Water Foliar spray        | 2.12                 | 1.90 | 2.01 | 2.07                   | 1.14 | 1.61 | 1.33                  | 1.84 | 1.58 | 17.93               | 11.66 | 14.79 |
|                | Water Drenching           | 2.60                 | 1.91 | 2.25 | 1.76                   | 1.29 | 1.52 | 1.72                  | 1.84 | 1.78 | 17.10               | 12.70 | 14.9  |
|                | PBZ Foliar spray (60 ppm) | 2.62                 | 2.03 | 2.32 | 2.33                   | 1.37 | 1.85 | 3.17                  | 2.59 | 2.88 | 22.81               | 13.61 | 18.21 |
|                | PBZ Drenching (120 ppm)   | 2.95                 | 2.46 | 2.71 | 2.18                   | 1.92 | 2.05 | 4.93                  | 2.98 | 3.96 | 21.76               | 17.53 | 19.65 |
|                | Mean                      | 2.57                 | 2.07 | 2.32 | 2.09                   | 1.43 | 1.76 | 2.79                  | 2.31 | 2.55 | 19.92               | 13.87 | 16.90 |
|                | SEM                       | 0.707                |      |      | 0.497                  |      |      | 0.572                 |      |      | 1.834               |       |       |
|                | CD at 5 %                 |                      |      |      |                        |      |      |                       |      |      |                     |       |       |
|                | Water status (W)          | 0.704                |      |      | 0.495                  |      |      | 0.569                 |      |      | N/A                 |       |       |
|                | Treatment (T)             | N/A                  |      |      | N/A                    |      |      | 0.805                 |      |      | 2.582               |       |       |
|                | Varieties (V)             | 0.704                |      |      | 0.495                  |      |      | 0.569                 |      |      | 1.826               |       |       |
|                | W x T =                   | N/A                  |      |      | N/A                    |      |      | 1.139                 |      |      | NA                  |       |       |
|                | W x V =                   | N/A                  |      |      | N/A                    |      |      | N/A                   |      |      | N/A                 |       |       |
|                | Tx V =                    | N/A                  |      |      | N/A                    |      |      | N/A                   |      |      | 3.651               |       |       |
|                | W x Tx V=                 | N/A                  |      |      | N/A                    |      |      | 1.611                 |      |      | NA                  |       |       |

#### **4.3.1.2.7.14. Hundred Seed weight**

On an average under water deficit condition significant reduction was noted in both chickpea varieties. Foliar and drenching applications improved 100 seed weight of both varieties particularly under water stress conditions (Table 4.3.1.2.7. (c).).

#### **4.3.1.2.7.15. Dry matter partitioning**

In general, under water deficit stress condition, dry matter partitioning in pod significantly reduced in *desi* and *kabuli* chickpea while allocation of dry matter in shoot and leaves increased. Foliar application of PBZ enhanced the dry matter partitioning to pods under both well watered condition and under water deficit stress condition. PBZ drenching was found the most effective in enhancing the dry matter partitioning to pods in both *kabuli* and *desi* chickpea (Fig. 4.3.1.2.7.15. (a &b))

#### **4.3.1.2.7.16. Harvest Index (HI)**

Under water stress condition, a significant reduction in HI was recorded in both *desi* and *kabuli* chickpea varieties. Foliar and drenching applications of PBZ in general enhanced the harvest index under both well watered and water deficit conditions in both the chickpea varieties. However, their effect on harvest index was not very pronounced under water stress condition (Fig. 4.3.1.2.7.16.)

#### **4.3.1.2.7.17. Drought susceptibility index**

Foliar and drenching applications of PBZ significantly reduced drought susceptibility index in both *desi* and *kabuli* varieties. In both varieties the lowest drought susceptibility index was estimated with PBZ drenching application (Fig. 4.3.1.2.7.17.)

#### **4.3.1.2.7.18. Drought tolerance efficiency**

In general, the foliar and drenching applications of PBZ significantly enhanced the drought tolerance efficiency (DTE) in both *desi* and *kabuli* chickpea. In *desi* chickpea, the highest drought tolerance efficiency was estimated with drenching treatment of PBZ (Fig. 4.3.1.2.7.18.)

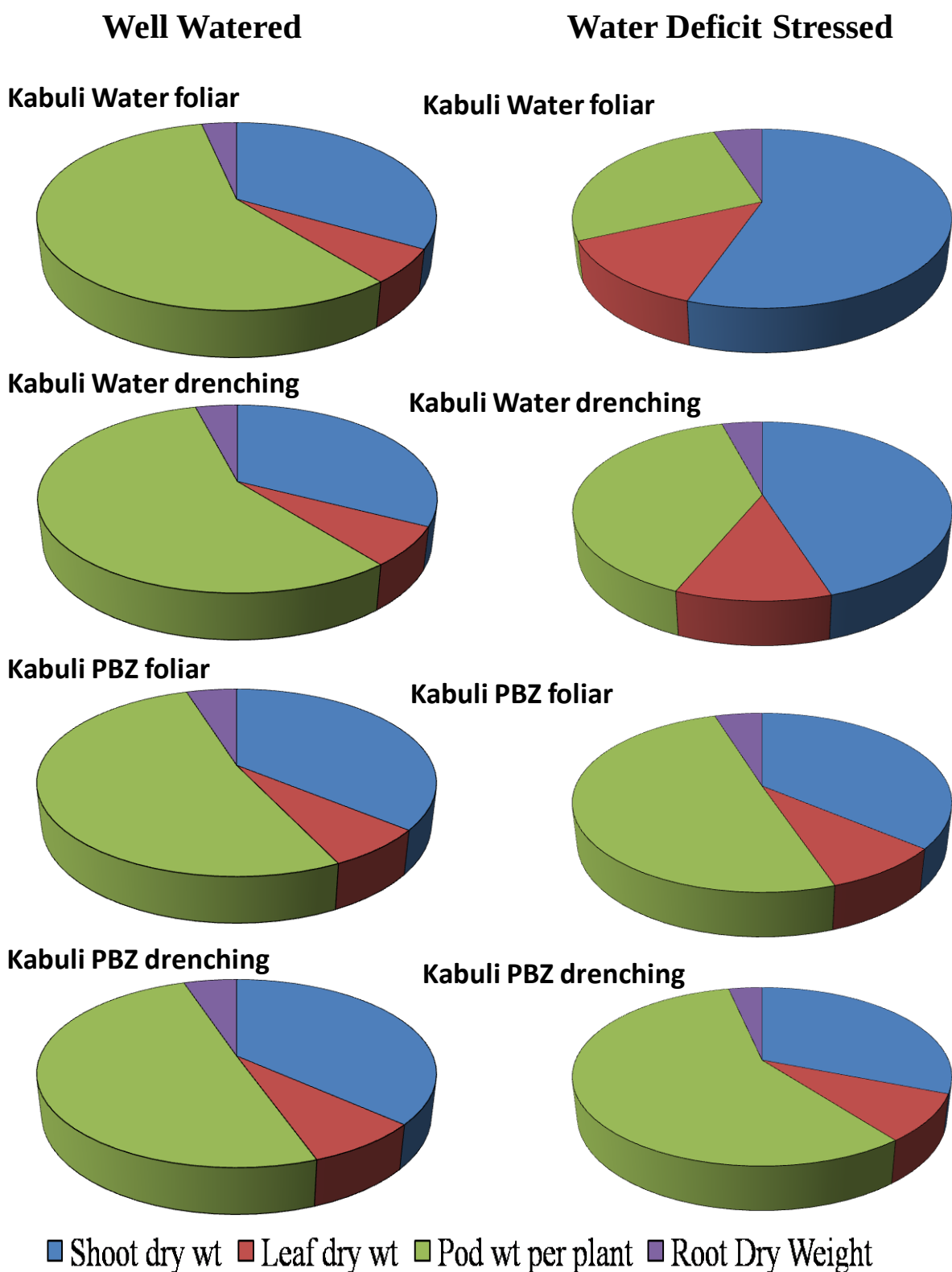


Fig. 4.3.1.2.7.15.(a). Effect of PBZ on dry matter partitioning in *Kabuli* chickpea variety under well watered and imposed water deficit stressed conditions.

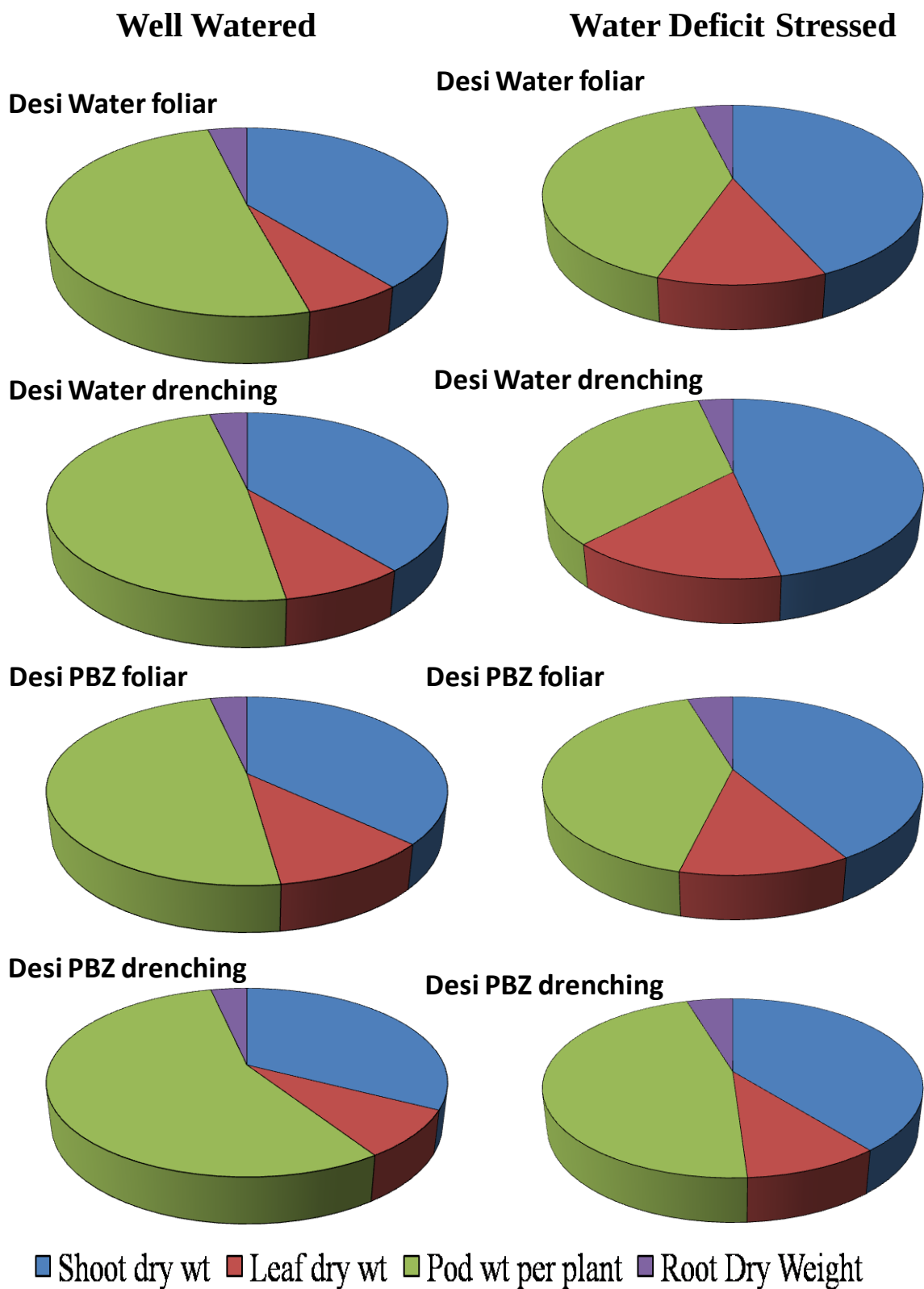


Fig. 4.3.1.2.7.15.(b). Effect of PBZ on dry matter partitioning in Desi chickpea variety under well watered and imposed water deficit stressed conditions.

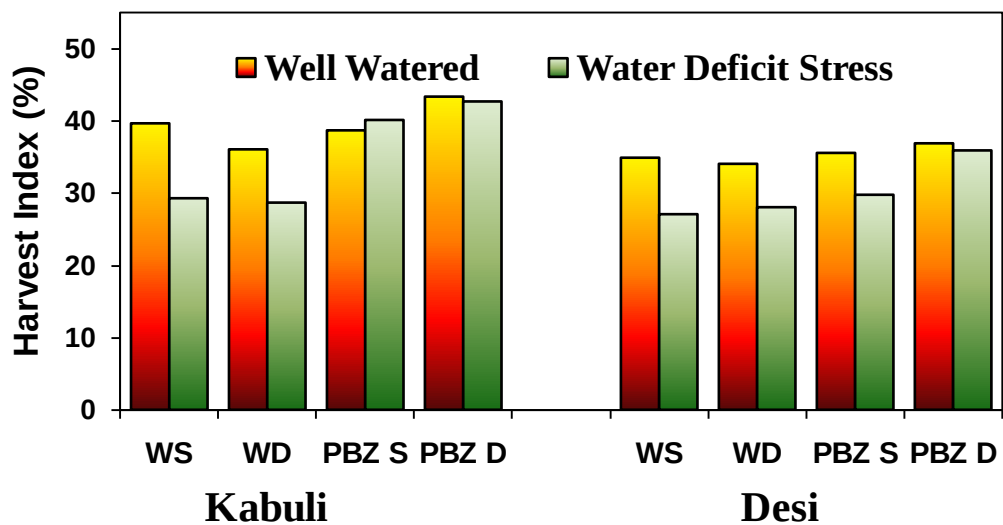


Fig. 4.3.1.2.7.16. Effect of PBZ on harvest index (HI) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

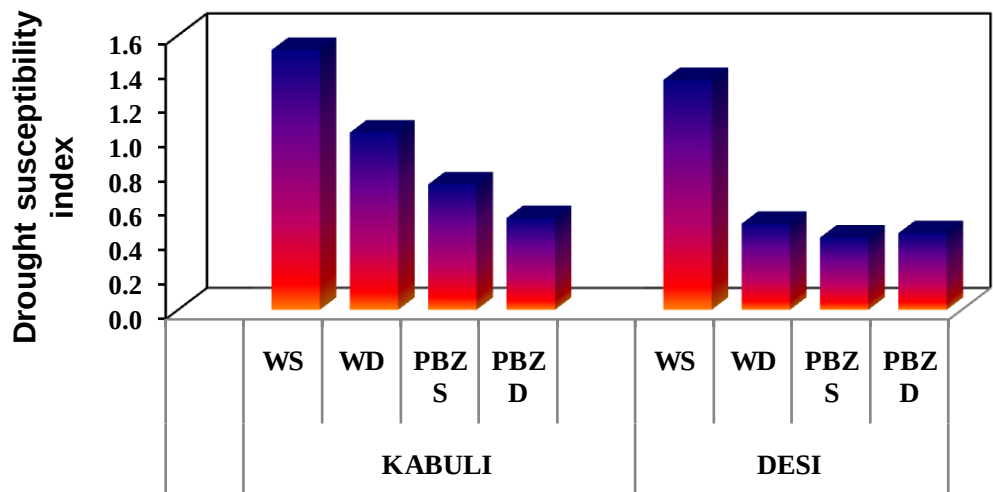


Fig. 4.3.1.2.7.17. Effect of PBZ on drought susceptibility index (DSI) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

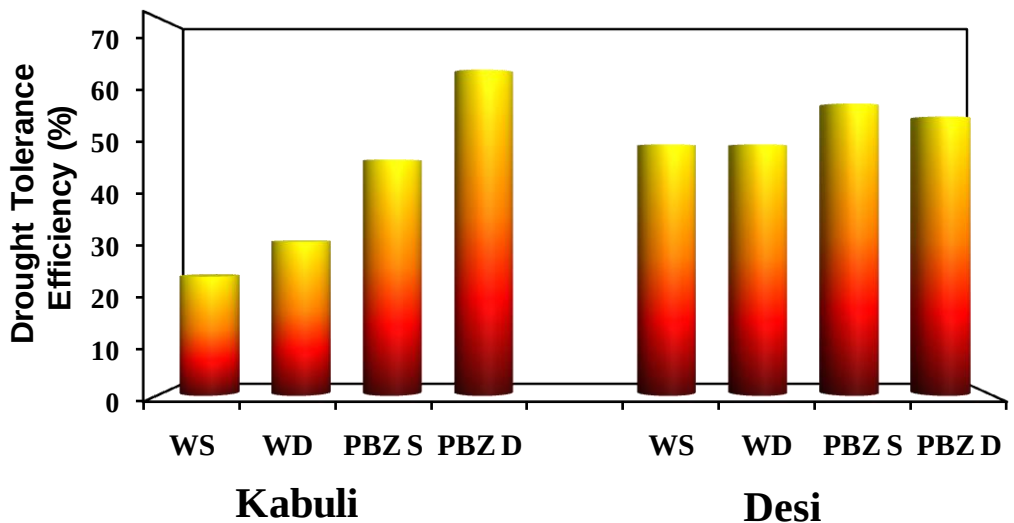


Fig. 4.3.1.2.7.18. Effect of PBZ on drought tolerance efficiency (DTE) in chickpea genotypes under well watered and imposed water deficit stressed conditions.

#### 4.4. DISCUSSION

Water deficit is a major constraint which reduces the productivity of crops globally (Bohnert *et al.*, 1995; Khamssi *et al.*, 2011). Water deficit is also a major limiting factor in chickpea production since most of the chickpea grown areas are in arid and semiarid regions and more than 90% of chickpea of the world is cultivated under marginal and rainfed conditions. Globally, water deficit reduces the chickpea yield by 40-50% (Ahmad *et al.*, 2005) by altering a variety of physiological and metabolic process in plants (Gunes *et al.*, 2006). Yield losses in chickpea are happened due to the intermittent water deficit during the vegetative phase or due to water deficit during reproductive development or due to terminal water deficit at the end of the crop cycle (Serraj *et al.*, 2004). In this crop, yield improvement through conventional breeding is difficult because of narrow genetic base (Udupa *et al.*, 1999). In the current scenario, an alternative approach which attracts more attention is application of bioregulators for the protection of the plant from the abiotic stresses (Jaleel *et al.*, 2007). Paclobutrazol, a triazole, is one of the strong candidates for stress amelioration, which induces numerous morphological, physiological and biochemical responses in plants, including inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses (Jaleel *et al.*, 2007). PBZ is considered as a gibberellin biosynthesis inhibitor. Its plant growth regulating properties are mediated by its ability to change the balance of important phytohormones including gibberellic acid, ABA and cytokinin (Kamounsis and Chronopoulson- Sereli, 1999). In the present investigation, an attempt has been made to analyze the mitigating effect of PBZ applications (foliar and drenching) on physiological, yield and its attributes in to diverse *desi* (Pusa362) and *kabuli* (Pusa1108) chickpea varieties under imposed water deficit stress.

In general, RWC, MSI, photosynthesis rate, chl<sub>a</sub>, chl<sub>b</sub>, total chlorophylls and total carotenoids increased with increase in the dose of PBZ used for foliar and drenching applications in both *desi* and *kabuli* varieties during water deficit stress condition as well as after water deficit termination during recovery except total carotenoids content in *desi* variety. Based on these parameters optimum dose of PBZ for foliar and drenching applications during water deficit and recovery were obtained 60 ppm and 120 ppm respectively. However beyond

these doses (optima) there was either stagnation or reduction in the values of aforementioned parameters probably due to the metabolic imbalances by vigorously altering the level of endogenous hormones at higher dose of PBZ. Similarly, higher dose of PBZ was also reported to reduce growth and yield of tomato (Rahman *et al.*, 1989). On an average, optimum dose of PBZ for foliar and drenching applications were estimated around 60 and 120 ppm respectively.

Water deficit alters several physiological and metabolic process in plants (Gunes *et al.*, 2006). In present study, RWC was decreased under water deficit condition and PBZ applications improved leaf RWC in both varieties. RWC is an important indicator of water stress in leaves (Merah, 2001). Reduction in RWC of leaves was observed in plants exposed to water deficit conditions. Plants exposed to stress immediately reduce RWC of their leaves (Grover *et al.*, 2004). Under water deficit, paclobutrazol by accelerating stomatal response and reducing transpiration rate, enhanced water retention and increased survival in jack pine (Mashall *et al.*, 1990), oak (Percival *et al.*, 2007), wheat (Sairam *et al.*, 1995; Aly *et al.*, 2011) and barley (Rady *et al.*, 2012). Paclobutrazol treated plants showed higher value of relative water content in *Curcuma alismatifolia* Gagnep (Jungklang *et al.*, 2012), triticale (Berova and Zlatev., 2003) and cucumber seedling (Baninasab *et al.*, 2011).

MSI was reduced under water deficit in both chickpea varieties and its reduction was reported higher in *kabuli* variety than *desi* one because *kabuli* type chickpea is more sensitive to water stress than *desi* variety. Cell membrane stability has been used as a drought tolerance test (Agarie *et al.*, 1995) and electrolyte leakage showed an increase with increasing water deficit (Gopi *et al.*, 2007). The inhibition of electrolyte leakage indicates ability of PBZ in maintaining the membrane integrity (Fletcher *et al.*, 2000) of plants. PBZ being a triazole altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane (Burden *et al.*, 1987). PBZ has been reported to reduce electrolyte leakage in wheat (Krans *et al.*, 1995; Aly *et al.*, 2011) and Horse chestnut (Percival *et al.*, 2008) during drought stress. In addition, upregulation of stress protective biomolecules, in PBZ treated plants have also enhanced the capacity to limit the damage caused by species of reactive oxygen.

