BENZYLADENINE INDUCED MODULATION OF PHOTOSYNTHESIS AND PRODUCTIVITY IN BARLEY (*Hordeum vulgare* L.) UNDER WATER STRESS CONDITIONS

THESIS

SUBMITTED TO THE
RAJASTHAN AGRICULTURAL UNIVERSITY, BIKANER
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF

MASTER OF SCIENCE
IN
AGRICULTURE (PLANT PHYSIOLOGY)

BY

SANTOSH KUMARI

2004

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This is to certify that **Mrs. Santosh Kumari** had successfully completed the comprehensive examination held on ---------2003 as required under the regulation for Master’s degree in Agriculture.

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LIST OF ABBREVIATIONS

BA	Benzyladenine
MI	Membrane Injury
PGRs	Plant Growth Regulators
CK	Cytokinin
ABA	Abscisic Acid
TR	Transpiration Rate
RWC	Relative Water Content
NS	Non-Significant
EC	Electrical conductivity
OP	Osmotic Potential
WUE	Water Use Efficiency

Benzyladenine Induced Modulation of Photosynthesis and Productivity in Barley (*Hordeum vulgare* L.) Under Water Stress Conditions

Santosh Kumari* **Dr. Sunita Gupta****
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ABSTRACT

The study entitled "Benzyladenine induced modulation of photosynthesis and productivity in barley under water stress condition" was conducted in the cage house at S.K.N. College of Agriculture, Jobner during the *rabi* season of 2002/2003 under pot culture experiments. (1) To observe influence of BA induced modulation of photosynthetic attributes, growth and yield of barley under water stress conditions. (2) To observe effect of BA on productivity in barley under water stress conditions.

Four barley cultivars namely RD-2052, RD-387, RD-2035 and RD-2552 were grown in ceramic pots under normal condition till anthesis stage and than plants were sprayed with Benzyladenine (0, 5, 10 and 15ppm concentrations) and than, half of the plants were subjected to water stress by withholding irrigation. The non-stressed plants were irrigated as frequently as needed and observations were recorded at 0,10, 20 and 30 days after anthesis.

BA significantly increased photosynthetic rate, transpiration rate, relative water content, stomatal conductance, total chlorophyll content and protein content whereas, significant decrease in membrane injury, leaf temperature and osmotic potential was recorded in all four cultivars under both non-stress and water stress conditions. The 10ppm concentration of BA was effective under both non-stress and water stress conditions.

Number of tillers, number of spikelets, grain yield, number of grain and 1000-seed weight were significantly increased on account of BA treatment both under non-stress and water stress conditions in all the four cultivars.

Most of the physiological parameters are positively and highly correlated with grain yield but leaf temperature and membrane injury was negatively and significantly correlated with grain yield.

It is concluded that among the four cultivars studied RD-2052 was drought tolerant and the tolerance was mediated by physiological characteristics. Benzyladenine increased productivity by reducing the adverse effect of drought stress on growth and yield attributes.

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1 INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the important cereals cultivated in most parts of the world. It is an important *rabi* cereals of India occupying 8.41 Lakh hectares of area and producing 15.83 Lakh tonnes of grain, with the average productivity of 1882 kg ha^{-1} (Anonymous 2002). It is cultivated chiefly in the states of U.P., Rajasthan and Bihar. Uttar Pradesh is the first and Rajasthan ranks second with regard to area and production. In Rajasthan it covers about 2.83 Lakh hectares with annual production of about 4.37 Lakh tonnes having average grain yield of 1850 kg ha^{-1}. Thus, Rajasthan alone contributes about 20 per cent to the national production of barely.

Barley is grown in all the districts of Rajasthan. Jaipur ranks first followed by Udaipur and Bhilwara in both area and production. The highest average yield of barley is in Chittaurgarh district (about 2507 kg ha^{-1}). Other important barley growing districts of the state are Ajmer, Alwar, Sikar and Tonk.

Barley is considered as an important food grain among poors and is used in the form of unleavened bread “Chapati” and as “Sattu” (flour of baked barley mixed in sugar and water). It is also used to prepare malt for manufacturing beer and whiskey and other products such as industrial alcohol and vinegar. Now a day, its demand for malting has increased considerably with the establishment of more malt industries. Malt sprout and by-products of breeding are used as a food for dairy cattle and surplus grain is fed to cattle. Biotic and abiotic factors cause losses in genetic yield potential of barley plants.