Water deficit affects plant growth at various levels (Blumwald *et al.*, 2004). The quantity and quality of plant growth depend on cell-division enlargement, and differentiation, and all of these events are affected by water deficit (Correia *et al.*, 2001). Under imposed water

deficit stress condition, in general, all plant growth parameters (shoot length, root length, leaf number, leaf area, root dry weight, leaf dry weight, shoot dry weight, specific leaf weight, specific leaf area) of both *desi* and *kabuli* genotypes showed a reduction. Drought stress decreases the rate of photosynthesis (e.g., Kawamitsu *et al.*, 2000). Plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO<sub>2</sub> fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants (Mafakheri *et al.*, 2010). Water deficit stress also affects total leaf area through its effect on initiation of new leaves, which is decreased under drought stress. Alves and Setter (2004) showed that reduction in both cell expansion and production of cells contributed to a loss in leaf area depending on the developmental stage at which the leaf was stressed. Continued drought stress can accelerate leaf senescence (de Souza *et al.*, 1997) and lead to death of leaf tissue, resulting in leaf drop, particularly old and mature leaves. PBZ applications in general enhanced all growth parameters in both chickpea genotypes under both well watered and water deficit conditions except shoot length under well watered condition in *Kabuli* variety. However, PBZ impact under water stress was more effective than well watered condition. In addition to this, PBZ drenching had better efficacy than its foliar application. PBZ is transportable and can move only through xylem (Buta and Spaulding, 1991) and therefore its efficacy primarily depend upon its uptake via root. Moreover, foliar PBZ cannot be transported out of the leaves to other parts that is why drenching application was found better over foliar application. Paclobutrazol has been reported to reduce plant growth, height, internodal distance with thicker and darker leaves in pepper and tomato (Rahman *et al.*, 1989), oilseed mustard (Setia *et al.*, 1996) and chinese potato (Kishorekumar *et al.*, 2006). Reduced height is a consequence of triazole induced GA inhibition exemplified by reduced internodal elongation (Fletcher *et al.*, 2000). However, PBZ application has been reported to improve the growth potential of *Catharanthus roseus* plants grown under stress conditions (Jaleel *et al.*, 2007). In present study, we also found relatively better performance of PBZ treated plants under water deficit stress in terms of growth parameters. Furthermore, PBZ identified selected doses used during the investigation for foliar and drenching applications were not sufficient to retard the growth parameters. In addition, we also observed that during our preliminary experiment higher doses of PBZ were able to reduce the plant height drastically.

Under imposed water deficit, destruction of thylakoid, disappearance of starch inclusions, changes in the lamellar structure, number of grana with loose type of thylakoid, large increase in osmiophilic granules and reduction of amount of starch in the bundle sheath chloroplast have also been reported earlier (Giles *et al.*, 1976, Vapaavuori *et al.*, 1984 and Utrillas and Alegre, 1997). However, water deficit with foliar and drenching applications of PBZ maintained the integrity of the chloroplast structure and its grana were recorded with compact type of thylakoid. Moreover, PBZ treatment had large and higher number of starch grains and low number of osmiophilic granules in chloroplast. PBZ applications maintained chloroplast structure integrity under water deficit probably by enhancing the activity of antioxidants and restricting lipid peroxidation (Research Paper II). Besides this, PBZ also maintained integrity of chloroplast structure under water deficit probably by increasing the level of cytokinins as reported earlier (Fletcher *et al.*, 2000; Zhu *et al.*, 2004). Moreover, application of BA has also been reported to improve chloroplast integrity under water stress (Haisel *et al.*, 2006).

In the present study, rate of photosynthesis, transpiration and stomatal conductance gradually decreased with increase in the period of water stress and on rehydration, gradually showed their recovery. PBZ applications, maintained the higher rate of photosynthesis during water deficit and after water deficit stress termination and thus, showed the fast recovery. Photosynthesis is very sensitive physiological process to water deficit. Even a small decrease in the relative water content of a plant causes its stomata to close, and decreases the intensity of photosynthetic assimilation of CO<sub>2</sub>. Water deficit reduces the rate of photosynthesis (Kawamitso *et al.*, 2000; Lawlor and Cornic, 2002; Rahbarian *et al.*, 2011). During water stress most plants avoid shoot desiccation by closing their stomata to decrease transpiration and decreased stomatal conductance is accompanied by a reduction in internal CO<sub>2</sub> concentration and decreased diffusion of CO<sub>2</sub> via Mesophyll cell walls, membranes, cytoplasm and chloroplast envelope leading to decreased chloroplast CO<sub>2</sub> concentration which decreases CO<sub>2</sub> fixation rate. Further, inhibition of ribulose-1, 5-bisphosphate carboxylase/oxygenase enzyme activity and ATP synthesis also lead to a decrease of net photosynthetic rate under water stress (Dulai *et al.*, 2006). However, many other processes such as contents of photosynthetic pigments, photochemical efficiency of photo systems and ultrastructure of chloroplast can also

be affected by severe water deficit stress (Athar and Ashraf, 2005; Dubey, 2005). Diffusive resistance of the stomata to CO<sub>2</sub> entry probably is the main factor limiting photosynthesis under drought (Boyer, 1970; Chaves, 1991; Cornic, 2000; Flexas *et al.*, 2004). Drought stress inhibits photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe *et al.*, 1998). Reduction in photosynthesis as a result of water stress can be attributed to both stomatal and non- stomatal limitations (Graan and Boyer, 1990, Shangguan, 1999).

Paclobutrazol has been reported to enhance net photosynthesis rate in triticale (Berova and Zlatev, 2003), mustard (Zhou *et al.*, 1993), *Setaria italic* (Bisht *et al.*, 2007) horse chestnut (Percival and Noviss, 2008) and *Withania somnifera* (Jaleel *et al.*, 2008). The higher chlorophyll content in the leaves, leading to higher photosynthesis in the triazole treated plants (Feng *et al.*, 2003). There is report that PBZ also increased activity of RUBP-carboxylase and thus rate of photosynthesis in peanut (Yan and Pan, 1992). Further, Paclobutrazol was reported to significantly enhance zeatin and zeatin riboside (Zhu *et al.*, 2004) and cytokinins protect photosynthetic machinery. PBZ application improved water use efficiency by reducing transpiration rate. PBZ affected transpiration rate by increasing the level of ABA (Research paer II). Our results are in agreement of earlier report (Asare-Boamah *et al.*, 1986).

Under water deficit, quantum yield (Fv/Fm) was decreased and PBZ applications improved this ratio. Water deficit stress affects photosystem efficiency (Fv/Fm) and decreases the electron transport rate (ETR) and the effective quantum yield of photosystem II photochemistry (Y) (Ahmed *et al.*, 2002). Several studies have demonstrated that damage to the PSII oxygen-evolving complex and the PSII reaction centers in turn caused degradation of D1 protein under drought stress (Lu and Zhang, 1998; Maxwell and Johnson, 2000; Galle *et al.*, 2002). Photoinhibition is represented by decreasing Fv/Fm ratio, effective quantum yield of photosystem II photochemistry and electron transport rate (Piper *et al.*, 2007). PBZ application reduced photoinhibition by protecting PS II (Baninasab *et al.*, 2009). Similarly, in Horse chestnut, chlorophyll fluorescence (Fv/Fm), photosynthetic rate were higher in trees treated with paclobutrazol (Percival *et al.*, 2008).

The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). A reduction in photosynthetic

pigments content was reported in water stressed cotton (Massacci *et al.*, 2008), Horse chestnut and *Catharanthus roseus* (Jaleel *et al.*, 2008) sunflower (Kiani *et al.*, 2008) and *Vaccinium myrtillus* (Tahkokorpi *et al.*, 2007). However, in present study, photosynthetic pigments (Chlorophylls and carotenoids) content showed variable response which is likely due to the increase in pigment density or reduction in tissue volume under water deficit because their levels were calculated on fresh weight basis. PBZ applications improved level of photosynthetic pigments under both well watered and water stressed condition probably by increasing level of cytokinins and antioxidant activity. PBZ application has been reported to increase level of pigments in oak (Percival *et al.*, 2007), *Setaria italica* (Bisht *et al.*, 2007), *Amorphophallus campanulatus* (Gopi and Jaleel., 2009), cucumber seedling (Baninasab and Ghobadi, 2011), *Triticum aestivum* (Aly *et al.*, 2011; Nouriyani *et al.*, 2012) and *Stevia rebaudiana* (Hajihashemi and Ehsanpour, 2013). In general, under the imposed water stress condition, density of all the pigments in profile was reduced as compared to the well watered one. Furthermore, the plants treated with PBZ treatments showed more dense photosynthetic pigments particularly carotenoids belonging to xanthophyll cycle under normal condition and also maintained the photosynthetic pigment profile under water stress condition which in turn indicated that the PBZ applications stimulated the xanthophyll cycle and thus contributed in the protection of the photosynthetic machinery. Paclobutrazol induced a suit of physiological adaptation to tolerate drought more especially, increased total leaf content of carotenoids (Lutein, beta-carotene, alpha carotene, neoxanthin) and xanthophylls (Antheraxanthin, zeaxanthin, violaxanthin) in Evergreen oak (Percival *et al.*, 2007).

Imposed water deficit showed the significant reduction in plant height, root length, number of main branches, pods per plant, shoot dry weight, leaf dry weight, root dry weight, pod weight, seed yield and test weight at harvest. PBZ applications in general, enhanced all the growth parameters and also maintained the yield and its attributes even under imposed water deficit stress condition. The present study, confirmed earlier field reports in chickpea that water deficit reduced grain and biological yield (Behboudian *et al.*, 2001; Bahavar *et al.*, 2009). Finding of present investigation indicated that PBZ enhanced grain yield by enhancing photosynthetic gas exchange, higher chlorophyll content, photosynthetic activity for a longer period and as a result facilitated higher photo assimilation. Similarly, PBZ has also

been reported to enhance yield in mustard (Setia *et al.*, 1996), *Brassica napus* (Zhou and Xi, 1993), barley (Rady and Gaballah., 2012) tomato (Mohamed *et al.*, 2011) and *Camelina sativa* (Kumar *et al.*., 2012). Its role as anti-gibberellin is considered sufficient for the induction of higher yield and for improvement of the quality in cucurbits (Hedden and Graebe, 1985), wheat (Reynolds and Wardle, 1990) and rice (Pan *et al.*, 2013). Furthermore, PBZ drenching had better yield efficacy than its foliar application because PBZ is xylem mobile (Buta and Spaulding, 1991) and therefore its efficacy primarily depend upon its uptake via root. In addition, foliar PBZ cannot be transported out of the leaves to other parts. That is why drenching application was found better over foliar application.

Water deficit reduced dry matter allocation to economic sink. PBZ applications improved Harvest index and dry matter partitioning to economic sink probably by enhancing the level of cytokinins. PBZ was also reported earlier to overall increase in dry matter per plant and partitioning coefficient in *Brassica juncea* and *Brassica carinata* (Setia *et al.*, 1995) altered sink strength within the plant resulting in redistribution of assimilates to meristematic regions, other than the shoot apices, and also improved the flow of assimilates towards reproductive structures (Setia *et al.*, 1996). Kumar *et al.*, 2012 linked PBZ enhanced yield in *Camelina sativa* with better partitioning coefficient.

PBZ applications significantly reduced the drought susceptibility index and PBZ drenching application showed the lowest values of the drought susceptibility index. Further, highest drought tolerance efficiency was recorded with application of drenching than foliar application. These findings suggested that PBZ applications enhanced the yield by improving the tolerance of chickpea varieties and PBZ drenching is better than its foliar application.

#### **4.5. CONCLUSION**

Optimum dose for foliar and drenching applications were identified 60 and 120 ppm respectively. Based on physiological responses *Kabuli* variety (Pusa 1108) was found more sensitive to water deficit stress than *desi* one (Pusa 362). PBZ drenching application was found better over its foliar application. Under water deficit, PBZ applications enhanced the yield and water deficit tolerance of both chickpea varieties by improving water relation, reducing membrane damage, enhancing photosynthetic activity and protecting photosynthetic machinery.

## 5. RESEARCH PAPER II

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### **Biochemical responses of chickpea (*Cicer arietinum* L.) genotypes to Paclobutrazol under water deficit condition**

**Soumya, P. R. and Pramod Kumar**

*Division of Plant Physiology, Indian Agriculture Research Institute, New Delhi 110012*

#### **ABSTRACT**

Chickpea is an important winter season pulse crop of arid and semi arid regions and mainly grown in marginal and rainfed areas. Therefore, water deficit is one of the important factors limiting its production. Paclobutrazol (PBZ) has been reported to induce the water deficit tolerance in some plants by altering the biochemical changes. Thus, a pot experiment was carried out to analyze the biochemical alterations for understanding the paclobutrazol induced mechanism of water deficit stress tolerance using two chickpea varieties i.e. *kabuli* (Pusa 1108) and *desi* (Pusa 362). Several biochemical parameters namely lipid peroxidation, total free amino acids, proline, total soluble sugars, total soluble proteins, antioxidant enzyme activities (catalase, peroxidase, glutathione reductase and superoxide dismutase) and abscisic acid content were estimated and studied. Lipid peroxidation and proline were increased with increase in water deficit duration and PBZ applications prevented the lipid peroxidation and increased the proline content in both chickpea varieties. Levels of the total amino acids and total soluble sugars were enhanced under water deficit condition. Furthermore, PBZ applications enhanced their levels under water stress and thus provided osmotic protection. Similarly all the antioxidant enzyme activities were stimulated by the application of PBZ. Absciscic acid content was increased under water deficit condition and PBZ applications enhanced the ABA level under both well watered and water deficit conditions. In general, PBZ drenching in terms of biochemical responses was found better over the foliar application of PBZ. Based on present findings it may be concluded that PBZ application induced the water deficit stress tolerance by increasing the content of osmolytes, ABA and activity of antioxidant enzymes.

**Key words:** *Chickpea, Drought, Antioxidant enzymes, Paclobutrazol*

### **5.1. INTRODUCTION**

Chickpea is grown in many part of the world. In the major chickpea growing countries India, Pakistan, Turkey, Iran chickpea is generally grown under rainfed conditions. Water deficit during reproductive stage is one of the most important constraint for chickpea production (Soltani *et al.*, 2001; Yadav *et al.*, 2006; Toker *et*

*al.*,2007). Water is essential for the plant survival as it provides the medium with in which most cellular function take place (Condon *et al.*,2002). Water deficit triggers deleterious responses ranging from cellular metabolism to alteration in growth, development and crop yield. When a plant is grown under abiotic stress condition some reactive oxygen species (i.e. super oxide, hydrogen peroxide, hydroxyl radical and singlet oxygen) are produced. However, various abiotic stresses extend the ROS production that exceeds the antioxidant defense capability of the cell resulting in cellular damage (Almeselmani *et al.*, 2006). Plants have developed complex antioxidant system to mitigate and repair damage initiated by ROS (Rio *et al.*, 2002). In addition to this water deficit significantly decreased the soluble protein content, increased soluble carbohydrate, free amino acid, proline content, lipid peroxidation and activity of antioxidant enzymes (Yadav *et al.*, 1996, Sairam *et al.*,2002, Sharma and Dubey,2005, Mafakheri *et al.*,2011). Under water deficit, marked enhancement in the level of endogenous ABA has been reported by several workers (Dodd *et al.*,2006; Verslues *et al.*,2006 and Kudoyarova *et al.*,2007).

At present, use of plant bioregulators attracts more attention to protect plants from abiotic stresses (Jaleel *et al.*, 2007) Paclobutrazol (PBZ), a derivative of triazole group interferes with gibberellins biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P 450-dependent oxygenase. PBZ has been used to provide plant protection against water stress (Banon *et al.* 2001; Berova *et al.*, 2003 and Zhu *et al.*, 2004). PBZ has biochemical effects on plants, such as detoxification of active oxygen (Upadhyaya *et al.*, 1989 Kraus and Fletcher, 1994) and reduced the decrease in leaf  $\psi_w$  (Naor, 1998) and increased levels of amino acid, proline (Mackay *et al.*, 1990, Rady and Gaballah, 2012) antioxidants (Senaratna *et al.*, 1988; Rady and Gaballah, 2012) and chlorophyll contents (Fletcher and Hofstra, 1988). Keeping above facts in mind it was thought interesting to analyze biochemical alterations for understanding the paclobutrazol induced mechanism of water deficit stress tolerance.

## **5.2. MATERIALS AND METHODS**

During *Rabi* season (2013-2014) main pot experiment was conducted in factorial CRD using two *Desi* type (Pusa 362) , *Kabuli* type (Pusa 1108) chickpea varieties to

biochemical responses of chickpea to paclobutrazol under water deficit stress conditions. In one half of the total number of pots optimal soil moisture conditions were maintained by watering the plants. While in the remaining half set of pots water stress was imposed by withholding water until the severe symptoms of wilting were visible at reproductive stage. Before imposition of water stress, plants of both sets were treated with PBZ foliar spray @ 60 ppm and its drenching application @120 ppm and for controls plants were treated with water spray & drenching with water.

#### **5.2.1. Soil moisture content**

Soil moisture content was estimated periodically during imposed water stress treatment at reproductive by gravimetric method. Soil samples were taken from pot at 10 cm depth using augur. For each samples 10 g fresh weight of soil was recorded using a weighing balance. Then the samples were kept in the oven at 105<sup>0</sup>C, and dried till constant weight was achieved. The soil moisture content was calculated by the formula as described elsewhere (Faulkner *et al.*, 1989) and expressed in percentage (%) (Fig. 3)

#### **5.2.2. Sample collection:**

During experiment water deficit stress treatment was imposed at reproductive stage. Plant samples were collected at 0<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days after withholding/ restricting water supply. After sampling, water deficit stress was terminated by irrigating plants and subsequent plant sampling during recovery period was done on 7<sup>th</sup> and 14<sup>th</sup>. Post harvest observations were recorded at harvest.

#### **5.2.3. Biochemical parameters**

Observations were recorded on lipid peroxidation (Heath and Packer, 1968), Total soluble sugars (McCready *et al.*, 1950), proline (Bates *et al.*, 1973), total free amino acids (Lee and Takahashi, 1966), total Soluble Proteins (Lowry *et al.*, 1951), catalase activity (Aebiet *et al.*, 1984), peroxidase activity (Castillo *et al.*, 1984), glutathione reductase activity (Smith *et al.*, 1988), superoxide dismutase (SOD) activity (Dhindsa *et al.*, 1981), abscisic acid (ABA) by HPLC (Zeevart, 1980).

## **5.3. RESULTS**

### **5.3.1. Biochemical Responses of Chickpea variety to PBZ applications under well watered and impose water deficit condition.**

#### **5.3.1.1. Lipid peroxidation**

Under imposed water deficit condition, lipid peroxidation gradually increased with advancement in water deficit period and maximum lipid peroxidation was recorded on fourteenth day of water deficit. Further, after water stress termination lipid peroxidation was recorded to decrease gradually. Foliar and drenching applications of PBZ restricted the lipid peroxidation under water deficit condition in both *desi* and *Kabuli* chickpea varieties. Maximum prevention of lipid peroxidation in both varieties was estimated with the treatment of PBZ drenching (Fig. 5.3.1.1. Table 5.3.1.1.)

#### **5.3.1.2. Proline content**

Imposed water deficit at reproductive stage in both *kabuli* and *desi* varieties significantly enhanced the level of proline. Comparatively higher level of proline content was analysed under water deficit in *kabuli* variety than *desi* one. Plants treated with foliar and drenching application of PBZ increased the level of proline content under water deficit condition and maximum enhancement in proline content was found with foliar application of PBZ (Fig. 5.3.1.2. Table 5.3.1.2.).

#### **5.3.1.3. Total free amino acids:**

In both chickpea varieties, imposed water stress increased the level of total free amino acids. Foliar and drenching application for PBZ enhanced the level of total free amino acids in both chickpea varieties under both well watered and water deficit conditions. Maximum enhancement of total free amino acids was obtained with the drenching application of PBZ followed by foliar application of PBZ. After water stress termination, total free amino acid content decreased gradually during recovery (Fig. 5.3.1.3. Table 5.3.1.3. ).