The physio-chemical factors include water and nutrient availability, temperature, day length, soil pH, aeration and excessive salt content ratio in the soil. Physio-chemical environment, especially the effect of drought is the dominant factors suppressing the productivity of land in present world (Kramer and Boyer, 1995). Almost forty per cent of world's arable land resource is under the influence of drought (Ashley, 1993; Chapman and Stone, 1997).

Plants in general cannot store moisture, hence, a control and tolerance mechanism is required to regulate the plant water status. Levitt (1982) have defined the mechanisms of escape, avoidance and drought tolerance. Different morpho-physiological and biochemical parameters in relation to drought tolerant have also been reviewed (Richards, 1982; Boyer, 1996). Hayek *et al.* (2000) and Baalbaki *et al.* (1999) have also reported that tolerance to drought could not be ascribed to one or two traits but was due to ability of the plant to develop a high number of tolerance mechanisms.

Drought tolerance is a complex character and can be associated with thickness of cuticle, opening and closing of stomata, root depth and extension, hormone composition, osmotic adjustment and antioxidant production (Szegletes *et al.* 2000)

Leaf area, fresh weight, dry weight, water content, leaf rolling, colour (Medhabati Devi *et al.* 1996), plant growth and root system (Volkmar *et al.* 1996), water relation parameters like relative water content, transpiration and stomatal regulation (Collaz *et al.* 1991), water potential and its components (Jat *et al.* 1991; Srivastava and Chaturvedi, 1996) have also been reported to affect under abiotic stress conditions. Osmoregulation and water stress (Morgan, 1984), osmo-stress proteins such as LEA's and dehydrins (Close and Lammers, 1993) osmatically active sugars (manitol, sorbitol, fructans and raffinose) are also produced under water stress conditions. Amino acids like proline, glycine, betaine as well as polyamines also contribute towards osmotic adjustment besides providing protection to macromolecules such as enzymes and proteins from electrolytes and temperature. Membrane stability and changes in composition of plasma membrane under stress has been reviewed by Navari-Izzo *et al.* (1993). Photosynthesis and yield attributes under drought condition are studied by Nilsen (1992) and Srivastava and Soni (1995).

Photosynthesis is the key to dry matter production and hence yield of economic organ. Thus increasing photosynthetic efficiency is the most important way to enhance the productivity (Gupta, 1992). However, water stress decrease the efficiency of photosystem-II and potential photosynthetic quantum and hence strongly reduce the photosynthetic activity at a light saturation level (Shangguan *et al.* 2000). The decrease in photosynthetic rate under water stress condition is also due to a decrease in stomatal conductance and other non-stomatal factors as reported by Iqbal and Wright, (1998) and Siddique *et al.* (1999).

Advances in the improvement of drought tolerance in crops are described in relation to the physiology of water efficiency, importance of harvest index, plant growth metabolism and gene expression. Therefore, an approach to improve drought tolerance includes breeding programme, which exploit and investigate the relationship between yield and favourable conditions, physiological correlations and morphological characteristics (Boyer, 1996). Drought tolerant crop plant show improved growth with limited water.

There are certain chemicals, which have potential to induce drought tolerance in crop plants. They are associated with several cellular and physiological processes and may be useful for protecting crops against abiotic stresses. Plant growth regulators (PGR's) showed tremendous potential in modulating growth, productivity and related processes (Gupta *et al.* 2000, 2003). They are considered as bio-softwares which elicit rapid as well as long term responses in crop plants (Yadav *et al.* 1994 and Malik *et al.* 1996). They enhance crop production through redirecting the metabolism balance of growth and partitioning of assimilate which result in the increase in quantity as well as quality of desired economic product (Nickel, 1982; Nowak and Lowson, 1983). Banzyladenine is a cytokinin which has been found important in growth and development of plant organs, retention of chlorophyll, translocation of nutrients and organic substances, nucleic acid metabolism and alternative pathway of respiration. Arteca (1995) and Gupta *et al.* (2003) reported that BA has great implication in water stress physiology. It is well documented that BA synthesis is enhanced under water stress, which is responsible for development of water stress syndrome in plants. Cytokinins (eg Benzyladenine) also protect cell membrane against degradation by preventing oxidation of unsaturated fatty acids and hence increase drought tolerance in plants (Salisbury and Ross, 1992). Cytokinins inhibit formation and break down of free radicals such as superoxide (O^-) and hydroxy radical (OH^-) that otherwise oxidize membrane lipids. Banzyladenine also increase photosynthetic rate by increasing activities of RUBISCO and PEP carboxylase enzymes under water stress conditions (Yonghua *et al.* 1997). Drought generally causes a reduction in cytokinin levels in leaves.