#### **5.3.1.4. Total Soluble Sugar**

Under imposed water deficit condition, level of total soluble sugar increased only in *kabuli* type and no significant alteration was noted in *desi* one. However, both applications of PBZ i.e., foliar and drenching significantly enhanced the level of total

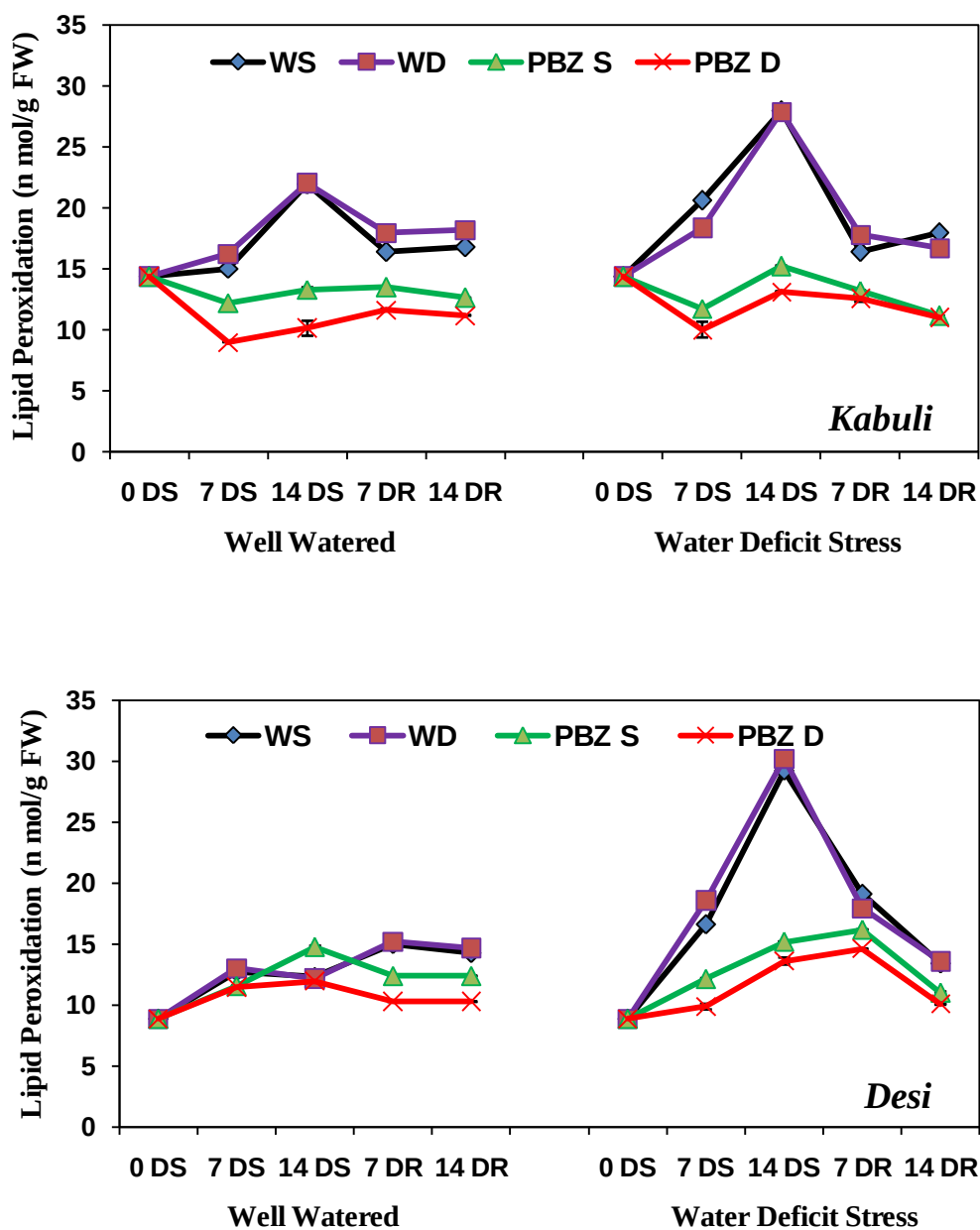


Fig. 5.3.1.1. Effect of PBZ on lipid peroxidation rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.1.Effect of PBZ on lipid peroxidation rate in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Lipid peroxidation (n mol/g FW) |      |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|---------------------------------|------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress              |      |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                          | Desi | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 14.33                           | 8.84 | 11.59 | 15.01              | 12.72 | 13.86 | 21.91               | 12.26 | 17.08 | 16.37          | 14.98 | 15.68 | 16.77           | 14.24 | 15.50 |
|                | Water Drenching           | 14.33                           | 8.84 | 11.59 | 16.25              | 12.99 | 14.62 | 21.99               | 12.15 | 17.07 | 17.97          | 15.17 | 16.57 | 18.23           | 14.66 | 16.45 |
|                | PBZ Foliar spray (60 ppm) | 14.33                           | 8.84 | 11.59 | 12.18              | 11.55 | 11.86 | 13.30               | 14.76 | 14.03 | 13.48          | 12.37 | 12.93 | 12.63           | 12.37 | 12.50 |
|                | PBZ Drenching (120 ppm)   | 14.33                           | 8.84 | 11.59 | 8.99               | 11.46 | 10.23 | 10.12               | 11.94 | 11.03 | 11.65          | 10.26 | 10.95 | 11.18           | 10.26 | 10.72 |
|                | Mean                      | 14.33                           | 8.84 | 11.59 | 13.11              | 12.18 | 12.64 | 16.83               | 12.78 | 14.80 | 14.87          | 13.19 | 14.03 | 14.70           | 12.88 | 13.79 |
| Water stressed | Water Foliar spray        | 14.33                           | 8.84 | 11.59 | 20.61              | 16.60 | 18.60 | 27.95               | 29.22 | 28.59 | 16.39          | 19.10 | 17.74 | 17.95           | 13.39 | 15.67 |
|                | Water Drenching           | 14.33                           | 8.84 | 11.59 | 18.34              | 18.57 | 18.45 | 27.92               | 30.19 | 29.05 | 17.84          | 17.90 | 17.87 | 16.71           | 13.57 | 15.14 |
|                | PBZ Foliar spray (60 ppm) | 14.33                           | 8.84 | 11.59 | 11.72              | 12.13 | 11.92 | 15.22               | 15.19 | 15.21 | 13.21          | 16.18 | 14.70 | 11.14           | 11.00 | 11.07 |
|                | PBZ Drenching (120 ppm)   | 14.33                           | 8.84 | 11.59 | 10.01              | 9.86  | 9.93  | 13.14               | 13.59 | 13.36 | 12.55          | 14.59 | 13.57 | 10.97           | 10.06 | 10.52 |
|                | Mean                      | 14.33                           | 8.84 | 11.59 | 15.17              | 14.29 | 14.73 | 21.06               | 22.05 | 21.55 | 15.00          | 16.94 | 15.97 | 14.19           | 12.00 | 13.10 |
|                | SEM                       | 0.267                           |      |       | 0.11               |       |       | 0.89                |       |       | 0.039          |       |       | 0.063           |       |       |
|                | CD at 5 %                 |                                 |      |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                             |      |       | 0.112              |       |       | 0.907               |       |       | 0.04           |       |       | 0.065           |       |       |
|                | Treatment (T)             | N/A                             |      |       | 0.158              |       |       | 1.282               |       |       | 0.056          |       |       | 0.091           |       |       |
|                | Varieties (V)             | 0.272                           |      |       | 0.112              |       |       | 0.907               |       |       | 0.04           |       |       | 0.065           |       |       |
|                | W x T =                   | N/A                             |      |       | 0.224              |       |       | 1.813               |       |       | 0.08           |       |       | 0.129           |       |       |
|                | W x V =                   | 0.385                           |      |       | N/A                |       |       | 1.282               |       |       | 0.056          |       |       | 0.091           |       |       |
|                | Tx V =                    | N/A                             |      |       | 0.224              |       |       | 1.813               |       |       | 0.08           |       |       | 0.129           |       |       |
|                | W x Tx V=                 | 0.77                            |      |       | 0.316              |       |       | 2.564               |       |       | 0.112          |       |       | 0.183           |       |       |

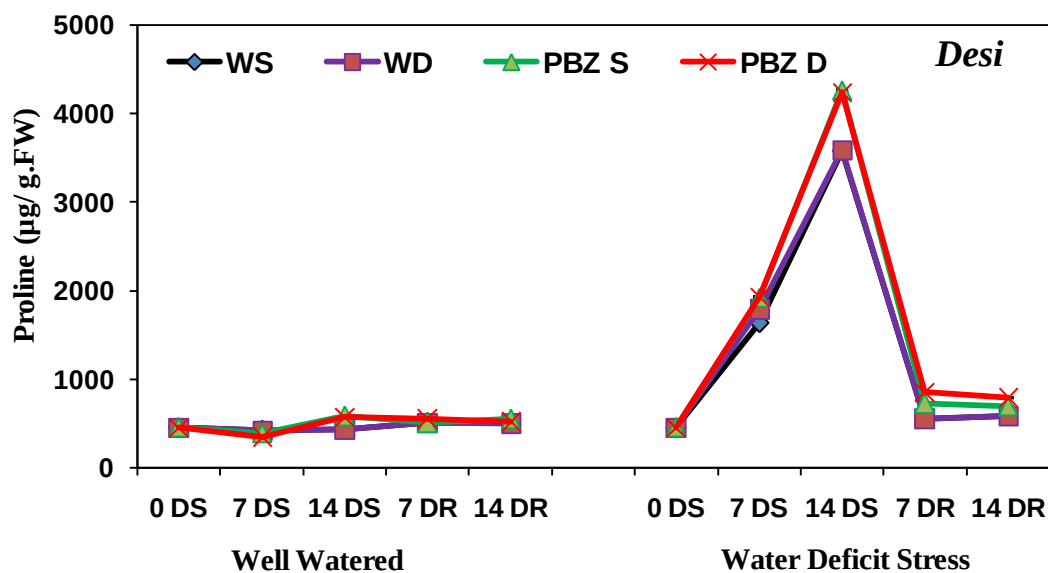
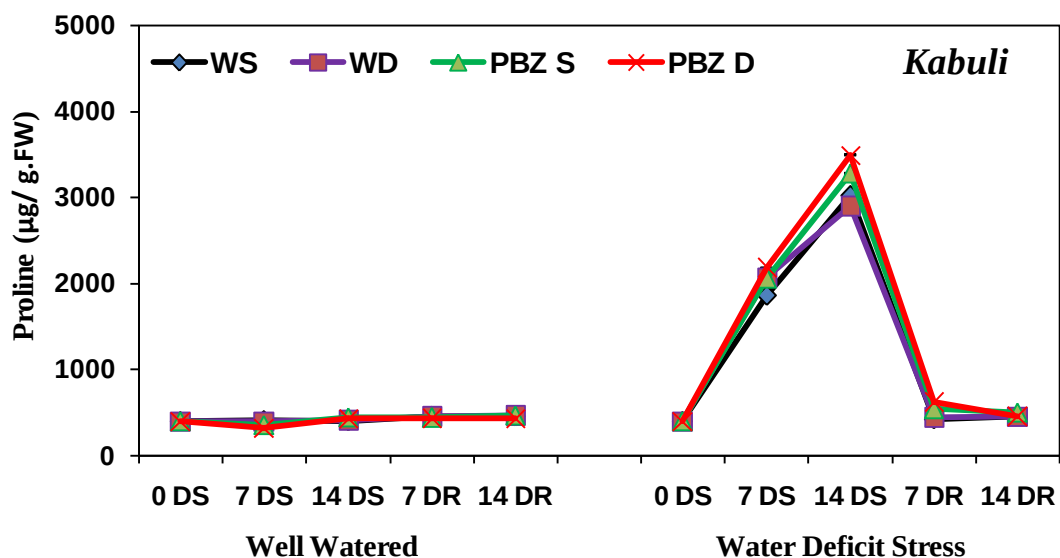


Fig 5.3.1.2.. Effect of PBZ on proline content in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.2. Effect of PBZ on proline content in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Proline (µg/ g.FW) |        |        |                    |         |         |                     |         |         |                |        |        |                 |        |        |
|----------------|---------------------------|--------------------|--------|--------|--------------------|---------|---------|---------------------|---------|---------|----------------|--------|--------|-----------------|--------|--------|
|                |                           | 0 day water stress |        |        | 7 day water stress |         |         | 14 day water stress |         |         | 7 day recovery |        |        | 14 day recovery |        |        |
|                |                           | Kabuli             | Desi   | Mean   | Kabuli             | Desi    | Mean    | Kabuli              | Desi    | Mean    | Kabuli         | Desi   | Mean   | Kabuli          | Desi   | Mean   |
| Well watered   | Water Foliar spray        | 402.38             | 459.62 | 431.00 | 413.86             | 421.86  | 417.86  | 400.71              | 433.93  | 417.32  | 451.43         | 508.64 | 480.04 | 456.21          | 514.00 | 485.11 |
|                | Water Drenching           | 402.38             | 459.62 | 431.00 | 399.43             | 422.29  | 410.86  | 406.29              | 434.29  | 420.29  | 452.43         | 513.29 | 482.86 | 460.36          | 493.79 | 477.07 |
|                | PBZ Foliar spray (60 ppm) | 402.38             | 459.62 | 431.00 | 353.07             | 391.93  | 372.50  | 445.86              | 581.79  | 513.82  | 439.50         | 508.14 | 473.82 | 466.93          | 548.43 | 507.68 |
|                | PBZ Drenching (120 ppm)   | 402.38             | 459.62 | 431.00 | 323.86             | 345.64  | 334.75  | 435.36              | 577.43  | 506.39  | 428.71         | 549.50 | 489.11 | 434.43          | 519.36 | 476.89 |
|                | Mean                      | 402.38             | 459.62 | 431.00 | 372.55             | 395.43  | 383.99  | 422.05              | 506.86  | 464.46  | 443.02         | 519.89 | 481.46 | 454.48          | 518.89 | 486.69 |
| Water stressed | Water Foliar spray        | 402.38             | 459.62 | 431.00 | 1855.64            | 1635.43 | 1745.54 | 3015.36             | 3581.36 | 3298.36 | 422.21         | 553.29 | 487.75 | 458.36          | 580.14 | 519.25 |
|                | Water Drenching           | 402.38             | 459.62 | 431.00 | 2074.14            | 1788.50 | 1931.32 | 2891.93             | 3591.79 | 3241.86 | 437.64         | 551.21 | 494.43 | 458.57          | 586.64 | 522.61 |
|                | PBZ Foliar spray (60 ppm) | 402.38             | 459.62 | 431.00 | 2056.50            | 1924.76 | 1990.63 | 3277.21             | 4264.21 | 3770.71 | 541.71         | 723.14 | 632.43 | 499.57          | 694.57 | 597.07 |
|                | PBZ Drenching (120 ppm)   | 402.38             | 459.62 | 431.00 | 2177.57            | 1932.36 | 2054.96 | 3490.00             | 4241.71 | 3865.86 | 619.07         | 859.50 | 739.29 | 451.36          | 789.36 | 620.36 |
|                | Mean                      | 402.38             | 459.62 | 431.00 | 2040.96            | 1820.26 | 1930.61 | 3168.63             | 3919.77 | 3544.20 | 505.16         | 671.79 | 588.47 | 466.96          | 662.68 | 564.82 |
|                | SEM                       | 1.671              |        |        | 8.89               |         |         | 226.095             |         |         | 0.286          |        |        | 0.852           |        |        |
|                | CD at 5 %                 |                    |        |        |                    |         |         |                     |         |         |                |        |        |                 |        |        |
|                | Water status (W)          | N/A                |        |        | 9.057              |         |         | 230.334             |         |         | 0.291          |        |        | 0.868           |        |        |
|                | Treatment (T)             | N/A                |        |        | 12.809             |         |         | N/A                 |         |         | 0.412          |        |        | 1.227           |        |        |
|                | Varieties (V)             | 1.702              |        |        | 9.057              |         |         | 230.334             |         |         | 0.291          |        |        | 0.868           |        |        |
|                | W x T =                   | N/A                |        |        | 18.114             |         |         | N/A                 |         |         | 0.583          |        |        | 1.736           |        |        |
|                | W x V =                   | N/A                |        |        | 12.809             |         |         | 325.742             |         |         | 0.412          |        |        | 1.227           |        |        |
|                | Tx V =                    | N/A                |        |        | 18.114             |         |         | N/A                 |         |         | 0.583          |        |        | 1.736           |        |        |
|                | W x Tx V=                 | 4.815              |        |        | 25.617             |         |         | N/A                 |         |         | 0.824          |        |        | 2.454           |        |        |