In view of the research projection cited above in relation to photosynthesis and productivity at crop plants, the study entitled- "Benzyladenine induced modulation of photosynthesis and productivity in barley under water stress conditions" was undertaken with the following objectives:

(1) To observe influence of BA induced modulation on photosynthetic attributes, growth and yield of barley under water stress conditions.

To observe effect of BA on productivity in barley under water stress conditions.

2 REVIEW OF LITERATURE

Water stress affects about 40% of the world arable land (Oerlti, 1983; Chapman and Stone, 1997). It affects almost every physiological and biochemical plant processes. Hayek *et al.* (2000) and Baalbaki *et al.* (1999) have also reported that tolerance to water stress could not ascribed to one or two traits but was due to the ability of the plant to develop a high number of tolerance mechanism. Water stress is a complex character and can be associated with thickness of cuticle, opening and closing of stomata, root depth and extension, hormone composition, osmotic adjustment and antioxidant production (Szegletes *et al.* 2000).

A number of important physiological processes like photosynthesis, respiration (Bjork man *et al.* 1980), osmotic adjustment, transpiration, water relation parameters and conversion of sucrose to starch in developing grains of wheat have been reported as selection traits for genotypes suitable for stress conditions (Bhullar and Jenner, 1986; Srivastava and Chaturvedi, 1989). The different mechanism of tolerance in terms of growth, plant water relation parameters, photosynthetic activity, membrane injury and role of Benzyladenine (BA) in inducing water stress tolerance are briefed below:

2.1 Water stress in relation to growth, yield and yield attributes

Plant response to drought stress is a complex phenomenon that appears to involve synthesis of osmolytes, polyamines and a new set of protein (Caplan *et al.* 1990). Wet *et al.* (1999) reported that drought inhibits the growth of coleoptiles in wheat, but the degree of inhibition of susceptible varieties was greater than resistance verities. Moisture stress at vegetative stage irreversibly reduced plant height, number of branches, number of leaves per plant, leaf area, number of pods and dry matter accumulation (Sadashivam *et al.* 1988). Anthesis stage have been found to be most critical stage to moisture stress as far as yield is concerned. Baalbaki *et al.* (1999) reported that drought resistant bread wheat cultivars have higher germination percentage and germination spread under moisture stress than drought susceptible cultivars but the germination spread was more sensitive to changes in osmotic potential than germination percentage. Growth and other associated characters also get influenced by moisture stress. Medhabati *et al.* (1996) described that seedling fresh weight, dry weight, relative water content, leaf area, leaf rolling and leaf area were dependent upon moisture content. Volkmar *et al.* (1996) also observed that plant growth and root systems are positively correlated by moisture level of plant and soil. Drought at the period between stem elongation and heading seriously affects the leaf area index, radiation use efficiency and biomass production of wheat (Giunta *et al.* 1995).

Oztruk (1999) reported that early drought primarily limited the grain number per unit area while the late drought affected the grain weight. The negative effect of early drought on grain yield was more significant than late drought. Water stress decreased number of grains/spike (Wang *et al.* 1993), spike length (Jamal *et al.* 1996; Klar *et al.* 1990; Weber and Hrynczuk, 1999), number of spikes and tiller number/plant (Jat *et al.* 1990; Saha and Paul, 1997; Weber and Hrynczuk, 1999), plant height and weight of grains per ear (Jat *et al.* 1990; Weber and Hrynczuk, 1999), shoot dry matter production (Klar *et al.* 1990; and Hegazi *et al.* 1999) and leaf area (Jat *et al.* 1990). Water stress also decreased harvest index (Moreira *et al.* 1999).

Seed yield and leaf area were decreased as a result of drought stress (Jones *et al.* 1982). Seed yield was reported to be decrease by 50 per cent by water deficit occurring at the flowering and pod filling stages and it was linearly related to an integrated value of the predawn leaf water potential from bud formation to mid pod filling (Shouse, 1979). Soil water deficit decreased number of seed, seed dry weight and yield per plant. Decrease in seed yield per capitula/plant has also been reported on account of soil water deficit (Hayashi and Hand, 1985).

The growth retardant increased protein content in barley but decreased in wheat (Krawczyk *et al.* 1996). It is also reported that growth retardant reduced the stem length by 13.15% and also affected mineral level in the grain. By scheduling irrigation water it was observed that increase in irrigation water resulted in increased yield (Hanks, 1983 and Panwar, 1987).

2.2 Water stress in relation to photosynthesis, chlorophyll and related parameters

Drought affects every phosio biochemical process but photosynthesis is of the first altered characteristic in C_3 and C_4 plants. (Donaldson, 1996 and Pena-Valdivia, 1994). Water stress decrease photosynthetic rate significantly (Shangguan *et al.* 2000; Reddy, 2000; Siddique *et al.* 2000; Janacek, 1997; Iqbal and Wright, 1998 and Li *et al.* 1992).

Rekika *et al.* (1998) reported that the decrease in photosynthetic rate under mild and severe water stress is mainly due to stomatal and non-stomatal factors, respectively. Xue *et al.* (1992) observed half reduction in photosynthetic rate under mild water stress in susceptible but it was less affected in tolerant wheat cultivars under water stress in addition to the significant decrease in net photosynthesis.

Nyachiro *et al.* (2001) reported that water deficit decreased photosynthetic efficiency (PSC) by 44.5 to 55.7 per cent and caused a decrease in chlorophyll a, b and total chlorophyll content in wheat.

Murthy and Singh (1979) studied the effect of water deficit on chlorophyll content, chlorophyll fluorescence and photosynthetic oxygen evolution in wheat cultivars under non-stress and water stress conditions. Chlorophyll content has been used to evaluate the effect of drought in C_3 plants (Gummuluru *et al.* 1989; Mayoral *et al.* 1981; Pena-Valdivia, 1994), C_4 plant (Pena Valdivia *et al.* 1997; Raya *et al.* 1997) and CAM (Flores *et al.* 1999). The stability of chlorophyll may be considered a trait for tolerance to abiotic factors (Gupta *et al.* 2000). Estill evaluated the effect of water stress in plant physiology and bio chemistry (Bichler *et al.* 1996; Cerovic *et al.* 1996; Flores *et al.* 1999; Raya *et al.* 1997). Association of chlorophyll content with grain yield potential under stress has been reported by Reynolds *et al.* (1994).

Gorny (2001) studied the effect of water stress on photosynthetic rate, transpiration rate, stomatal conductance and photosynthetic water use efficiency in diallel crosses of barley. Photosynthesis and transpiration of leaves are the major growth and yield supportive processes. The efficiency of the leaf gas exchange, defined as the photosynthetic efficiency or water use efficiency (WUE) i.e. the ratio of assimilated CO_2 per unit transpired H_2O, may be considered as an important component of plant productivity under diverse environmental habitats (Sinclair *et al.* 1984; Ehleringer *et al.* 1993 and Vanden Boogoard, 1995).

2.3 Water relation parameters

Water stress decreased the leaf water potential and relative water content of wheat (Singh *et al.* 1993; Li *et al.* 1992; Jat *et al.* 1991; Singh and Patel, 1996). The tolerant varieties show less reduction as compared with susceptible varieties. Water stress affects the rate of transpiration which is reduced in water

stress condition due to stomatal closure (Singh, 1989; and Nathawat, 1991). Relative water content and leaf water potential were decreased by water stress (Singh *et al.* 1993 and Deshmukh *et al.* 1991), while leaf diffusive resistance (Kumar and Gour, 1992 and Strauss and Agenbag, 2000) were enhanced by water stress.