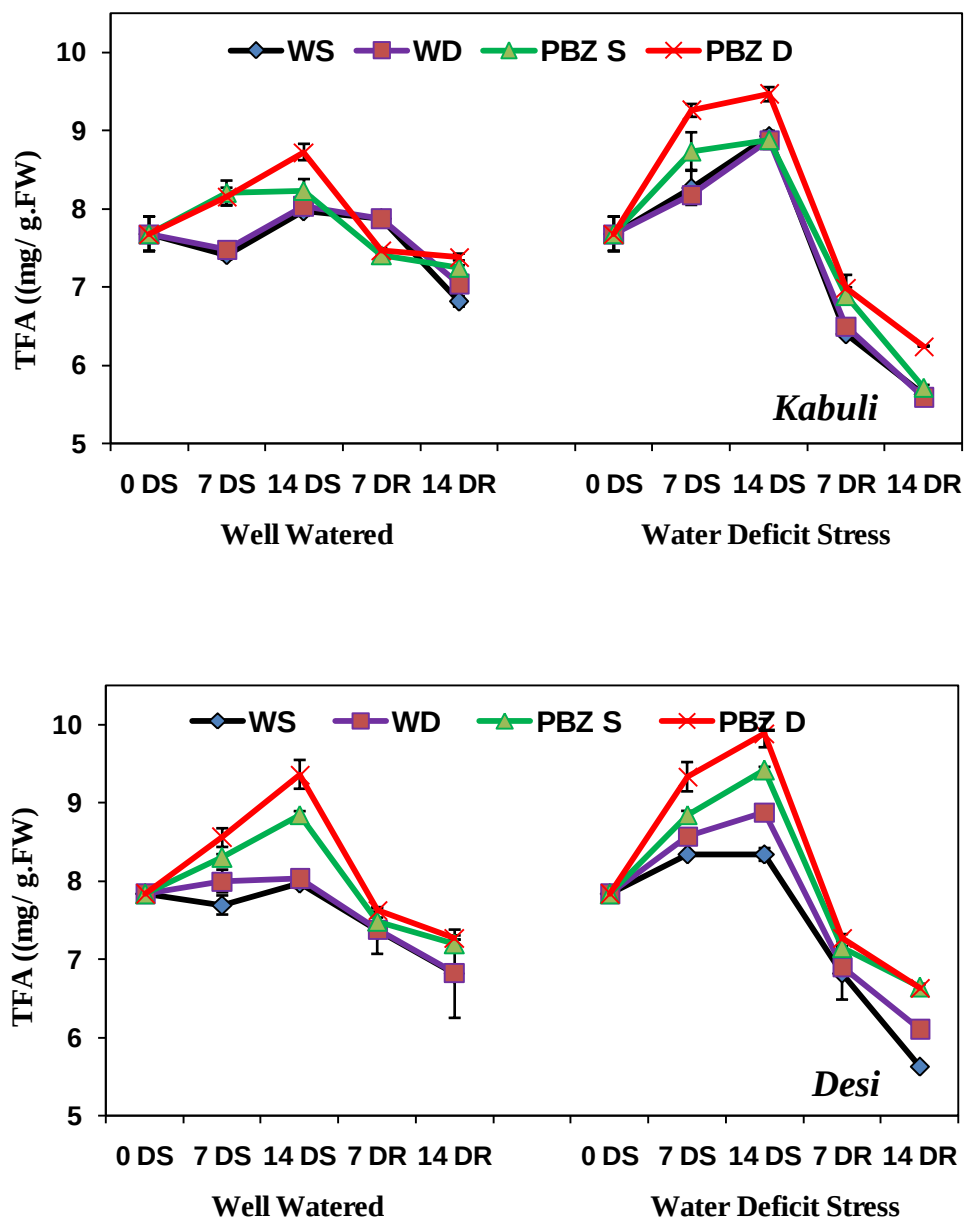


Fig 5.3.1.3. Effect of PBZ on total free aminoacid (TFA) content in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.3. Effect of PBZ on total free aminoacid content in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status              | PBZ Treatments            | Total Free amino acid (mg/ g.FW) |      |      |                    |       |      |                     |       |      |                |       |      |                 |       |      |
|---------------------------|---------------------------|----------------------------------|------|------|--------------------|-------|------|---------------------|-------|------|----------------|-------|------|-----------------|-------|------|
|                           |                           | 0 day water stress               |      |      | 7 day water stress |       |      | 14 day water stress |       |      | 7 day recovery |       |      | 14 day recovery |       |      |
|                           |                           | Kabuli                           | Desi | Mean | Kabuli             | Desi  | Mean | Kabuli              | Desi  | Mean | Kabuli         | Desi  | Mean | Kabuli          | Desi  | Mean |
| Well watered              | Water Foliar spray        | 7.68                             | 7.83 | 7.76 | 7.41               | 7.69  | 7.55 | 7.97                | 7.97  | 7.97 | 7.87           | 7.37  | 7.62 | 6.81            | 6.81  | 6.81 |
|                           | Water Drenching           | 7.68                             | 7.83 | 7.76 | 7.48               | 8.00  | 7.74 | 8.03                | 8.03  | 8.03 | 7.87           | 7.38  | 7.63 | 7.04            | 6.83  | 6.93 |
|                           | PBZ Foliar spray (60 ppm) | 7.68                             | 7.83 | 7.76 | 8.20               | 8.30  | 8.25 | 8.23                | 8.84  | 8.54 | 7.41           | 7.49  | 7.45 | 7.25            | 7.19  | 7.22 |
|                           | PBZ Drenching (120 ppm)   | 7.68                             | 7.83 | 7.76 | 8.15               | 8.55  | 8.35 | 8.72                | 9.36  | 9.04 | 7.47           | 7.63  | 7.55 | 7.38            | 7.28  | 7.33 |
|                           | Mean                      | 7.68                             | 7.83 | 7.76 | 7.81               | 8.14  | 7.97 | 8.24                | 8.55  | 8.39 | 7.65           | 7.47  | 7.56 | 7.12            | 7.03  | 7.07 |
|                           | Water stressed            | Water Foliar spray               | 7.68 | 7.83 | 7.76               | 8.27  | 8.34 | 8.30                | 8.93  | 8.34 | 8.63           | 6.40  | 6.82 | 6.61            | 5.63  | 5.63 |
| Water Drenching           |                           | 7.68                             | 7.83 | 7.76 | 8.18               | 8.58  | 8.38 | 8.88                | 8.88  | 8.88 | 6.50           | 6.90  | 6.70 | 5.58            | 6.10  | 5.84 |
| PBZ Foliar spray (60 ppm) |                           | 7.68                             | 7.83 | 7.76 | 8.74               | 8.84  | 8.79 | 8.88                | 9.42  | 9.15 | 6.89           | 7.14  | 7.02 | 5.72            | 6.64  | 6.18 |
| PBZ Drenching (120 ppm)   |                           | 7.68                             | 7.83 | 7.76 | 9.26               | 9.33  | 9.29 | 9.46                | 9.89  | 9.68 | 6.99           | 7.27  | 7.13 | 6.24            | 6.63  | 6.44 |
| Mean                      |                           | 7.68                             | 7.83 | 7.76 | 8.61               | 8.77  | 8.69 | 9.04                | 9.13  | 9.09 | 6.69           | 7.03  | 6.86 | 5.79            | 6.25  | 6.02 |
|                           |                           | SEM                              | 0.09 |      |                    | 0.109 |      |                     | 0.059 |      |                | 0.073 |      |                 | 0.084 |      |
|                           | CD at 5 %                 |                                  |      |      |                    |       |      |                     |       |      |                |       |      |                 |       |      |
|                           | Water status (W)          | N/A                              |      |      | 0.111              |       |      | 0.06                |       |      | 0.075          |       |      | 0.085           |       |      |
|                           | Treatment (T)             | N/A                              |      |      | 0.157              |       |      | 0.085               |       |      | 0.106          |       |      | 0.121           |       |      |
|                           | Varieties (V)             | 0.092                            |      |      | 0.111              |       |      | 0.06                |       |      | 0.075          |       |      | 0.085           |       |      |
|                           | W x T =                   | N/A                              |      |      | 0.221              |       |      | N/A                 |       |      | N/A            |       |      | N/A             |       |      |
|                           | W x V =                   | N/A                              |      |      | 0.157              |       |      | N/A                 |       |      | 0.106          |       |      | 0.121           |       |      |
|                           | Tx V =                    | N/A                              |      |      | N/A                |       |      | 0.12                |       |      | N/A            |       |      | 0.171           |       |      |
|                           | W x Tx V=                 | 0.261                            |      |      | 0.313              |       |      | 0.169               |       |      | 0.211          |       |      | N/A             |       |      |

soluble sugar in both *kabuli* and *desi* varieties under both well watered and water deficit conditions. Maximum enhancement of total soluble sugar in both chickpea varieties was recorded with drenching application of PBZ (Fig. 5.3.1.4. Table 5.3.1.4. ).

#### **5.3.1.5. Total Soluble Proteins**

Level of total soluble proteins was found to decrease under water deficit condition in both *kabuli* and *desi* chickpea varieties. However, PBZ applications i.e., foliar and drenching significantly enhanced the level of total soluble proteins in both *kabuli* and *desi* varieties under both well watered and water deficit conditions. Under both well watered and water stress conditions drenching application of PBZ had maximum enhancement of total soluble proteins in both chickpea varieties (Fig. 5.3.1.5. Table 5.3.1.5. ).

#### **5.3.1.6. Catalase (CAT) activity:**

In both the chickpea varieties, there was significant alteration in catalase activity under water deficit conditions. However, foliar and drenching application significantly enhanced the catalase activity in both the chickpea varieties. Drenching application of PBZ enhanced the maximum activity of catalase in both *kabuli* and *desi* varieties under water deficit conditions (Fig. 5.3.1.6. Table 5.3.1.6. ).

#### **5.3.1.7. Peroxidase (POX) activity**

Under water deficit condition, peroxidase activity was increased in both varieties. PBZ treatments enhanced the activity of peroxidase in both *kabuli* and *desi* chickpea varieties. Maximum enhancement of peroxidase activity was obtained with drenching application of PBZ in both varieties under water deficit conditions (Fig. 5.3.1.7. Table 5.3.1.7.).

#### **5.3.1.8. Superoxide dismutase (SOD) activity**

Superoxide dismutase activity was increased gradually in both chickpea varieties during water deficit and further decreased after termination of water deficit. PBZ applications enhanced superoxide dismutase activity in both *kabuli* and *desi* varieties. Under water deficit condition, drenching application of PBZ had maximum enhancement of superoxide dismutase activity in both chickpea varieties than foliar application (Fig. 5.3.1.8. Table 5.3.1.8.).

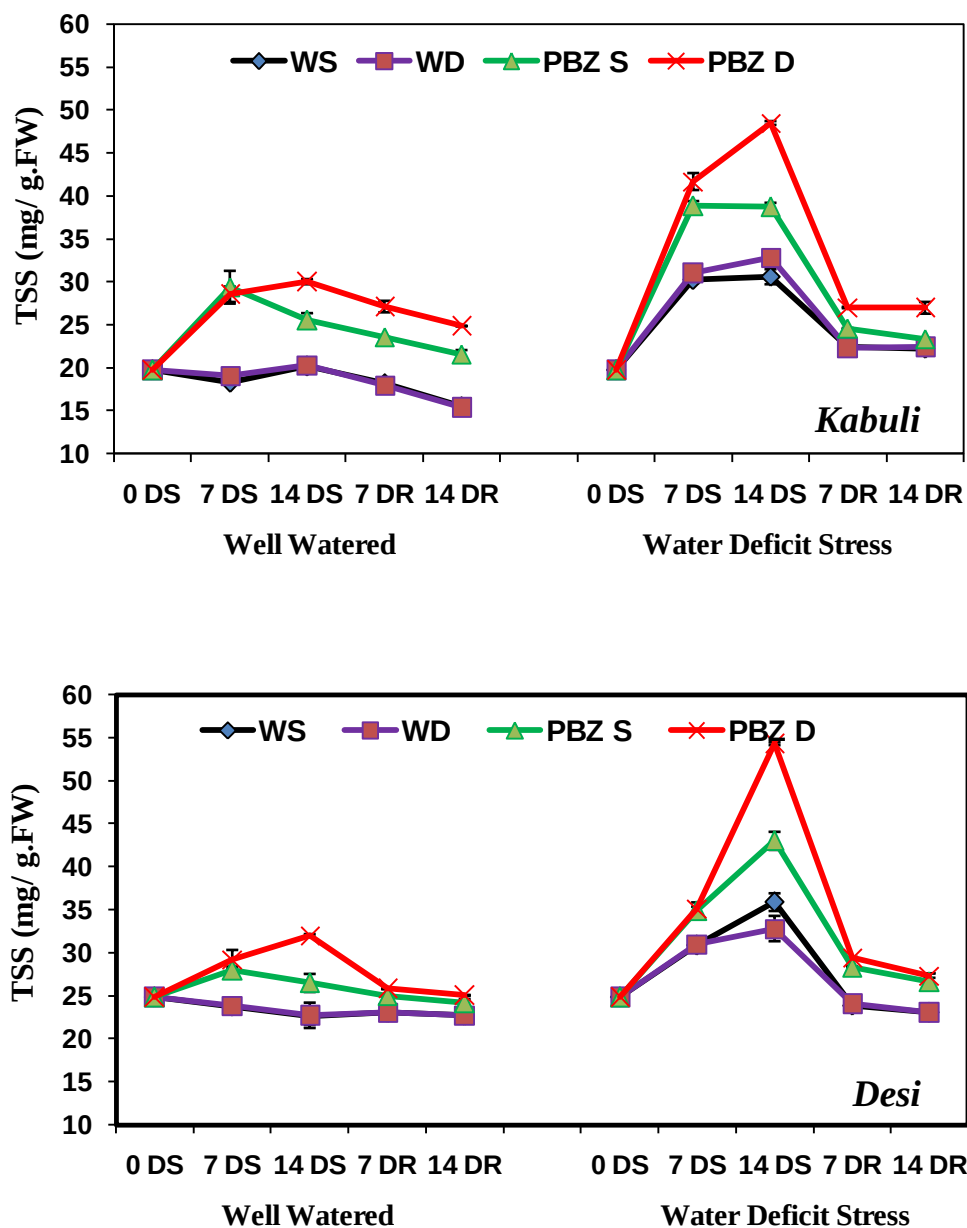


Fig. 5.3.1.4. Effect of PBZ on total soluble sugar (TSS) content in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.4. Effect of PBZ on total soluble sugars in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Total Soluble Sugars (mg/ g.FW) |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|---------------------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress              |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                          | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 19.69                           | 24.85 | 22.27 | 18.24              | 23.79 | 21.01 | 20.15               | 22.64 | 21.39 | 18.20          | 23.03 | 20.61 | 15.52           | 22.70 | 19.11 |
|                | Water Drenching           | 19.69                           | 24.85 | 22.27 | 19.11              | 23.89 | 21.50 | 20.30               | 22.73 | 21.52 | 17.96          | 23.09 | 20.53 | 15.36           | 22.79 | 19.08 |
|                | PBZ Foliar spray (60 ppm) | 19.69                           | 24.85 | 22.27 | 29.35              | 27.96 | 28.66 | 25.52               | 26.49 | 26.01 | 23.54          | 25.02 | 24.28 | 21.56           | 24.15 | 22.85 |
|                | PBZ Drenching (120 ppm)   | 19.69                           | 24.85 | 22.27 | 28.54              | 29.21 | 28.87 | 30.00               | 32.00 | 31.00 | 27.10          | 25.83 | 26.46 | 24.84           | 25.04 | 24.94 |
|                | Mean                      | 19.69                           | 24.85 | 22.27 | 23.81              | 26.21 | 25.01 | 23.99               | 25.96 | 24.98 | 21.70          | 24.24 | 22.97 | 19.32           | 23.67 | 21.50 |
| Water stressed | Water Foliar spray        | 19.69                           | 24.85 | 22.27 | 30.23              | 30.87 | 30.55 | 30.57               | 35.88 | 33.23 | 22.40          | 23.91 | 23.15 | 22.19           | 23.06 | 22.62 |
|                | Water Drenching           | 19.69                           | 24.85 | 22.27 | 31.00              | 31.00 | 31.00 | 32.81               | 32.81 | 32.81 | 22.28          | 24.06 | 23.17 | 22.40           | 23.02 | 22.71 |
|                | PBZ Foliar spray (60 ppm) | 19.69                           | 24.85 | 22.27 | 38.79              | 34.85 | 36.82 | 38.70               | 43.00 | 40.85 | 24.56          | 28.31 | 26.43 | 23.34           | 26.61 | 24.98 |
|                | PBZ Drenching (120 ppm)   | 19.69                           | 24.85 | 22.27 | 41.65              | 35.16 | 38.41 | 48.47               | 54.29 | 51.38 | 27.00          | 29.45 | 28.22 | 26.96           | 27.35 | 27.15 |
|                | Mean                      | 19.69                           | 24.85 | 22.27 | 35.42              | 32.97 | 34.19 | 37.64               | 41.50 | 39.57 | 24.06          | 26.43 | 25.24 | 23.72           | 25.01 | 24.37 |
|                | SEM                       | 0.17                            |       |       | 0.463              |       |       | 0.717               |       |       | 0.103          |       |       | 0.103           |       |       |
|                | CD at 5 %                 |                                 |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                             |       |       | 0.471              |       |       | 0.73                |       |       | 0.105          |       |       | 0.213           |       |       |
|                | Treatment (T)             | N/A                             |       |       | 0.667              |       |       | 1.033               |       |       | 0.149          |       |       | 0.301           |       |       |
|                | Varieties (V)             | 0.173                           |       |       | 0.471              |       |       | 0.73                |       |       | 0.105          |       |       | 0.213           |       |       |
|                | W x T =                   | N/A                             |       |       | 0.943              |       |       | 1.461               |       |       | 0.21           |       |       | 0.426           |       |       |
|                | W x V =                   | N/A                             |       |       | 0.667              |       |       | 1.033               |       |       | N/A            |       |       | 0.301           |       |       |
|                | Tx V =                    | N/A                             |       |       | 0.943              |       |       | 1.461               |       |       | 0.21           |       |       | 0.426           |       |       |
|                | W x Tx V=                 | 0.491                           |       |       | 1.333              |       |       | 2.066               |       |       | 0.297          |       |       | 0.603           |       |       |

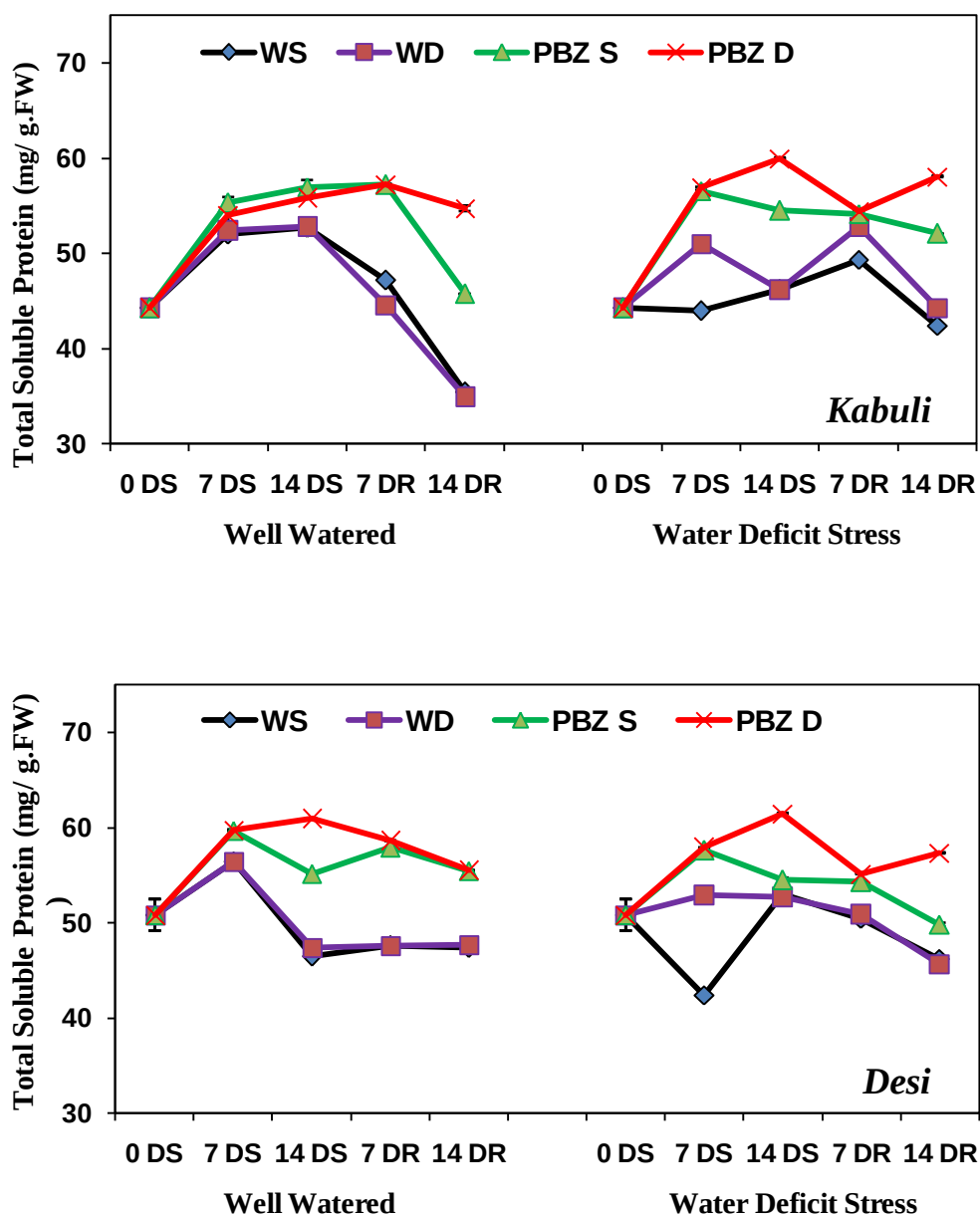


Fig. 5.3.1.5. Effect of PBZ on total soluble protein in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.5. Effect of PBZ on total soluble protein in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Total Soluble Protein (mg/ g.FW) |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|----------------------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress               |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                           | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 44.21                            | 50.82 | 47.51 | 51.97              | 56.42 | 54.19 | 52.64               | 46.43 | 49.54 | 47.16          | 47.53 | 47.35 | 35.40           | 47.36 | 41.38 |
|                | Water Drenching           | 44.21                            | 50.82 | 47.51 | 52.36              | 56.42 | 54.39 | 52.75               | 47.40 | 50.07 | 44.56          | 47.58 | 46.07 | 34.88           | 47.70 | 41.29 |
|                | PBZ Foliar spray (60 ppm) | 44.21                            | 50.82 | 47.51 | 55.30              | 59.65 | 57.47 | 56.94               | 55.15 | 56.04 | 57.19          | 57.89 | 57.54 | 45.72           | 55.42 | 50.57 |
|                | PBZ Drenching (120 ppm)   | 44.21                            | 50.82 | 47.51 | 54.00              | 59.76 | 56.88 | 55.78               | 60.90 | 58.34 | 57.25          | 58.61 | 57.93 | 54.71           | 55.50 | 55.10 |
|                | Mean                      | 44.21                            | 50.82 | 47.51 | 53.41              | 58.06 | 55.73 | 54.53               | 52.47 | 53.50 | 51.54          | 52.90 | 52.22 | 42.68           | 51.49 | 47.09 |
| Water stressed | Water Foliar spray        | 44.21                            | 50.82 | 47.51 | 43.94              | 42.40 | 43.17 | 46.15               | 53.04 | 49.60 | 49.29          | 50.43 | 49.86 | 42.36           | 46.21 | 44.28 |
|                | Water Drenching           | 44.21                            | 50.82 | 47.51 | 50.99              | 52.89 | 51.94 | 46.13               | 52.67 | 49.40 | 52.84          | 50.88 | 51.86 | 44.29           | 45.68 | 44.99 |
|                | PBZ Foliar spray (60 ppm) | 44.21                            | 50.82 | 47.51 | 56.55              | 57.60 | 57.08 | 54.48               | 54.46 | 54.47 | 54.10          | 54.29 | 54.20 | 52.04           | 49.79 | 50.92 |
|                | PBZ Drenching (120 ppm)   | 44.21                            | 50.82 | 47.51 | 56.92              | 57.87 | 57.40 | 59.97               | 61.47 | 60.72 | 54.45          | 55.14 | 54.79 | 58.06           | 57.32 | 57.69 |
|                | Mean                      | 44.21                            | 50.82 | 47.51 | 52.10              | 52.69 | 52.40 | 51.68               | 55.41 | 53.55 | 52.67          | 52.68 | 52.68 | 49.19           | 49.75 | 49.47 |
|                | SEM                       | 0.06                             |       |       | 0.132              |       |       | 0.182               |       |       | 0.057          |       |       | 0.063           |       |       |
|                | CD at 5 %                 |                                  |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                              |       |       | 0.135              |       |       | N/A                 |       |       | 0.058          |       |       | 0.064           |       |       |
|                | Treatment (T)             | N/A                              |       |       | 0.19               |       |       | 0.263               |       |       | 0.083          |       |       | 0.09            |       |       |
|                | Varieties (V)             | 0.709                            |       |       | 0.135              |       |       | 0.186               |       |       | 0.058          |       |       | 0.064           |       |       |
|                | W x T =                   | N/A                              |       |       | 0.269              |       |       | 0.372               |       |       | 0.117          |       |       | 0.128           |       |       |
|                | W x V =                   | N/A                              |       |       | 0.19               |       |       | 0.263               |       |       | 0.083          |       |       | 0.09            |       |       |
|                | Tx V =                    | N/A                              |       |       | 0.269              |       |       | 0.372               |       |       | 0.117          |       |       | 0.128           |       |       |
|                | W x Tx V=                 | 2.005                            |       |       | 0.381              |       |       | 0.525               |       |       | 0.165          |       |       | 0.181           |       |       |

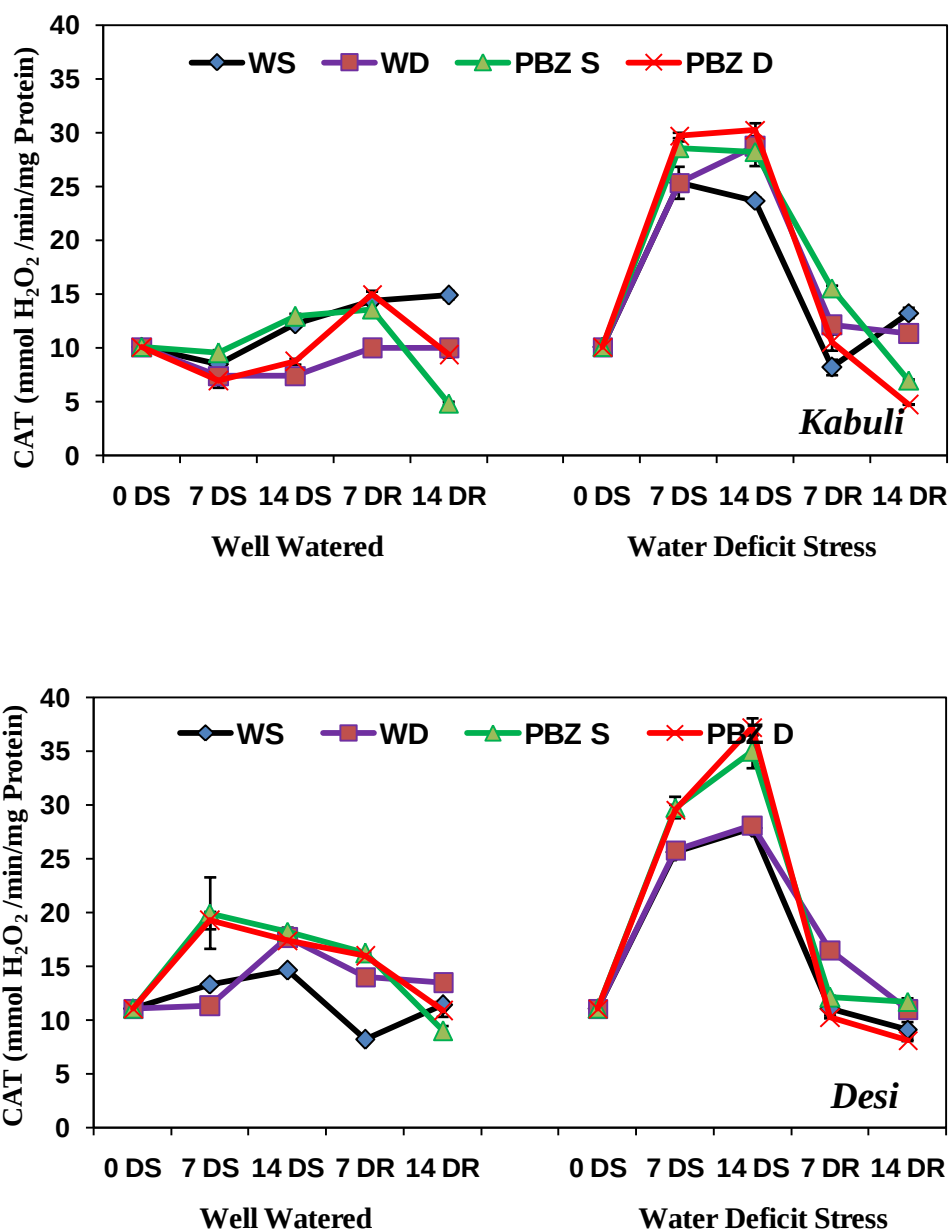


Fig. 5.3.1.6. Effect of PBZ on catalase (CAT) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.6. Effect of PBZ on catalase (CAT) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Catalase (mmol H <sub>2</sub> O <sub>2</sub> /min/mg Protein) |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|----------------|---------------------------|---------------------------------------------------------------|-------|-------|--------------------|-------|-------|---------------------|-------|-------|----------------|-------|-------|-----------------|-------|-------|
|                |                           | 0 day water stress                                            |       |       | 7 day water stress |       |       | 14 day water stress |       |       | 7 day recovery |       |       | 14 day recovery |       |       |
|                |                           | Kabuli                                                        | Desi  | Mean  | Kabuli             | Desi  | Mean  | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean  | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 10.12                                                         | 11.08 | 10.60 | 8.45               | 13.34 | 10.89 | 12.19               | 14.60 | 13.39 | 14.34          | 8.18  | 11.26 | 14.89           | 11.40 | 13.15 |
|                | Water Drenching           | 10.12                                                         | 11.08 | 10.60 | 7.41               | 11.31 | 9.36  | 7.43                | 17.64 | 12.53 | 9.96           | 14.01 | 11.99 | 9.97            | 13.44 | 11.71 |
|                | PBZ Foliar spray (60 ppm) | 10.12                                                         | 11.08 | 10.60 | 9.54               | 19.94 | 14.74 | 12.92               | 18.23 | 15.58 | 13.59          | 16.21 | 14.90 | 4.85            | 8.97  | 6.91  |
|                | PBZ Drenching (120 ppm)   | 10.12                                                         | 11.08 | 10.60 | 6.94               | 19.24 | 13.09 | 8.70                | 17.44 | 13.07 | 15.00          | 15.96 | 15.48 | 9.34            | 10.88 | 10.11 |
|                | Mean                      | 10.12                                                         | 11.08 | 10.60 | 8.09               | 15.96 | 12.02 | 10.31               | 16.98 | 13.64 | 13.22          | 13.59 | 13.41 | 9.76            | 11.17 | 10.47 |
| Water stressed | Water Foliar spray        | 10.12                                                         | 11.08 | 10.60 | 25.34              | 25.66 | 25.50 | 23.67               | 27.86 | 25.76 | 8.17           | 11.06 | 9.62  | 13.25           | 9.13  | 11.19 |
|                | Water Drenching           | 10.12                                                         | 11.08 | 10.60 | 25.37              | 25.80 | 25.58 | 28.70               | 28.15 | 28.43 | 12.10          | 16.51 | 14.31 | 11.33           | 10.97 | 11.15 |
|                | PBZ Foliar spray (60 ppm) | 10.12                                                         | 11.08 | 10.60 | 28.58              | 29.76 | 29.17 | 28.23               | 34.98 | 31.60 | 15.53          | 12.09 | 13.81 | 6.98            | 11.73 | 9.35  |
|                | PBZ Drenching (120 ppm)   | 10.12                                                         | 11.08 | 10.60 | 29.74              | 29.57 | 29.65 | 30.28               | 37.25 | 33.76 | 10.49          | 10.29 | 10.39 | 4.72            | 8.12  | 6.42  |
|                | Mean                      | 10.12                                                         | 11.08 | 10.60 | 27.26              | 27.70 | 27.48 | 27.72               | 32.06 | 29.89 | 11.57          | 12.49 | 12.03 | 9.07            | 9.99  | 9.53  |
|                | SEM                       | 0.105                                                         |       |       | 0.171              |       |       | 0.439               |       |       | 0.899          |       |       | 0.339           |       |       |
|                | CD at 5 %                 |                                                               |       |       |                    |       |       |                     |       |       |                |       |       |                 |       |       |
|                | Water status (W)          | N/A                                                           |       |       | 0.174              |       |       | 0.448               |       |       | 0.916          |       |       | 0.346           |       |       |
|                | Treatment (T)             | N/A                                                           |       |       | 0.246              |       |       | 0.633               |       |       | 1.295          |       |       | 0.489           |       |       |
|                | Varieties (V)             | 0.107                                                         |       |       | 0.174              |       |       | 0.448               |       |       | N/A            |       |       | 0.346           |       |       |
|                | W x T =                   | N/A                                                           |       |       | 0.348              |       |       | 0.895               |       |       | 1.831          |       |       | 0.691           |       |       |
|                | W x V =                   | N/A                                                           |       |       | 0.246              |       |       | 0.633               |       |       | N/A            |       |       | N/A             |       |       |
|                | Tx V =                    | N/A                                                           |       |       | 0.348              |       |       | 0.895               |       |       | 1.831          |       |       | 0.691           |       |       |
|                | W x Tx V=                 | 0.303                                                         |       |       | 0.492              |       |       | 1.266               |       |       | 2.59           |       |       | 0.977           |       |       |

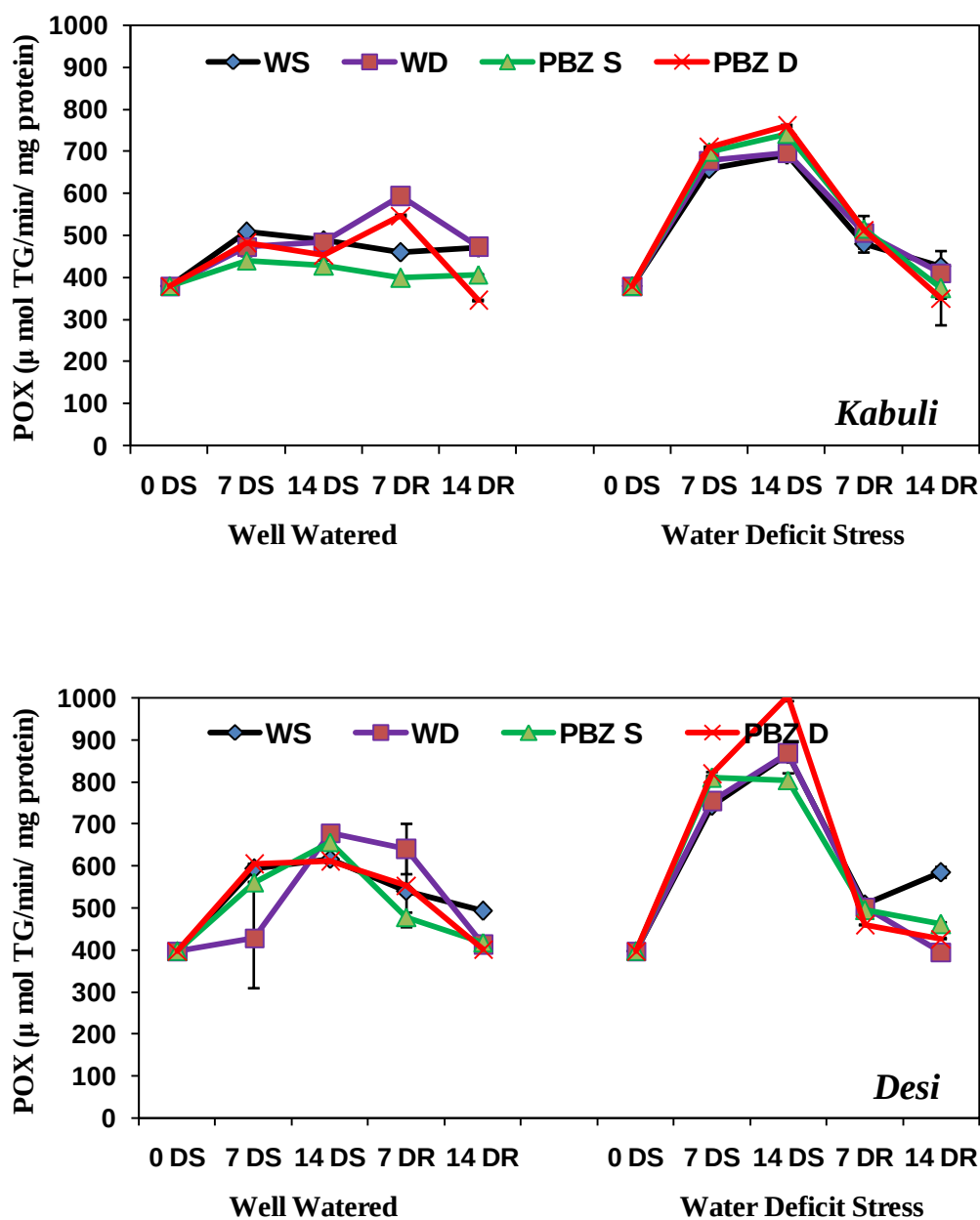


Fig. 5.3.1.7. Effect of PBZ on peroxidase (POX) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.7. Effect of PBZ on peroxidase (POX) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Peroxidase (μ mol TG/min/ mg protein) |        |        |                    |        |        |                     |         |        |                |        |        |                 |        |        |
|----------------|---------------------------|---------------------------------------|--------|--------|--------------------|--------|--------|---------------------|---------|--------|----------------|--------|--------|-----------------|--------|--------|
|                |                           | 0 day water stress                    |        |        | 7 day water stress |        |        | 14 day water stress |         |        | 7 day recovery |        |        | 14 day recovery |        |        |
|                |                           | Kabuli                                | Desi   | Mean   | Kabuli             | Desi   | Mean   | Kabuli              | Desi    | Mean   | Kabuli         | Desi   | Mean   | Kabuli          | Desi   | Mean   |
| Well watered   | Water Foliar spray        | 380.40                                | 397.54 | 388.97 | 508.88             | 593.14 | 551.01 | 490.44              | 615.90  | 553.17 | 459.70         | 540.85 | 500.28 | 471.15          | 492.30 | 481.73 |
|                | Water Drenching           | 380.40                                | 397.54 | 388.97 | 474.13             | 429.37 | 451.75 | 485.27              | 679.27  | 582.27 | 593.53         | 640.00 | 616.77 | 473.54          | 412.22 | 442.88 |
|                | PBZ Foliar spray (60 ppm) | 380.40                                | 397.54 | 388.97 | 441.43             | 560.99 | 501.21 | 428.42              | 657.12  | 542.77 | 399.45         | 477.86 | 438.65 | 407.63          | 418.12 | 412.88 |
|                | PBZ Drenching (120 ppm)   | 380.40                                | 397.54 | 388.97 | 482.59             | 604.21 | 543.40 | 454.19              | 610.51  | 532.35 | 547.54         | 553.79 | 550.67 | 345.70          | 402.14 | 373.92 |
|                | Mean                      | 380.40                                | 397.54 | 388.97 | 476.76             | 546.93 | 511.84 | 464.58              | 640.70  | 552.64 | 500.06         | 553.13 | 526.59 | 424.50          | 431.20 | 427.85 |
| Water stressed | Water Foliar spray        | 380.40                                | 397.54 | 388.97 | 658.54             | 742.31 | 700.42 | 691.51              | 865.65  | 778.58 | 479.74         | 509.50 | 494.62 | 427.77          | 584.44 | 506.11 |
|                | Water Drenching           | 380.40                                | 397.54 | 388.97 | 680.05             | 753.36 | 716.70 | 697.83              | 867.97  | 782.90 | 508.03         | 501.99 | 505.01 | 411.60          | 395.69 | 403.65 |
|                | PBZ Foliar spray (60 ppm) | 380.40                                | 397.54 | 388.97 | 700.11             | 809.24 | 754.68 | 741.89              | 804.08  | 772.98 | 516.21         | 494.57 | 505.39 | 374.89          | 461.59 | 418.24 |
|                | PBZ Drenching (120 ppm)   | 380.40                                | 397.54 | 388.97 | 709.65             | 818.76 | 764.21 | 761.02              | 1003.50 | 882.26 | 514.90         | 459.36 | 487.13 | 351.46          | 427.17 | 389.32 |
|                | Mean                      | 380.40                                | 397.54 | 388.97 | 687.09             | 780.92 | 734.00 | 723.06              | 885.30  | 804.18 | 504.72         | 491.36 | 498.04 | 391.43          | 467.22 | 429.33 |
|                | SEM                       | 2.102                                 |        |        | 5.175              |        |        | 3.592               |         |        | 18.083         |        |        | 12.863          |        |        |
|                | CD at 5 %                 |                                       |        |        |                    |        |        |                     |         |        |                |        |        |                 |        |        |
|                | Water status (W)          | N/A                                   |        |        | 7.318              |        |        | 3.659               |         |        | N/A            |        |        | N/A             |        |        |
|                | Treatment (T)             | N/A                                   |        |        | 10.349             |        |        | 5.175               |         |        | 26.053         |        |        | 18.532          |        |        |
|                | Varieties (V)             | 2.142                                 |        |        | 5.175              |        |        | 3.659               |         |        | N/A            |        |        | 13.104          |        |        |
|                | W x T =                   | N/A                                   |        |        | 7.318              |        |        | 7.318               |         |        | 36.845         |        |        | 26.209          |        |        |
|                | W x V =                   | N/A                                   |        |        | 10.349             |        |        | 5.175               |         |        | 26.053         |        |        | 18.532          |        |        |
|                | Tx V =                    | N/A                                   |        |        | 5.175              |        |        | 7.318               |         |        | 36.845         |        |        | 26.209          |        |        |
|                | W x Tx V=                 | 6.058                                 |        |        | 7.318              |        |        | 10.349              |         |        | N/A            |        |        | 37.065          |        |        |

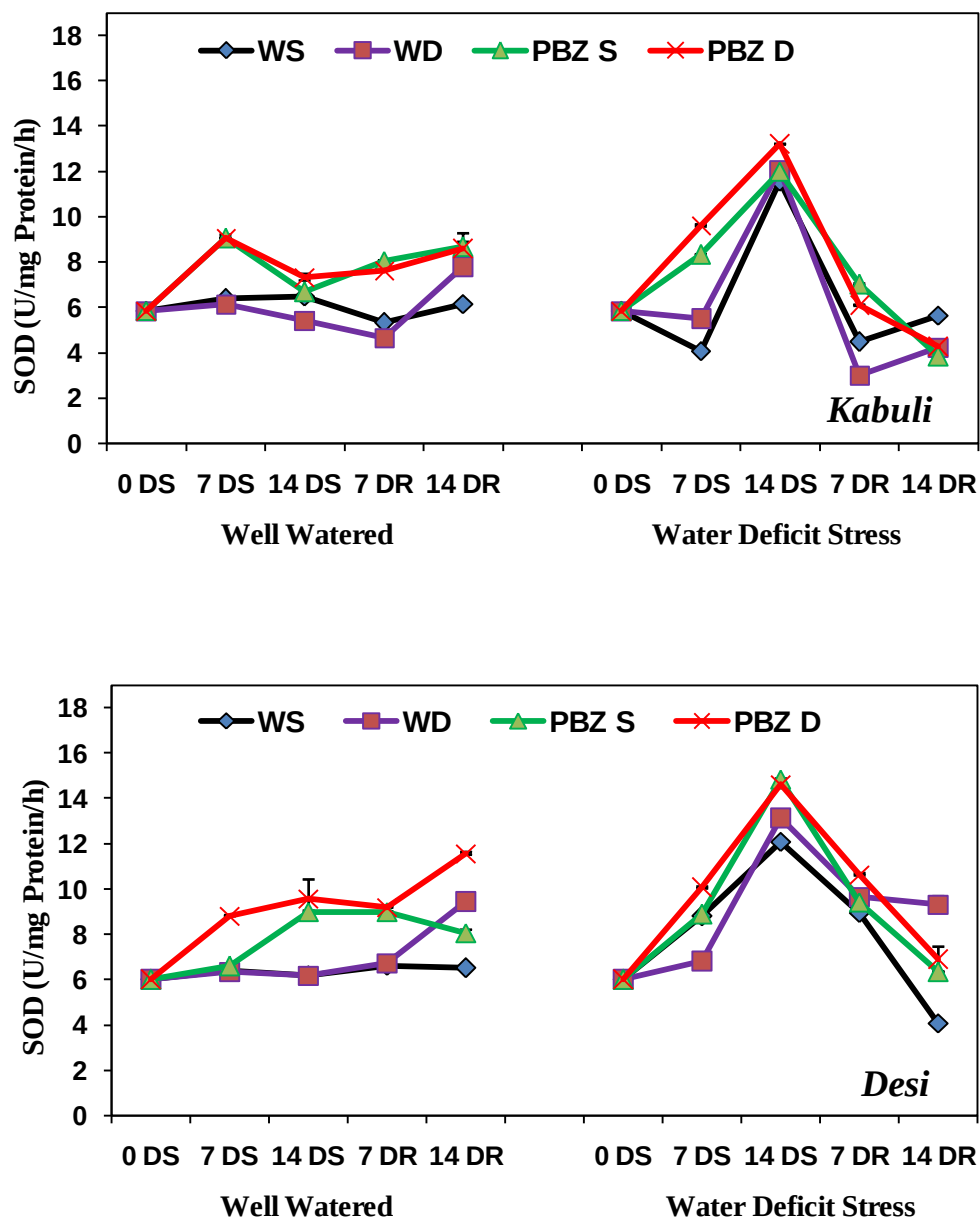


Fig. 5.3.1.8. Effect of PBZ on superoxide dismutase (SOD) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions

**Table 5.3.1.8. Effect of PBZ on superoxide dismutase (SOD) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Superoxide dismutase (U/mg Protein/h) |      |      |                    |       |      |                     |       |       |                |       |      |                 |       |       |
|----------------|---------------------------|---------------------------------------|------|------|--------------------|-------|------|---------------------|-------|-------|----------------|-------|------|-----------------|-------|-------|
|                |                           | 0 day water stress                    |      |      | 7 day water stress |       |      | 14 day water stress |       |       | 7 day recovery |       |      | 14 day recovery |       |       |
|                |                           | Kabuli                                | Desi | Mean | Kabuli             | Desi  | Mean | Kabuli              | Desi  | Mean  | Kabuli         | Desi  | Mean | Kabuli          | Desi  | Mean  |
| Well watered   | Water Foliar spray        | 5.86                                  | 6.02 | 5.94 | 6.39               | 6.39  | 6.39 | 6.49                | 6.20  | 6.35  | 5.33           | 6.61  | 5.97 | 6.17            | 6.51  | 6.34  |
|                | Water Drenching           | 5.86                                  | 6.02 | 5.94 | 6.16               | 6.38  | 6.27 | 5.43                | 6.20  | 5.81  | 4.67           | 6.74  | 5.71 | 7.79            | 9.44  | 8.62  |
|                | PBZ Foliar spray (60 ppm) | 5.86                                  | 6.02 | 5.94 | 9.07               | 6.60  | 7.83 | 6.69                | 9.00  | 7.85  | 8.06           | 9.00  | 8.53 | 8.69            | 8.07  | 8.38  |
|                | PBZ Drenching (120 ppm)   | 5.86                                  | 6.02 | 5.94 | 9.07               | 8.83  | 8.95 | 7.34                | 9.60  | 8.47  | 7.65           | 9.22  | 8.44 | 8.60            | 11.58 | 10.09 |
|                | Mean                      | 5.86                                  | 6.02 | 5.94 | 7.67               | 7.05  | 7.36 | 6.49                | 7.75  | 7.12  | 6.43           | 7.89  | 7.16 | 7.81            | 8.90  | 8.36  |
| Water stressed | Water Foliar spray        | 5.86                                  | 6.02 | 5.94 | 4.07               | 8.81  | 6.44 | 11.56               | 12.09 | 11.82 | 4.48           | 8.95  | 6.71 | 5.64            | 4.08  | 4.86  |
|                | Water Drenching           | 5.86                                  | 6.02 | 5.94 | 5.50               | 6.81  | 6.15 | 12.09               | 13.13 | 12.61 | 3.01           | 9.68  | 6.35 | 4.25            | 9.34  | 6.79  |
|                | PBZ Foliar spray (60 ppm) | 5.86                                  | 6.02 | 5.94 | 8.34               | 8.90  | 8.62 | 12.02               | 14.86 | 13.44 | 7.02           | 9.41  | 8.22 | 3.85            | 6.35  | 5.10  |
|                | PBZ Drenching (120 ppm)   | 5.86                                  | 6.02 | 5.94 | 9.62               | 10.07 | 9.85 | 13.22               | 14.65 | 13.93 | 6.10           | 10.66 | 8.38 | 4.28            | 6.90  | 5.59  |
|                | Mean                      | 5.86                                  | 6.02 | 5.94 | 6.88               | 8.65  | 7.77 | 12.22               | 13.68 | 12.95 | 5.15           | 9.67  | 7.41 | 4.51            | 6.67  | 5.59  |
|                | SEM                       | 0.236                                 |      |      | 0.027              |       |      | 0.125               |       |       | 0.32           |       |      | 0.145           |       |       |
|                | CD at 5 %                 |                                       |      |      |                    |       |      |                     |       |       |                |       |      |                 |       |       |
|                | Water status (W)          | N/A                                   |      |      | 0.028              |       |      | 0.128               |       |       | 0.326          |       |      | 0.148           |       |       |
|                | Treatment (T)             | N/A                                   |      |      | 0.039              |       |      | 0.181               |       |       | 0.461          |       |      | 0.209           |       |       |
|                | Varieties (V)             | 0.24                                  |      |      | 0.028              |       |      | 0.128               |       |       | 0.326          |       |      | 0.148           |       |       |
|                | W x T =                   | 0.481                                 |      |      | 0.055              |       |      | 0.255               |       |       | N/A            |       |      | 0.296           |       |       |
|                | W x V =                   | 0.34                                  |      |      | 0.039              |       |      | 0.181               |       |       | 0.461          |       |      | 0.209           |       |       |
|                | Tx V =                    | 0.481                                 |      |      | 0.055              |       |      | 0.255               |       |       | 0.652          |       |      | 0.296           |       |       |
|                | W x Tx V=                 | N/A                                   |      |      | 0.078              |       |      | 0.361               |       |       | 0.922          |       |      | 0.419           |       |       |

#### **5.3.1.9. Glutathione reductase (GR) activity**

Water deficit condition, enhanced the glutathione reductase activity in chickpea varieties. Foliar and drenching applications enhanced the GR activity under both well watered and water deficit conditions. The plants treated with the drenching application of PBZ had at the highest activity of GR in both varieties under well water and stress conditions (Fig. 5.3.1.9 Table 5.3.1.9.).

#### **5.3.1.10. Absciscic acid (ABA) content**

During imposed water deficit condition at reproductive stage in both *desi* and *kabuli* chickpea varieties ABA content was enhanced gradually as compared to well watered conditions. Further, after water deficit stressed termination, level of ABA was decreased gradually. Foliar and drenching applications of PBZ effectively enhanced the level of ABA in both varieties under water stress as well as well water. Plants treated with PBZ drenching were consisting the highest level of ABA content in both *desi* and *kabuli* varieties. In general, *desi* variety had higher content of ABA than *kabuli* one (Fig. 5.3.1.10.).

### **5.4. DISCUSSION**

Modern agriculture is influenced by environmental stresses such as water, drought, temperature, light and salt stress. Sustainable agriculture in harsh environments needs an understanding of the ways that plant genes respond to both biotic and abiotic factors. Water deficit is one of the abiotic stresses which adversely affect the crop physiology all around the world (Bohnert *et al.*, 1995). Water deficit is also a major yield-limiting factor in chickpea (*Cicer arietinum* L.) production as the large chickpea growing areas are in the arid and semi-arid zones and about 90% of world's chickpea is grown under rain fed conditions (Kumar and Abbo, 2001). Crop production in the rainfed areas is often very difficult and risky because of environmental stress. In chickpea crop, yield losses might be the result of intermittent drought either during the vegetative or reproductive phase or due to terminal drought at the end of the crop cycle (Serraj *et al.*, 2004). Water deficit decreases the rate of photosynthesis (Kawamitsu *et al.*, 2000). One of the main reasons why environmental stress inhibits growth and photosynthetic abilities of plants is the breakdown of the balance between the production of reactive oxygen

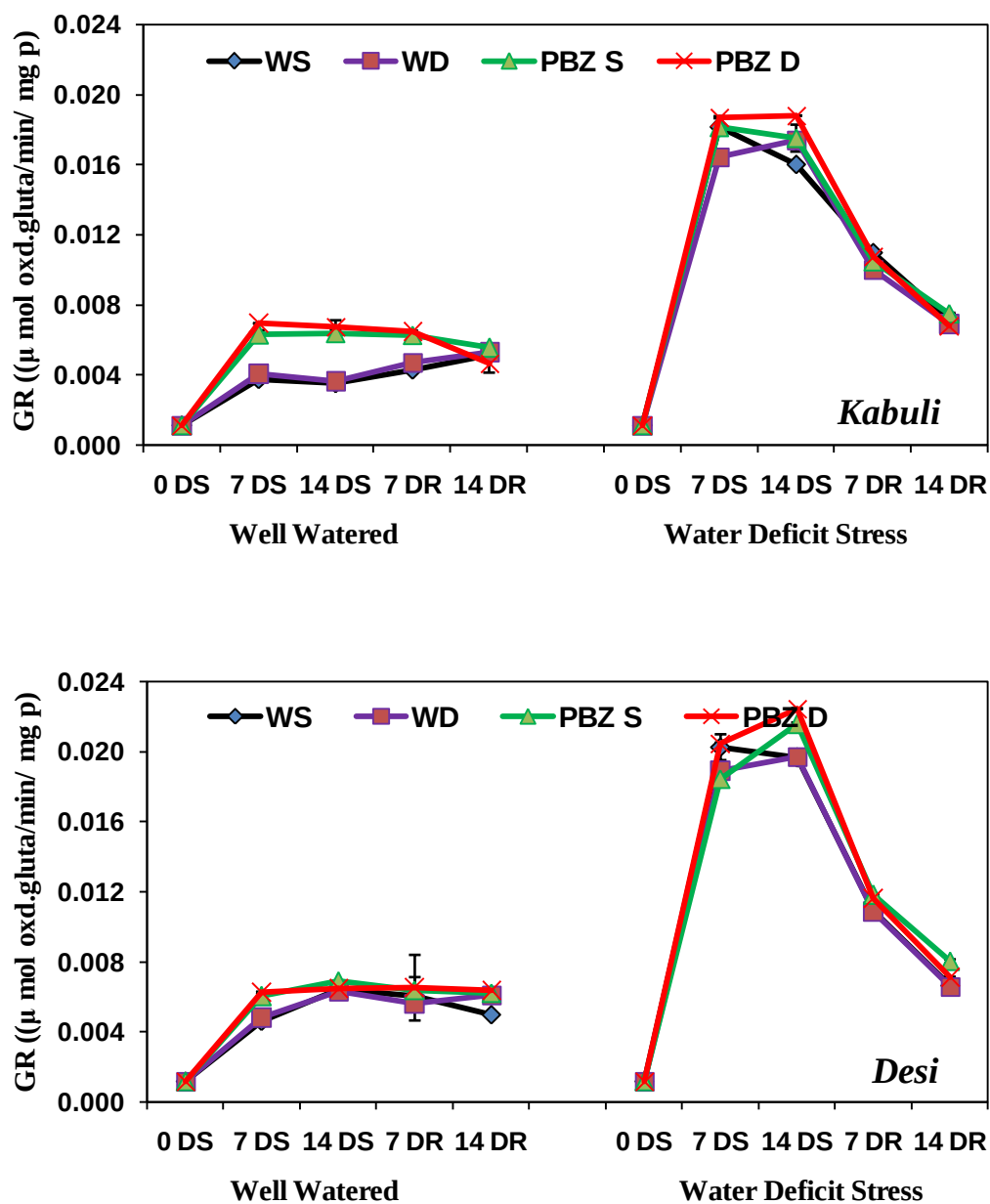


Fig. 5.3.1.9. Effect of PBZ on glutathione reductase (GR) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.

**Table 5.3.1.9. Effect of PBZ on glutathione reductase (GR) activity in chickpea genotypes under well watered and imposed water deficit stressed conditions.**

| Water status   | PBZ Treatments            | Glutathione reductase(μ mol oxd.gluta/min/ mg protein) |             |             |                    |             |             |                     |             |             |                |             |             |                 |             |             |
|----------------|---------------------------|--------------------------------------------------------|-------------|-------------|--------------------|-------------|-------------|---------------------|-------------|-------------|----------------|-------------|-------------|-----------------|-------------|-------------|
|                |                           | 0 day water stress                                     |             |             | 7 day water stress |             |             | 14 day water stress |             |             | 7 day recovery |             |             | 14 day recovery |             |             |
|                |                           | Kabuli                                                 | Desi        | Mean        | Kabuli             | Desi        | Mean        | Kabuli              | Desi        | Mean        | Kabuli         | Desi        | Mean        | Kabuli          | Desi        | Mean        |
| Well watered   | Water Foliar spray        | 0.00                                                   | 0.00        | <b>0.00</b> | 0.00               | 0.00        | <b>0.00</b> | 0.00                | 0.01        | <b>0.00</b> | 0.00           | 0.01        | <b>0.01</b> | 0.01            | 0.00        | <b>0.01</b> |
|                | Water Drenching           | 0.00                                                   | 0.00        | <b>0.00</b> | 0.00               | 0.00        | <b>0.00</b> | 0.00                | 0.01        | <b>0.00</b> | 0.00           | 0.01        | <b>0.01</b> | 0.01            | 0.01        | <b>0.01</b> |
|                | PBZ Foliar spray (60 ppm) | 0.00                                                   | 0.00        | <b>0.00</b> | 0.01               | 0.01        | <b>0.01</b> | 0.01                | 0.01        | <b>0.01</b> | 0.01           | 0.01        | <b>0.01</b> | 0.01            | 0.01        | <b>0.01</b> |
|                | PBZ Drenching (120 ppm)   | 0.00                                                   | 0.00        | <b>0.00</b> | 0.01               | 0.01        | <b>0.01</b> | 0.01                | 0.01        | <b>0.01</b> | 0.01           | 0.01        | <b>0.01</b> | 0.00            | 0.01        | <b>0.01</b> |
|                | Mean                      | <b>0.00</b>                                            | <b>0.00</b> | <b>0.00</b> | <b>0.01</b>        | <b>0.01</b> | <b>0.01</b> | <b>0.01</b>         | <b>0.01</b> | <b>0.01</b> | <b>0.01</b>    | <b>0.01</b> | <b>0.01</b> | <b>0.01</b>     | <b>0.01</b> | <b>0.01</b> |
| Water stressed | Water Foliar spray        | 0.00                                                   | 0.00        | <b>0.00</b> | 0.02               | 0.02        | <b>0.02</b> | 0.02                | 0.02        | <b>0.02</b> | 0.01           | 0.01        | <b>0.01</b> | 0.01            | 0.01        | <b>0.01</b> |
|                | Water Drenching           | 0.00                                                   | 0.00        | <b>0.00</b> | 0.02               | 0.02        | <b>0.02</b> | 0.02                | 0.02        | <b>0.02</b> | 0.01           | 0.01        | <b>0.01</b> | 0.01            | 0.01        | <b>0.01</b> |
|                | PBZ Foliar spray (60 ppm) | 0.00                                                   | 0.00        | <b>0.00</b> | 0.02               | 0.02        | <b>0.02</b> | 0.02                | 0.02        | <b>0.02</b> | 0.01           | 0.01        | <b>0.01</b> | 0.01            | 0.01        | <b>0.01</b> |
|                | PBZ Drenching (120 ppm)   | 0.00                                                   | 0.00        | <b>0.00</b> | 0.02               | 0.02        | <b>0.02</b> | 0.02                | 0.02        | <b>0.02</b> | 0.01           | 0.01        | <b>0.01</b> | 0.01            | 0.01        | <b>0.01</b> |
|                | Mean                      | <b>0.00</b>                                            | <b>0.00</b> | <b>0.00</b> | <b>0.02</b>        | <b>0.02</b> | <b>0.02</b> | <b>0.02</b>         | <b>0.02</b> | <b>0.02</b> | <b>0.01</b>    | <b>0.01</b> | <b>0.01</b> | <b>0.01</b>     | <b>0.01</b> | <b>0.01</b> |
|                | SEM                       | 0.001                                                  |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |
|                | CD at 5 %                 |                                                        |             |             |                    |             |             |                     |             |             |                |             |             |                 |             |             |
|                | Water status (W)          | N/A                                                    |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |
|                | Treatment (T)             | N/A                                                    |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |
|                | Varieties (V)             | N/A                                                    |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |
|                | W x T =                   | N/A                                                    |             |             | 0                  |             |             | 0                   |             |             | N/A            |             |             | 0               |             |             |
|                | W x V =                   | N/A                                                    |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |
|                | T x V =                   | N/A                                                    |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |
|                | W x Tx V=                 | 0.002                                                  |             |             | 0                  |             |             | 0                   |             |             | 0              |             |             | 0               |             |             |

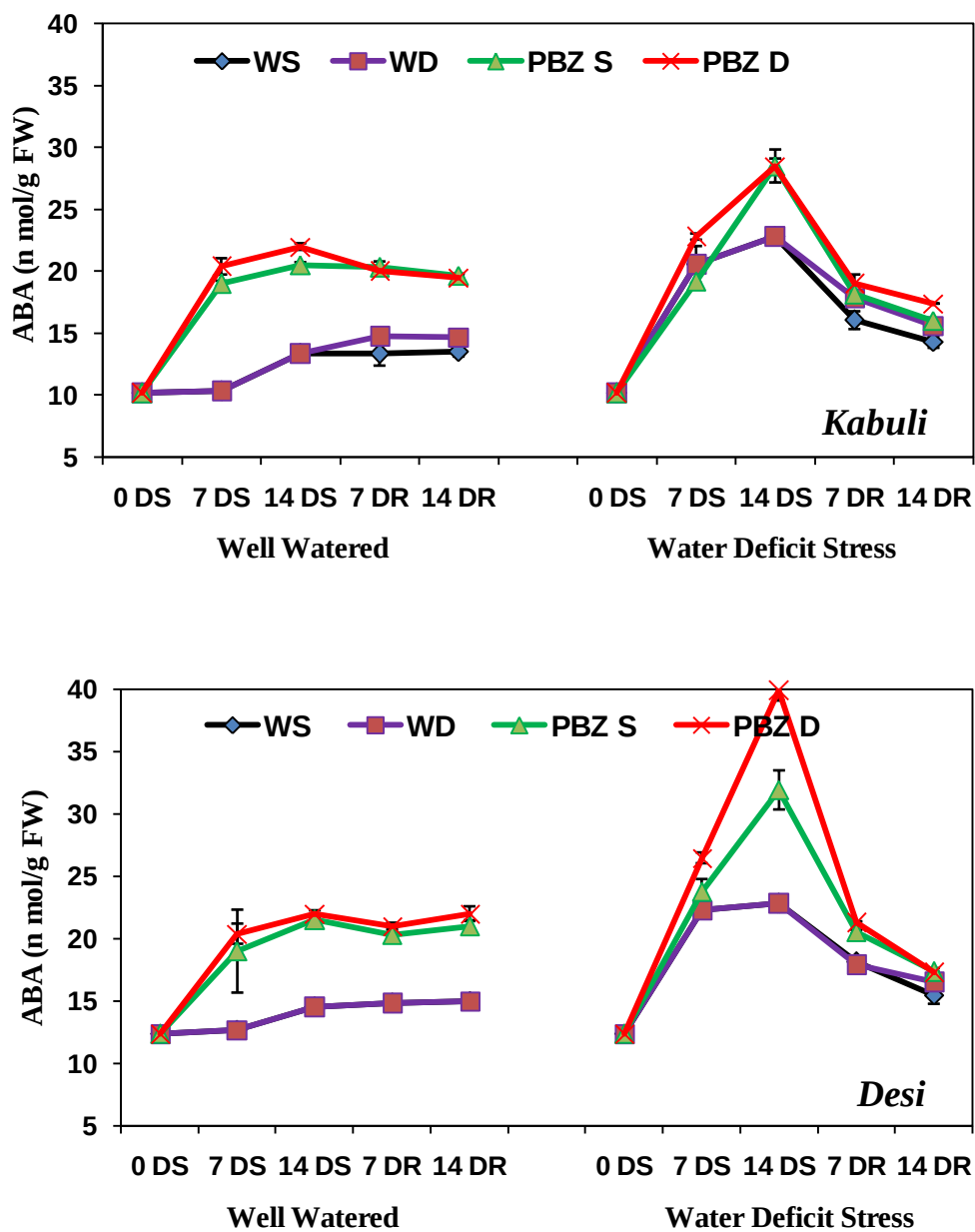


Fig. 5.3.1.10. Effect of PBZ on Absciscic acid (ABA) content in chickpea genotypes under well watered and imposed water deficit stressed conditions.

species (ROS) and the antioxidant defense (Iturbe- Ormaetxe *et al.*, 1998) These activated oxygens injure the cellular components of proteins, membrane lipids and nucleic acids (Foyer *et al.*, 1994). To cope with water stress, plants have developed a complex antioxidant system (Rio *et al.*, 2002). Water deficit also alters a variety of biochemical and physiological processes and accumulates solute (osmolytes) for protecting them under stress environment (Hu and Schmidhalter 1998).

In present scenario, application of plant growth regulators (PGR) for the protection of plants from abiotic stresses attracts more attention (Jaleel *et al.*, 2007). Attempts have been made in the past to overcome the adverse effects of water deficit by using paclobutrazol, which have potential to minimize the water stress. (Banon *et al.* 2001; Berova *et al.*, 2003 and Zhu 2004). PBZ has biochemical effects on plants, e.g. detoxification of active oxygen (Upadhyaya *et al.*, 1989 Kraus *et al.*, 1994) and induces levels of amino acids and proline (Mackay *et al.*, 1990, Rady and Gaballah, 2012), antioxidants (Senaratna *et al.*, 1988; Rady and Gaballah, 2012) and chlorophyll contents (Fletcher *et al.*, 1988).

Lipid peroxidation is principally associated with oxidative damage and hence is used as an indicator of stress. Significant enhancement in lipid peroxidation was found under water stress in both chickpea genotypes. However, *desi* chickpea had comparatively lower value of lipid peroxidation under water stress. In the present study, PBZ applications, in general, lowered the value of lipid peroxidation under water stress. Drought stress substantially increased the malonaldehyde content in green bean genotypes (Yaser *et al.*, 2010). It was reported that wheat genotypes with higher stress tolerance have less MDA and less lipid peroxidation, and those with higher lipid peroxidation are more susceptible (Karanlik, 2001). Decreased lipid peroxidation in PBZ treated plants is correlated with the proved ability of triazoles in maintaining the membrane integrity (Fletcher *et al.*, 2000; Hajhashemi and Ehsanpour, 2013; Pan *et al.*, 2013). The increased malonyldialdehyde (MDA) with drought stress in wheat seedlings, but uniconazole reduced MDA accumulation of stressed soybean (Zhang *et al.*, 2007). The reduction of lipid peroxidation in paclobutrazol-exposed Patumma plants was recorded due to an increase in the antioxidant potential system (Jungklang and Saengnil, 2012).