Singh *et al.* (1993) reported significant parabolic correlations between leaf water potential and canopy temperature, linear reciprocal relations between leaf water potential and stomatal resistance and a liner correlation between stomatal resistance and canopy temperature. The varieties are also found to differ with respect to their transpiration and stomatal behavior under moisture stress conditions. Drought resistant varieties employed both water saving and water conserving mechanism to avoid drought (Levitt, 1982). The stomatal control is also the basis of drought avoidance through saving and spending strategies (Jat *et al.* 1991; Lal, 1991). Closure of stomata on accounts of water stress is well documented response (Hsiao, 1973; Begg and Turner, 1976; Farquhar and Sharkey, 1982).

Collaz *et al.* (1991) studied the effect of water stress on plant relation parameters like relative water content, transpiration and stomatal regulation. Similarly water potential and it components have been investigated by Jat *et al.* (1991); Srivastava and Chaturvedi (1989) and Gupta *et al.* (2000).

2.4 Water stress in relation to membrane stability

The role of cell membrane remains to be more critical for adaptation under temperature and moisture stress conditions (Ren, 1985; Singh *et al.* 1992; Prasad and Srivastava, 1999).

Blum and Ebercon (1981) described that under water stress conditions, measurement of electrolyte leakage can be used to estimate the water stress tolerance. Heat tolerant genotypes were found to possess higher membrane stability (Sairam *et al.* 1997; Xu *et al.* 1997). Higher membrane stability in drought tolerant genotypes under stress was due to increased activities of antioxidative enzymes which prevent damage of membrane by active oxygen species produced under stress (Sairam and Saxena, 2000 and Sairam *et al.* 1998). Membrane stability at drought and high temperature, estimated by conductivity measurement of solute leakage, has been proposed as a criterion of tolerance (Sullivan and Ross, 1979; Blum and Ebercon, 1981; Deshmukh *et al.* 1991 and Gupta *et al.* 2000).

2.5 Role of Benzyladenine in increasing drought tolerance

Various workers have reported the beneficial effect of BA under moisture stress. Yonghua *et al.* (1997) have reported that BA increased RUBISCO activities under moisture stress in maize. Application of polystimulin K (a polymer from of BA) increased leaf relative water content, grain yield, grain protein content

and glutein content in wheat (Grigoryuk *et al.* 1990). The BA increased dry weight and chlorophyll concentration in leaves of wheat (Xian *et al.* 1995), tiller number, biomass and re-growth after cutting in spring barley (Christiansen *et al.* 1995) and yield in pigeon pea (Singh and Kakralya, 1992). The BA treatment increased the stability of chloroplast shape and ultra structure during senescence (Zhenyvan *et al.* 1998).

Cytokinins are believed to offset the harmful effect of water stress and ABA during seed germination therefore, it has been described as playing a permissive role in seed germination by allowing gibberellic acid to function (Khan, 1971). Seed germination by red light treatment may also be increased by cytokinin treatment (Vanstaden *et al.* 1982).

Cytokinin affects the opening and closing of stomata. A continuous supply of cytokinin sustain maximum stomatal opening, an indication of inhibited root activity, resulting in restricted stomatal opening and water use (Blackman and Davies, 1985).

It has been known from some years that external application of cytokinins can increase transpiration from intact leaves of several graminaceous species (Livne and Vaadia, 1972; Biddington and Thomas, 1978).

2.6 Role of Benzyladenine in increasing productivity

Kinetin and closely related hormones, generally termed cytokinin, were primarily discovered as growth promoting PGR specifically through induction of cell division (Skoog *et al.* 1965). Later on, cytokinins have been found important in cell and organ enlargement, seed germination, root initiation and growth, bud development, shoot growth, retention of chlorophyll, delayed senescence in leaves, translocation of nutrients and organic substances, nucleic acid metabolism and alternative pathway of respiration (Arteca, 1995).

Kinetin treatment of sunflower hypocotyls increased fresh and dry weight more than double of the control although no elongation occurred (De Ropp, 1956).