Proline, a compatible osmolytes was increased under water deficit in both *desi* and *kabuli* chickpea varieties. Proline as an osmoprotectant compound plays a major role in osmoregulation and osmotolerance (Jaleel *et al.*, 2007). Leaf proline concentration increased sharply during the stress period and a significant correlation between enhanced drought tolerance and proline accumulation in plants has been found (Ashraf and Foolad, 2006). Increased level of proline was found associated with less membrane damage of *Mangifera indica* leaves (Srivastava *et al.*, 2010), *Festuca arundinacea* and *Lolium perenne* (Shahrokhi *et al.*, 2011) and Horse chestnut (Percival *et al.*, 2008). Applications of paclobutrazol enhanced level of proline under water deficit. Similar observations were also made by Upadhyaya *et al.*, 1989 in cucumber, Jaleel *et al.*, 2008 in *Cajanus cajan* and Farooqi *et al.*, 2010 in *Vetiveria zizanioides*.

Total free amino acids increased gradually with increase in period of water stress in chickpea genotypes. Gorbani *et al.*, 1998 also reported increased free amino acid content in chickpea cultivars under water deficit condition. PBZ application increased the level of free amino acids probably by increasing level of ABA and cytokinins. Total amino acid content increased as the stress condition prolonged and it increased significantly due to ABA treatment in chickpea (Kumar *et al.*, 2001). Application of BAP stimulated accumulation of proline, amino acids and soluble sugars under water stress that provides osmotic adjustment (Yadav *et al.*, 1997). Yadav *et al.*, 1996 and Gorbani *et al.*, 1998 also reported increased free amino acid content in chickpea cultivars under water deficit condition. PBZ application was also reported earlier to increase amino acid content in carrot (Gopi *et al.*, 2007).

Water deficit treatment increased the level of total soluble sugars and the applications of the PBZ enhanced the level of total soluble sugars. Higher enhancement of soluble sugars was recorded with the PBZ drenching and *desi* variety accumulated higher level of soluble sugars than *kabuli*. During the course of drought stresses active solute accumulation of compatible solutes such as carbohydrates is claimed to be an effective stress tolerance mechanism (McKersie and Leshem, 1994). The tolerant variety accumulated more soluble carbohydrate than the sensitive one (Mafakheri *et al.*, 2011). ). PBZ enhanced the levels of total soluble sugars, starch and proteins silique wall and seed

of brasicca (Setia *et al.*, 1996). PBZ treatments significantly enhanced the accumulation of dry matter in stem when compared to control in *Camelina sativa* (Kumar *et al.*, 2012).

Treatment of imposed water deficit reduced the level of total soluble proteins in both the chickpea varieties and the applications of the PBZ enhanced the level of total soluble proteins under well watered and water deficit conditions. The highest level of total soluble proteins was maintained by application of PBZ drenching. The accumulation of soluble proteins in leaves of chickpea genotypes under moisture stress condition helps in maintaining turgor (Chopra *et al.*, 1994). One of the mechanisms by which PBZ prevented leaf senescence is through the promotion of protein synthesis as it induces the synthesis of cytokinins (Chernyadev', 2005 and Davies, 2010). PBZ treatment has also been reported to enhance protein content of the leaves and tubers in carrot (Gopi *et al.*, 2007). Further, increased protein content under triazole treatment can be correlated to the increased production of cytokinins by triazoles (Davis *et al.*, 1988).

Water deficit stimulated the oxidative stress and augmented the activity of various antioxidant enzymes (catalase, peroxidase, glutathion reductase and superoxide dismutase) have also been reported earlier in rice (Sharma and Dubey, 2005 and Mafhakeri *et al.*, 2011). PBZ application has been reported to minimize water stress by enhancing antioxidant enzymes activity in oak (Percival, 2007), *Arachis hypogea* (Sankar *et al.*, 2007), *Daucus carota* (Gopi *et al.*, 2007), *Sesamum indicum* (Somasundaram *et al.*, 2009), horse chestnut (Percival, 2008), *Festuca arundinacea* and *Lolium perenne* (Shahrokhi *et al.*, 2011), tomato (Mohamed *et al.*, 2011).

Imposed water deficit sharply increased the ABA content in both the chickpea genotypes. Further, PBZ applications also enhanced the level of ABA. Under water stress, marked increase in content of endogeneous ABA has been repeatedly described (Dodd *et al.*, 2006; Verslues *et al.*, 2006 and Kudoyarova *et al.*, 2007). Absciscic acid is defined as a stress phytohormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that help plant survival over the stresses (Zhang *et al.*, 2006). The accumulation of ABA due to water stress has been reported in plants such as rice (Yang *et al.*, 2001), cucumber (Pustovoitova *et al.*, 2004) and bean (Domash *et al.*, 2006). Absciscic acid plays a critical role in regulating corn

water status through guard cells and growth (Luan, 2002; Zhu, 2002). ABA is produced under stress conditions in dehydrated roots in drying soil is transported to the xylem and regulates stomatal opening and leaf growth in the shoots (Zhang *et al.*, 2006). It has also been shown that paclobutrazol stimulated accumulation of ABA in the leaves (Asare-Boamah *et al.*, 1986). Triazole compounds including paclobutrazol (PBZ), were considered a gibberellin biosynthesis inhibitor. It blocks three separate steps in the biosynthetic pathway for the production of gibberellins (Zeevaart *et al.*, 1993). Thus, by interfering with GA production, PBZ affects the synthesis of ABA.

## **5.6. CONCLUSION**

In both *desi* (Pusa 362) and *kabuli* (Pusa 1108) chickpea varieties, PBZ applications induced the water stress tolerance by enhancing the level of osmolytes, antioxidant enzymes activity and thereby reduced lipid peroxidation. PBZ applications induced the level of ABA under water stress and because of its rapid accumulation it mediated many biochemical responses that helped plant survival under water deficit stress in chickpea. In general, both chickpea varieties in term of biochemical alterations responded better to PBZ drenching than PBZ foliar application.

## 6. DISCUSSION

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Globally, water deficit is a major limiting factor which reduces the productivity of crops (Bohnert *et al.*, 1995; Khamssi *et al.*, 2011). Water deficit is also a major constraint in chickpea production since most of the chickpea grown areas are in arid and semiarid regions and more than 90% of chickpea of the world is cultivated under marginal and rainfed conditions. Water deficit reduces the chickpea yield by 40-50% (Ahmad *et al.*, 2005) by altering a variety of physiological and metabolic processes in plants (Gunes *et al.*, 2006). Yield losses in chickpea takes place due to the intermittent water deficit during the vegetative or reproductive phase or due to terminal water deficit at the end of the crop cycle (Serraj *et al.*, 2004). One of the main reasons why environmental stress inhibits growth, photosynthetic abilities and yield of plants is the breakdown of the balance between the production of reactive oxygen species (ROS) and the antioxidant defense (Iturbe- Ormaetxe *et al.*, 1998) These activated oxygens injure the cellular components like proteins, membrane lipids and nucleic acids (Foyer *et al.*, 1994). To cope with water stress, plants have developed a complex antioxidant system (Rio *et al.*, 2002). Water deficit also alters a variety of biochemical and physiological processes and accumulates solute (osmolytes) for protecting them under stress environment (Hu and Schmidhalter 1998).

Yield improvement in chickpea, through conventional breeding is difficult because of narrow genetic base (Udupa *et al.*, 1999). Furthermore, Conventional plant breeding attempts are time consuming and rely on present genetic variability (Zhu, 2002). In the current scenario, an alternative approach which attracts more attention is application of bioregulators for the protection of the plant from the environmental stresses (Jaleel *et al.*, 2007). Paclobutrazol, a triazole, is one of the strong candidates for stress amelioration, which induces numerous morphological, physiological and biochemical responses in plants, including inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses (Jaleel *et al.*, 2007). PBZ is considered as a gibberellin biosynthesis inhibitor. Its plant growth regulating properties are mediated by its ability to change the balance of important phytohormones including gibberellic acid, ABA and cytokinin

(Kamounsis and Chronopoulson- Sereli, 1999). In the present investigation, an attempt was made to analyze the mitigating effect of PBZ applications (foliar and drenching) on physiological, biochemical and yield traits in two diverse *desi* (Pusa362) and *kabuli* (Pusa1108) chickpea varieties under imposed water deficit stress. Based on physiological responses (RWC, MSI, photosynthesis rate, chl<sub>a</sub>, chl<sub>b</sub>, total chlorophylls and total carotenoids) of both chickpea varieties to varying doses of PBZ under water deficit, optimum dose of PBZ for foliar and drenching applications were selected 60 ppm and 120 ppm respectively.

Water deficit stress affects several physiological and metabolic processes in plants (Gunes *et al.*, 2006). In present study, RWC was decreased under water deficit condition and PBZ applications improved leaf RWC in both varieties. RWC is an important indicator of water stress in leaves (Merah, 2001). Reduction in RWC of leaves was observed in plants exposed to water deficit conditions. Plants exposed to stress immediately reduce RWC of their leaves (Grover *et al.*, 2004). Under water deficit, paclobutrazol by accelerating stomatal response and reducing transpiration rate, enhanced water retention and increased survival in jack pine (Mashall *et al.*, 1990), oak (Percival *et al.*, 2007), wheat (Sairam *et al.*, 1995; Aly *et al.*, 2011) and barley (Rady *et al.*, 2012). Paclobutrazol treated plants showed higher value of relative water content in *Curcuma alismatifolia* Gagnep (Jungklang *et al.*, 2012), triticale (Berova and Zlatev., 2003) and cucumber seedling (Baninasab *et al.*, 2011).

Under water deficit, MSI was reduced in both chickpea varieties and its reduction was reported higher in *kabuli* variety than *desi* one because *kabuli* type chickpea is more sensitive to water stress than *desi* variety. Cell membrane stability has been used as a drought tolerance test (Agarie *et al.*, 1995) and electrolyte leakage showed an increase with increasing water deficit (Gopi *et al.*, 2007). The inhibition of electrolyte leakage indicates ability of PBZ in maintaining the membrane integrity (Fletcher *et al.*, 2000) of plants. PBZ being a triazole altered the sterol biosynthesis and changed the composition of sterol in the plasma membrane (Burden *et al.*, 1987). PBZ has been reported to reduce electrolyte leakage in wheat (Kraus and Fletcher, 1994; Aly *et al.*, 2011) and Horse chestnut (Percival *et al.*, 2008) during drought stress. In addition, upregulation of stress protective

biomolecules, in PBZ treated plants have also enhanced the capacity to limit the damage caused by species of reactive oxygen.

Water deficit influences plant growth at various levels (Blumwald *et al.*, 2004). The quantity and quality of plant growth depend on cell-division enlargement, and differentiation, and all of these events are affected by water deficit (Correia *et al.*, 2001). Under imposed water deficit stress condition, in general, all plant growth parameters (shoot length, root length, leaf number, leaf area, root dry weight, leaf dry weight, shoot dry weight, specific leaf weight, specific leaf area) of both *desi* and *kabuli* genotypes showed a reduction. Drought stress decreases the rate of photosynthesis (Kawamitsu *et al.*, 2000). Plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO<sub>2</sub> fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants (Mafakheri *et al.*, 2010). Water deficit stress also affects total leaf area through its effect on initiation of new leaves, which is decreased under drought stress. Alves and Setter (2004) showed that reduction in both cell expansion and production of cells contributed to a loss in leaf area depending on the developmental stage at which the leaf was stressed. Continued drought stress can accelerate leaf senescence (de Souza *et al.*, 1997) and lead to death of leaf tissue, resulting in leaf drop, particularly old and mature leaves. PBZ applications in general enhanced all growth parameters in both chickpea genotypes under both well watered and water deficit conditions except shoot length under well watered condition in *Kabuli* variety. However, PBZ influenced under water stress was more effective than well watered condition. In addition to this, PBZ drenching had better efficacy than its foliar application. PBZ is transportable and can move only through xylem (Buta and Spaulding, 1991) therefore its efficacy primarily depend upon its uptake via root. Moreover, foliar PBZ cannot be transported out of the leaves to other parts that is why drenching application was found better over foliar application. Paclobutrazol has been reported to reduce plant growth, height, internodal distance with thicker and darker leaves in pepper and tomato (Rahman *et al.*, 1989), oilseed mustard (Setia *et al.*, 1996) and chinese potato (Kishorekumar, 2006). Reduced height is a consequence of triazole induced GA inhibition exemplified by reduced internodal elongation (Fletcher, 2000). However, PBZ application has been reported to improve the growth potential of *Catharanthus roseus* plants grown under stress conditions

(Jaleel *et al.*, 2007). In present study too, PBZ treated plants had better growth performance under water deficit. Probably, PBZ identified selected doses used during the investigation for foliar and drenching applications were not sufficient to retard the growth parameters. In addition, we also observed during our preliminary experiment that higher doses of PBZ were able to reduce the plant height drastically.

Under imposed water deficit condition, destruction of thylakoid, disappearance of starch inclusions, changes in the lamellar structure, number of grana with loose type of thylakoid, large increase in osmiophilic granules and reduction of amount of starch in the bundle sheath chloroplast have also been reported earlier (Giles *et al.*, 1976, Vapaavuori *et al.*, 1984 and Utrillas and Alegre, 1997). However, water deficit with foliar and drenching applications of PBZ maintained the integrity of the chloroplast structure and its grana were recorded with compact type of thylakoid. Moreover, PBZ treated plants had large and higher number of starch grains and low number of osmiophilic granules in chloroplast. PBZ applications maintained chloroplast structure integrity under water deficit probably by enhancing the activity of antioxidants and restricting lipid peroxidation (Research Paper II). Besides this, PBZ also maintained integrity of chloroplast structure under water deficit by increasing the level of cytokinins (Fletcher *et al.*, 2000; Zhu *et al.*, 2004). Application of BA has been reported to improve Chloroplast integrity under water stress (Haisel *et al.*, 2006).

During the present study, rate of photosynthesis, transpiration and stomatal conductance were gradually decreased with increase in the period of water stress and on rehydration, gradually showed their recovery. PBZ applications, maintained the higher rate of photosynthesis during water deficit and after water deficit stress termination and thus, showed the fast recovery. Photosynthesis is very sensitive physiological process to water deficit. Even a small decrease in the relative water content of a plant causes its stomata to close, and decreases the intensity of photosynthetic assimilation of CO<sub>2</sub>. Water deficit reduces the rate of photosynthesis (Kawamitso *et al.*, 2000; Chaver and Oliveira, 2004; Lawlor and Cornic, 2002; Rahbarian *et al.*, 2011). During water stress most plants avoid shoot desiccation by closing their stomata to decrease transpiration and decreased stomatal conductance is accompanied by a reduction in internal CO<sub>2</sub> concentration and decreased diffusion of CO<sub>2</sub> via Mesophyll cell walls, membranes, cytoplasm and chloroplast envelope

leading to decreased chloroplast CO<sub>2</sub> concentration which decreases CO<sub>2</sub> fixation rate. Further, inhibition of ribulose-1, 5-bisphosphate carboxylase/oxygenase enzyme activity and ATP synthesis also lead to a decrease of net photosynthetic rate under water stress (Dulai *et al.*, 2006). However, many other processes such as contents of photosynthetic pigments, photochemical efficiency of photo systems and ultrastructure of chloroplast can also be affected by severe water deficit stress (Athar and Ashraf, 2005; Dubey, 2005). Diffusive resistance of the stomata to CO<sub>2</sub> entry probably is the main factor limiting photosynthesis under drought (Boyer, 1970; Chaves, 1991; Cornic, 2000; Flexas *et al.*, 2004). Drought stress inhibits photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe *et al.*, 1998). Reduction in photosynthesis as a result of water stress can be attributed to both stomatal and non- stomatal limitations (Graan and Boyer, 1990, Shangguan *et al.*, 1999).

Paclobutrazol has been reported to enhance net photosynthesis rate in triticale (Berova and Zlatev, 2003), mustard (Zhou *et al.*, 1993), *Setaria italic* (Bisht *et al.*, 2007) horse chestnut (Percival *et al.*, 2008) and *Withania somnifera* (Jaleel *et al.*, 2008). The higher chlorophyll content in the leaves, leading to higher photosynthesis in the triazole treated plants (Feng *et al.*, 2003). There is report that PBZ also increased activity of RUBP-carboxylase and thus rate of photosynthesis in peanut (Yan and Pan, 1992). Further, Paclobutrazol was reported to enhance zeatin and zeatin riboside (Zhu *et al.*, 2004) and it is well known that cytokinins protect photosynthetic machinery. PBZ application improved water use efficiency by reducing transpiration rate. PBZ affected transpiration rate by increasing the level of ABA (Research paer II). Our results are in agreement of earlier reports (Asare-Boamah *et al.*, 1986).

Under water deficit, quantum yield (Fv/Fm) was decreased and PBZ applications improved this ratio. Photoinhibition is represented by decreasing Fv/Fm ratio, effective quantum yield of photosystem II photochemistry and electron transport rate (Piper *et al.*, 2007). PBZ application reduced photoinhibition by protecting PS II (Baninasab *et al.*, 2009). Similarly, in Horse chestnut, chlorophyll fluorescence (Fv/Fm), photosynthetic rate were higher in trees treated with paclobutrazol (Percival *et al.*, 2008).

The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). A reduction in photosynthetic pigments content was reported in water stressed cotton (Massacci *et al.*, 2008), Horse chestnut and *Catharanthus roseus* (Jaleel *et al.*, 2008) sunflower (Kiani *et al.*, 2008) and *Vaccinium myrtillus* (Tahkokorpi *et al.*, 2007). However, in present study, photosynthetic pigments (Chlorophylls and carotenoids) content showed variable response which is likely due to the increase in pigment density or reduction in tissue volume under water deficit because their levels were calculated on fresh weight basis. PBZ applications improved level of photosynthetic pigments under both well watered and water stressed condition probably by increasing level of cytokinins and antioxidant activity. PBZ application has been reported to increase level of pigments in oak (Percival *et al.*, 2007), *Setaria italica* (Bisht *et al.*, 2007), *Amorphophallus campanulatus* (Gopi *et al.*, 2009) and cucumber seedling (Baninasab *et al.*, 2011), *Triticum aestivum* (Aly *et al.*, 2011; Nouriyani *et al.*, 2012) and *Stevia rebaudiana* (Hajihashemi and Ehsanpour, 2013). In general, under the imposed water stress condition, density of all the pigments in profile reduced in comparison to the well watered one. Furthermore, the plants treated with PBZ treatments showed more dense photosynthetic pigments particularly carotenoids belonging to xanthophyll cycle under normal condition and also maintained the photosynthetic pigment profile under water stress condition which in turn indicated that the PBZ applications stimulated the xanthophyll cycle and thus contributed in the protection of the photosynthetic machinery. Paclobutrazol induced a suit of physiological adaptation to tolerate drought more especially, increased total leaf content of carotenoids (Lutein, beta-carotein, alpha carotein, neoxanthin) and xanthophylls (Antheraxanthin, zeaxanthin, violaxanthin) in Evergreen oak (Percival *et al.*, 2007).

Further, imposed water deficit exhibited the reduction in plant height, root length, number of main branches, pods per plant, shoot dry weight, leaf dry weight, root dry weight, pod weight, seed yield and test weight at harvest. PBZ applications in general, enhanced all the growth parameters and also maintained the yield and its attributes even under imposed water deficit stress condition. The present study, confirmed earlier field reports in chickpea that water deficit reduced grain and biological yield (Behboudian *et al.*, 2001; Bahavar *et al.*, 2009). Finding of present investigation, indicated that PBZ enhanced

grain yield by enhancing photosynthetic gas exchange, higher chlorophyll content, photosynthetic activity for a longer period and as a result facilitated higher photo assimilation. Similarly, PBZ has also been reported to enhance yield in mustard (Setia *et al.*, 1996), *Brassica napus* (Zhou *et al.*, 1993), barley (Rady *et al.*, 2012) tomato (Mohamed *et al.*, 2011) and *Camelina sativa* (Kumar *et al.*, 2012). Its role as anti-gibberellin is considered sufficient for the induction of higher yield and for improvement of the quality in cucurbits (Hedden and Graebe, 1985), wheat (Reynolds and Wardle, 1990) and rice (Pan *et al.*, 2013). Furthermore, PBZ drenching had better yield efficacy than its foliar application because PBZ is xylem mobile (Buta and Spaulding, 1991) therefore its efficacy primarily depends upon its uptake via root. In addition, PBZ entered by the means of foliar application cannot be transported out of the leaves to other parts. That is why drenching application was found better over foliar application.

Water deficit reduced dry matter allocation to economic sink. PBZ applications improved Harvest index and dry matter partitioning to economic sink probably by enhancing the level of cytokinins. PBZ was also reported earlier to overall increase in dry matter per plant and partitioning coefficient in *B. juncea* and *B. carinata* (Setia *et al.*, 1995) altered sink strength within the plant resulting in redistribution of assimilates to meristematic regions, other than the shoot apices, and also improved the flow of assimilates towards reproductive structures (Setia *et al.*, 1996). Kumar *et al.*, 2012 linked PBZ enhanced yield in *Camelina sativa* with better partitioning coefficient.

PBZ applications significantly reduced the drought susceptibility index and PBZ drenching application showed the lowest values of the drought susceptibility index. Further, highest drought tolerance efficiency was recorded with application of drenching than foliar application. These findings suggested that PBZ applications enhanced the yield by improving the tolerance of chickpea varieties and PBZ drenching is better than its foliar application.

Lipid peroxidation is fundamentally associated with oxidative damage and hence is used as an indicator of stress. Significant enhancement in lipid peroxidation was found under water stress in both chickpea genotypes. However, *desi* chickpea had comparatively lower value of lipid peroxidation under water stress. In the present study, PBZ applications, in

general reduced the value of lipid peroxidation under water stress. Drought stress substantially increased the malonaldehyde content in green bean genotypes (Yaser *et al.*, 2010). It was reported that wheat genotypes with higher stress tolerance have less MDA and less lipid peroxidation, and those with higher lipid peroxidation are more susceptible (Karanlik, 2001). Decreased lipid peroxidation in PBZ treated plants is correlated with the proved ability of triazoles in maintaining the membrane integrity (Fletcher *et al.*, 2000; Hajhashemi and Ehsanpour, 2013; Pan *et al.*, 2013). The increased malonyldialdehyde (MDA) with drought stress in wheat seedlings, but uniconazole reduced MDA accumulation of stressed soybean (Zhang *et al.*, 2007). The reduction of lipid peroxidation in paclobutrazol-exposed Patumma plants was caused by paclobutrazol that increases the antioxidant potential system (Jungklang *et al.*, 2003).

Proline, a compatible osmolytes was increased under water deficit in both *desi* and *kabuli* chickpea varieties. Proline as an osmoprotectant compound plays a major role in osmoregulation and osmotolerance (Jaleel *et al.*, 2007). Leaf proline concentration increased sharply during the stress period and a significant correlation between enhanced drought tolerance and proline accumulation in plants has been found (Ashraf and Foolad, 2006). Increased level of proline was found associated with less membrane damage of *Mangifera indica* leaves (Srivastava *et al.*, 2010), *Festuca arundinacea* and *Lolium perenne* (Shahrokhi *et al.*, 2011) and Horse chestnut (Percival *et al.*, 2008). Applications of paclobutrazole enhanced level of proline under water deficit. Similar observations were also made earlier in cucumber (Upadhyaya *et al.*, 1989), *Cajanus cajan* (Jaleel *et al.*, 2008) and *Vetiveria zizanioides* (Farooqi *et al.*, 2010).

Total free amino acids increased gradually with increase in period of water stress in chickpea genotypes. Gorbani *et al.*, 1998 also reported increased free amino acid content in chickpea cultivars under water deficit condition. PBZ application increased the level of free amino acids probably by increasing level of ABA and cytokinins. Total amino acid content increased as the stress condition prolonged and it increased significantly due to ABA treatment in chickpea (Kumar *et al.*, 2001). Application of BAP stimulated accumulation of proline, amino acids and soluble sugars under water stress that provides osmotic adjustment (Yadav *et al.*, 1997). Yadav *et al.*, 1996 and Gorbani *et al.*, 1998 also reported increased free

amino acid content in chickpea cultivars under water deficit condition. PBZ application was also reported to increase amino acid content in carrot (Gopi *et al.*, 2007).

Water deficit treatment increased the level of total soluble sugars and the applications of the PBZ enhanced the level of total soluble sugars. Higher enhancement of soluble sugars was recorded with the PBZ drenching and *desi* variety accumulated higher level of soluble sugars than *kabuli*. During the course of drought stresses active solute accumulation of compatible solutes such as carbohydrates is claimed to be an effective stress tolerance mechanism (McKersie and Leshem, 1994). The tolerant variety accumulated more soluble carbohydrate than the sensitive one (Mafakheri *et al.*, 2011). ). PBZ enhanced the levels of total soluble sugars, starch and proteins silique wall and seed of brasicca (Setia *et al.*, 1996). PBZ treatments significantly enhanced the accumulation of dry matter in stem when compared to control in *Camelina sativa* (Kumar *et al.*, 2012).

Treatment of imposed water deficit had lower content of total soluble proteins in both the chickpea varieties and the applications of the PBZ enhanced the level of total soluble proteins under well watered and water deficit conditions. The highest level of total soluble proteins was maintained by application of PBZ drenching. The accumulation of soluble protein in leaves of chickpea genotypes under moisture stress condition helps in maintaining turgor (Chopra *et al.*, 1995). One of the mechanisms by which PBZ prevented leaf senescence is through the promotion of protein synthesis as it induces the synthesis of cytokinins (Chernyadev, 2005 and Davies, 2010). PBZ treatment has also been reported to enhance protein content of the leaves and tubers in carrot (Gopi *et al.*, 2007). Further, increased protein content under triazole treatment can be correlated with increased production of cytokinins by triazoles (Davis *et al.*, 1988).

Water deficit stimulated the oxidative stress and augmented the activity of various antioxidant enzymes (catalase, peroxidase, glutathion reductase and superoxide dismutase) have also been reported earlier in rice (Sharma and Dubey, 2005 and Mafhakeri *et al.*, 2011). PBZ application has been reported to minimize water stress by enhancing antioxidant enzymes activity in oak (Percival, 2007), *Arachis hypogea* (Sankar *et al.*, 2007), *Daucus carota* (Gopi *et al.*, 2007), *Sesamum indicum* (Somasundaram *et al.*, 2009), horse chestnut (Percival, 2008), *Festuca arundinacea* and *Lolium perenne* (Maha shahrokhi *et al.*, 2011) and tomato (Mohamed *et al.*, 2011).

Imposed water deficit sharply increased the ABA content in both the chickpea genotypes. Further, PBZ applications also enhanced the level of ABA. Under water stress, marked increase in content of endogenous ABA has been repeatedly described earlier (Dodd *et al.*, 2006; Verslues and Bray, 2006 and Kudoyarova *et al.*, 2007). Absciscic acid is defined as a stress phytohormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that help plant survival over the stresses (Zhang *et al.*, 2006). The accumulation of ABA due to water stress has been reported in plants such as rice (Yang *et al.*, 2001), cucumber (Pustovoitova *et al.*, 2004) and bean (Domash *et al.*, 2006). Absciscic acid plays a critical role in regulating corn water status through guard cells and growth (Luan, 2002; Zhu, 2002). ABA is produced under stress conditions in dehydrated roots in drying soil is transported to the xylem and regulates stomatal opening and leaf growth in the shoots (Zhang *et al.*, 2006). It has also been found that paclobutrazol increased accumulation of ABA in the leaves (Asare-Boamah *et al.*, 1986). PBZ is considered a gibberellin biosynthesis inhibitor. It blocks three separate steps in the biosynthetic pathway for the production of gibberellins (Zeevaart *et al.*, 1993) as a result, by interfering with GA synthesis; PBZ enhances the synthesis of ABA.

## 6.1. CONCLUSION

Optimum dose for foliar and drenching applications were identified 60 and 120 ppm respectively. Based on physiological responses *Kabuli* variety (Pusa 1108) was found more sensitive to water deficit stress than *desi* one (Pusa 362). PBZ drenching application was found better over its foliar application. Under water deficit, PBZ improved photosynthetic activity, photosynthetic pigments & water relation, reduced membrane damage and protected the photosynthetic machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby induced water deficit stress tolerance and enhanced the yield.

## 7. SUMMARY

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The present investigation entitled “**Role of Paclobutrazol in Amelioration of Water Deficit Stress in Chickpea (*Cicer arietinum* L.)**” was conducted to identify the optimum dose of paclobutrazol for foliar and drenching applications under water deficit stress condition and to analyze physiological and biochemical alterations for understanding the Paclobutrazol (PBZ) induced mechanism of water deficit stress tolerance. For the present study two contrasting chickpea genotypes in terms of water deficit stress tolerance were selected viz. *desi* (Pusa362) and *kabuli* (Pusa1108). Plants were grown in pot culture, Division of Plant physiology, IARI, New Delhi from November 2013 to April 2014. Plants were subjected to water deficit stress by restricting water supply until severe symptoms of wilting were visible. Before imposition of water deficit, plants were pretreated with applications of PBZ.

Summary of the results obtained in the present investigation are given below:

Initially, a preliminary experiment was conducted with varying dose of PBZ i.e. 0, 30, 60, 90, 120, and 150 ppm for foliar application and 0, 60, 120, 180, 240 and 300 ppm for drenching application to identify the optimum dose of PBZ available for both drenching and foliar applications. Based on RWC, MSI, Photosynthetic rate, and Photosynthetic pigments response of treated plants, optimum doses of PBZ for foliar application 60 ppm and for drenching application 120 ppm were identified and used for main experiment.

Main experiment was consisted of two main treatments (i.e. well watered and water deficit condition at reproductive stage) and four sub-treatments (PBZ foliar spray (60 ppm), PBZ drenching (120 ppm), water spray and water drenching). The results of the main experiment in pot culture showed decrease in RWC and MSI under water deficit stress conditions. Both foliar and drenching applications of PBZ maintained higher RWC and MSI values over their respective controls. Furthermore, drenching application of PBZ maintained higher values of RWC and MSI during water deficit and after water stress termination during recovery.

In general, growth parameters like shoot length, root length, leaf number, leaf area, shoot dry weight, root dry weight, leaf dry weight were reduced under water deficit stress

conditions. However, root length was increased under water stress. PBZ applications maintained higher values of growth parameters during water deficit stress conditions and provoked fast recovery after water deficit stress termination.

Chloroplast ultrastructure under water deficit condition showed various alterations in terms of swelling and destruction of thylakoid, appearance of lipid droplets, disappearance of starch inclusions, changes in the lamellar structure, number of grana with loose type of thylakoid, large increase in osmophilic granules in the chloroplast in both varieties. However, water deficit stress with foliar and drenching applications of PBZ maintained integrity of the chloroplast structure and grana were recorded with compact type of thylakoid in both varieties.

Foliar and drenching application of PBZ maintained higher rates of photosynthesis, Fv/Fm, water use efficiency under water deficit condition and also showed the fast recovery after water stress termination. Under water deficit condition, both foliar and drenching applications of PBZ significantly reduced the rate of transpiration and stomatal conductance and improved leaf water use efficiency in both *desi* and *kabuli* varieties.

Plants treated with foliar and drenching applications of PBZ in both *kabuli* and *desi* varieties had higher Chla, chl<sub>b</sub>, total chlorophyll and total carotenoids contents under both well watered and water deficit stress conditions. Moreover, PBZ treated plants showed denser photosynthetic pigment band under imposed water deficit stress condition in both *desi* and *kabuli* varieties. Density of the carotenoids bands in PBZ treated plant was observed much sharper than that of untreated one. The photosynthetic pigments profile of PBZ treated plants under imposed water stress condition exhibited the distinct differences in terms of appearance of all the carotenoids particularly belonging to zeaxanthin cycle than their respective controls.

Yield and its attributes were reduced under imposed water stress conditions and applications of PBZ significantly enhanced the yield in both the chickpea varieties. PBZ applications reduced the drought susceptibility index and enhanced the drought tolerance efficiency. Highest drought tolerance efficiency was recorded with application of PBZ drenching application. In general, under water stress condition, dry matter partitioning in pod significantly reduced in *desi* and *kabuli* chickpea. PBZ applications enhanced the dry matter partitioning to pods particularly under well water deficit condition.

Biochemical analysis revealed that lipid peroxidation and proline were increased with increase in water deficit duration and PBZ applications prevented the lipid peroxidation and increased the proline content in both chickpea varieties. Levels of the total amino acids and total soluble sugars were enhanced under water deficit condition. Furthermore, PBZ applications enhanced their levels under water stress and thus provided osmotic protection. The antioxidant enzyme system was stimulated under induced water stress in both the chickpea varieties. The activities of major antioxidant enzymes (catalase, peroxidase, glutathione reductase and superoxide dismutase) were stimulated by the applications of PBZ.

During imposed water deficit condition ABA content was enhanced gradually and after water stressed termination during recovery ABA was decreased gradually. Foliar and drenching applications of PBZ effectively enhanced the level of ABA in both varieties under water stress as well as well water. Plants treated with PBZ drenching had the highest level of ABA content.

From the present study it can be concluded that optimum dose for foliar and drenching applications were identified 60 and 120 ppm respectively. *Kabuli* variety (Pusa 1108) was found more sensitive to water deficit stress than *desi* one (Pusa 362). PBZ drenching application was found better over its foliar application. Under water deficit, PBZ improved photosynthetic activity, photosynthetic pigments & water relation, reduced membrane damage and protected the photosynthetic machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby induced water deficit stress tolerance and enhanced the yield.

## 8. ABSTRACT

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### “Role of Paclobutrazol in Amelioration of Water deficit Stress in Chickpea (*Cicer arietinum* L.)”

Chickpea (*Cicer arietinum* L.) as a third major cool season grain legume in the world has an important role in poor people's diet. India is the largest producer of chickpea. Traditionally this crop is cultivated under marginal and rainfed areas. Therefore, drought is one of the major constraints in chickpea production. At present, application of bioregulators attracts more attention to protect plants from abiotic stresses. Paclobutrazol (PBZ) interferes with gibberellins biosynthesis and provides plant protection against water deficit stress. Thus, present study was undertaken using two contrasting chickpea varieties viz. *desi* (Pusa 362) and *kabuli* (Pusa1108) for identifying optimum dose of paclobutrazol for foliar and drenching applications under water deficit condition and to analyze the physiological and biochemical alterations to gain insight into the mechanism of paclobutrazol induced water deficit stress tolerance. Initially, a preliminary experiment was conducted with varying dose of paclobutrazol for both foliar and drenching applications and Water deficit stress was imposed by restricting water supply until severe symptoms of wilting were visible. Before imposition of water stress, plants were treated exogenously with identified optimum dose of paclobutrazol. Based on relative water content, membrane stability index, photosynthetic pigments and photosynthesis rate, optimum dose of PBZ for foliar spray (60 ppm) and drenching (120 ppm) were identified and used for main experiment. Main experiment was comprised two main treatments (i.e. well watered and water deficit condition) and four sub-treatments (PBZ foliar spray (60 ppm), PBZ drenching (120 ppm), water spray and water drenching).

The results of main experiment showed decrease in RWC, MSI, rate of photosynthesis, stomatal conductance, transpiration rate, Fv/Fm ratio, water use efficiency, chlorophylls, carotenoids, growth parameters, harvest index, drought tolerance efficiency, yield and its attributes and disrupted chloroplast grana under imposed water deficit stress condition. Applications of paclobutrazol maintained higher values of all these parameters and maintained the integrity of chloroplast structure under imposed water stress and also stimulated faster recovery after water deficit stress termination in both chickpea varieties. Biochemical analysis revealed that total soluble sugars, total free amino acids and proline content increased under imposed water deficit stress and paclobutrazol maintained these at higher level and hence has a role in osmo-protection. Paclobutrazol reduced the rate of lipid peroxidation and hence provided reduced membrane leakage. It also enhanced the activity of antioxidant enzymes and provided stress tolerance ability to both chickpea varieties. Paclobutrazol increased the level of abscisic acid and thus contributed in stress tolerance. In general PBZ drenching in terms of physio-biochemical responses was found better over the foliar application of PBZ.

From the present study it can be concluded that paclobutrazol maintained RWC, MSI, photosynthetic activity, photosynthetic pigments and protected the photosynthetic machinery by enhancing the level of osmolytes, antioxidant activities and level of endogenous hormones and thereby induced water deficit stress tolerance and enhanced the yield.

## 9. ABSTRACT (HINDI)

### चने (साइसर एराइटीनम एल.) में न्यून जल-तनाव के सुधार में पैक्लोब्यूट्राजोल की भूमिका

सार

विष्व की तीसरी प्रमुख सर्द ऋतु की दाने वाली दहलन फसल के रूप में चने (साइसर एराइटीनम एल.) की निर्धन व्यक्तियों के आहार में एक महत्वपूर्ण भूमिका है। भारत चने का सबसे बड़ा उत्पादक देश है। पारम्परिक रूप से यह फसल सीमान्त एवं असिंचित क्षेत्रों के अन्तर्गत बोई जाती है। इसलिए चने के उत्पादन में सूखा, प्रमुख बाधाओं में से एक है। वर्तमान में पौधों को अजैविक तनावों से सुरक्षा प्रदान करने हेतु जैव नियामकों के अनुप्रयोगों ने अधिक ध्यानाकर्षित किया है। पैक्लोब्यूट्राजोल (पी.बी.जैड.) जिब्वरेलिन्स के जैव संश्लेषण में बाधा उत्पन्न करता है और न्यून जल-तनाव के विरुद्ध पादप सुरक्षा उपलब्ध कराता है। इसलिए प्रस्तुत अध्ययन में चने की दो विपर्यासी किस्मों, देसी (पूसा 362) एवं काबुली (पूसा 1108) का जल तनाव अवस्था के अन्तर्गत पर्णिय छिड़काव एवं ड्रैचिंग अनुप्रयोगों के लिए इष्टतम मात्रा की पहचान हेतु तथा पैक्लोब्यूट्राजोल प्रेरित न्यून जल सहनशीलता की क्रिया विधि जानने के लिए तथा पादप कार्यिकीय एवं जैव रासायनिक परिवर्तनों के विश्लेषण के लिए उपयोग किया गया। शुरुआत में पर्णिय एवं ड्रैचिंग अनुप्रयोगों हेतु पैक्लोब्यूट्राजोल की भिन्न-भिन्न मात्राओं के साथ प्राथमिक प्रयोग किया गया तथा मुर्झाने के गंभीर लक्षण होने तक सीमित जल आपूर्ति द्वारा न्यून जल तनाव उत्पन्न किया गया। जल तनाव उत्पन्न करने से पहले पौधों को वाह्य रूप से पैक्लोब्यूट्राजोल की चयनित इष्टतम मात्रा के साथ उपचारित किया गया। आपेक्षित जल अंश, झिल्ली स्थायित्व घातांक, प्रकाश संश्लेषण संबंधी वर्णों, पादप संश्लेषण दर के आधार पर पी.बी.जैड. की, पर्णिय छिड़काव (60 पीपीएम) एवं ड्रैचिंग उपचार (120 पीपीएम) हेतु इष्टतम मात्रा की पहचान की गई और उसका मुख्य प्रयोग में उपयोग किया गया। मुख्य प्रयोग में दो मुख्य उपचार (सुसिंचित एवं न्यून जल अवस्था) तथा चार उप-उपचारों [पी.बी.जैड. पर्णिय छिड़काव (60 पीपीएम), पी.बी.जैड. ड्रैचिंग (120 पीपीएम), जल छिड़काव एवं जल ड्रैचिंग] थे।

मुख्य प्रयोग के परिणामों ने दर्शाया कि न्यून जल तनाव उत्पन्न अवस्था के अन्तर्गत आर.डब्ल्यू.सी., एम.एस.आई, प्रकाश संश्लेषण दर, स्टोमेटा संबंधी चालकता, वाष्पोत्सर्जन दर, एफवी/एफएम अनुपात, जल-उपयोग दक्षता, हरित वर्णों, कैरोटिनॉयड्स, वृद्धि प्राचलों, कटाई घातांक, सूखा सहनशील क्षमता, उपज एवं उपज संबंधी गुणों में कमी आई तथा हरितलवक-ग्रेना का फटना देखा गया। पैक्लोब्यूट्राजोल के अनुप्रयोगों द्वारा चने की दोनों किस्मों में न्यून जल तनाव विद्यमान होने पर भी इन सभी प्राचलों के उच्चतर मान बने रहे तथा इसके द्वारा हरितलवक-संरचना की अक्षुण्णता बनी रही तथा साथ ही न्यून जल-तनाव अवस्था समाप्त होने पर इसने तेजी से पूर्व-स्थिति प्राप्ति को भी प्रेरित किया। जैव रासायनिक विश्लेषण ने दर्शाया कि न्यून जल-तनाव होने पर कुल विलयणीय शर्कराओं, कुल स्वतंत्र अमीनो अम्लों एवं प्रोलीन अंश में बढ़ोतरी हुई तथा पैक्लोब्यूट्राजोल ने इन्हें उच्चतर स्तर पर बनाए रखा इसलिए ओस्मो प्रोटेक्शन में इसकी भूमिका है। पैक्लोब्यूट्राजोल ने लिपिड परॉक्सीडेशन की दर को कम किया इसलिए झिल्ली-क्षरण में कमी हुई। इसने चने की दोनों किस्मों में प्रतिऑक्सीकारक एंजायम्स की सक्रियता में बढ़ोतरी की और तनाव सहनशीलता की योग्यता उपलब्ध करायी। पैक्लोब्यूट्राजोल ने एब्सीसिक अम्ल के स्तर में बढ़ोतरी की और इस प्रकार से तनाव सहनशीलता में योगदान किया। सामान्य रूप से, भौतिक-जैवरासायनिक अनुक्रियाओं के सन्दर्भ में पी.बी.जैड. के पत्तियों पर अनुप्रयोग की तुलना में पी.बी.जैड. ड्रैचिंग को बेहतर पाया गया।

प्रस्तुत अध्ययन से यह निष्कर्ष निकाला जा सकता है कि पैक्लोब्यूट्राजोल ने आर.डब्ल्यू.सी., एम.एस.आई, प्रकाश संश्लेषण-सक्रियता एवं प्रकाश संश्लेषण संबंधी वर्णकों का रख-रखाव किया तथा ओस्मोलाइट्स, प्रति ऑक्सीकारक सक्रियताओं एवं अंतर्जात हॉर्मोन्स के स्तर को बढ़ाकर प्रकाश संश्लेषण संबंधी मशीनरी की सुरक्षा की और इस प्रकार से न्यून जल-तनाव सहनशीलता को प्रेरित किया एवं उपज में बढ़ोतरी की।

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