Yadav *et al.* (1997) also reported that BA partially alleviated the adverse effects of water stress on *Cicer arietinum* by stimulating the accumulation of metabolites in the leaves. Such stress induced increase in metabolites is responsible

for osmotic adjustment. The BA also increased the content of cytokinin and lowered the level of ABA in the leaves and spikes of wheat. As a result, photosynthetic activity in leaves increased leading to an increase in grain mass and productivity (Elagina and Yakushkina, 1997).

When kinetin is applied to excised barley or radish leaves under condition in which leaf water potential was not affected. The accumulation of proline is inhibited.

Gupta *et al.* (2000) reported exogenous application of cytokinin increased cell membrane and chlorophyll stability index in drought sensitive and drought tolerant water cultivars of wheat (C-306) at anthesis stage, which were significantly correlated with grain yield.

3 MATERIALS AND METHODS

A pot experiment was carried out in the cage house located in the vicinity of the department of Plant Physiology, SKN college of Agriculture, Jobner during *rabi* season of 2002-2003 to investigate "Benzyladenine induced modulation of photosynthesis and productivity in barley under water stress conditions".

3.1 CULTIVARS

Four cultivars of barley were used:

(1) RD – 2052

(2) RD – 2035

(3) RD – 2552

(4) RD – 387 (Raj Kiran)

These varieties were grown both under non-stress and water stress condition. The 4 barley cultivars have been selected on the basis of their drought tolerant characters namely RD-2052 (drought tolerant), RD-2035 (widely adopted but drought sensitive), RD-2552 (salt tolerant) and RD-387 (drought tolerant). Seeds of these cultivars were sown in ceramic pots containing well fertile loamy sand soil on November 14, 2002 under normal cultural practices.

3.2 METEOROLOGICAL CONDITIONS

The soil used was loamy sand having a bulk density of 1.5 g cm^{-1}, pH 8.2, ECe1.1 dsm^{-1}, SAR 12.5, field capacity 11.8% and permanent wilting point 2.8 %.

Initially 10 plants were raised in each pot but after thinning 5 plants were maintained in each pot.

3.3 TREATMENT

Benzyladenine (BA) of following concentrations was used for different treatments.

(1) 0 mg/litre (control)

(2) 5 mg/litre

(3) 10 mg/litre

(4) 15 mg/litre

Prior to stress the plants were sprayed with BA 0 mg/litre, 5 mg/litre, 10 mg/litre and 15 mg/litre under both non-stress and water stress conditions.

3.4 SCHEDULING OF IRRIGATION

All plants were grown under normal condition upto the 70 days after sowing (anthesis stage). Then the water stress condition was created by withholding irrigation for five days before the schedule date of observations. Non- stressed plants were irrigated as frequently as needed.

Observations were taken at 0, 10, 20 and 30 days after anthesis in between 10 to 12 am on the first fully expanded leaf from the top.

Observations including relative water content (RWC) membrane injury, chlorophyll content, protein content, osmotic potential, photosynthetic rate, stomatal conductance, leaf temperature, leaf transpiration rate were recorded on the flag leaf at 0, 10, 20 and 30 days after anthesis stage. Yield parameters such as total number of tillers, were taken at maturity stage. Number of grains/ear, number of spikelets/plant, grains yield/plant and 1000 grains weight were recorded after harvest.

3.5 PROCEDURES AND TECHNIQUES

3.5.1 Determination of relative water content

Fresh weight of the flag leaf was taken and then the flag leaf was kept in distilled water for 4 hours (Barrs and Weatherly, 1962) to obtain turgid weight. The turgid weight was recorded after blotting the excess water on the surfaces of the leaf. Dry weight was obtained after drying the leaf in an oven at 60^{0}C till content weight obtained. The relative water content (RWC) was then calculated by the formula (Slavik, 1974) as:

Fresh weight – Dry weight

R.W.C. (%) = --- x 100

Turgid weight – Dry weight

3.5.2 Cell membrane injury

Cell membrane injury was determined by the method of Sullivan (1972). A paired set of leaves (1 g each) under different treatments were washed thoroughly with three change of distilled water to remove electrolytes adhering to the surface of leaves and taken in test tubes. To each test tube, 25 ml of double distilled water (ddH_2O) was added. One set of test tubes (denoted as treatment) was covered with rubber cork and incubated in a water bath at 45^0C for one hour while the other set (denoted as control) was kept at room temperature.

Both the control and treatment sets were kept at 10^0C for 24 hours to allow diffusion of electrolytes from leaves. The test tubes were brought to 25^0C and shaken to mix the contents. The initial conductance of the test tubes contents was determined with conductivity meter (Systronics, India). Tubes of both the sets were boiled again for 30 minutes in boiling water bath. After cooling to room temperature the volume was made to 25 ml with dH_2O and the final conductance was measured.

The membrane injury (MI) was determined by the formula:

$$\text{MI } (\%) = \left\{ 1 - \frac{1 - T_1/T_2}{1 - C_1/C_2} \right\} \times 100$$

Where,

T and C refers to electrical conductivity of treatments and control and subscripts land 2 refers to initial and final conductance, respectively.

3.5.3 Estimation of total chlorophyll content (mg g^{-1} fresh weight)

Total chlorophyll content (the sum of chlorophyll ‘a’ and chlorophyll ‘b’) was measured by method of Witham *et al.* (1971).

Sample extract was prepared from 100 mg of leaf with 10 ml of 85% acetone and the homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was transferred to a 25 ml measuring cylinder. The residue was again re-extracted with 5 ml of acetone and centrifuged and then the supernatant was transferred to the measuring cylinder. The final volume of the supernatant was made to 20 ml with the acetone.

Finally, the optical density of chlorophyll ‘a’ and ‘b’ was measured at 663 and 645 nm, respectively using spectrophotometer, by taking 85% acetone as control.

The total chlorophyll content on mg l^{-1} was calculated by the formula:

$$\text{The total chlorophyll (mg } l^{-1}) = 20.2\ A_{645} + 8.02\ A_{663}$$

Where,

A_{645} and A_{663} were absorption (optical density) of chlorophyll 'b' and 'a', respectively. From this value, chlorophyll content is mg g^{-1} of fresh weight of the leaf sample was calculated using the expression:

$$\text{Total chlorophyll (mg g}^{-1}\text{)} = \frac{\text{Total chlorophyll (mg l}^{-1}\text{)}}{1000} \times \frac{\text{Total volume of extract}}{\text{Sample weight (g)}}$$

3.5.4 Soil moisture content

The soil moisture per cent was determined gravi-metrically and was expressed on per cent of dry weight (Singh, 1980). Collect soil samples by tube or auger from a number of points within the experimental site and mix thoroughly. Place composite sub samples of about 50 gm to 100 gm of soil in moisture can with tight fitting lids. Take at least three sub samples. The moist samples were weighed immediately, dried to constant weight in oven at 105-110^{0}C (for about 24 hr) and reweighed after cooling in a desicator. Determine the tare weight of the moisture cans. Calculated the soil moisture content by determining the loss in weight on drying and the weight of the oven dry soil as follows:

$$= \frac{\text{(Weight of wet soil + tare) – (Weight of dry soils + tare)}}{\text{(Weight of dry soil + tare) – (tare)}} \times 100$$

$$M_w\,(\%) = \frac{\text{Loss in weight on drying}}{\text{Weight of oven dry soil}} \times 100$$

3.5.5 Estimation of total soluble proteins (mg g^{-1} fresh weight)

For extraction of soluble proteins, 100 mg of leaf was homogenized in 0.1 M NaCl (pH 7.5). The homogenate was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and final volume was made to 5 ml with the 0.1 M NaCl. The proteins in the extract were estimated using the method of Lowry *et al.* (1951).

An extract volume of 0.2 ml was taken in test tube and at the same time a series of test tubes 0.2, 0.4, 0.6, 0.8 and 1.0 ml of protein solutions were prepared by dissolving 10 mg of bovin albumin serum protein in 100 ml of distilled water. In each test tube the volume was made to 1.0 ml with dH_2O. A tube with 1.0 ml of dH_2O served as blank.

After adding 5 ml of alkaline solution in each test tube, the mixture was kept at room temperature for 10 minutes. This was followed by addition of 0.5 ml of diluted folin-ciocalteau's phenol reagent.

The mixture was incubated at room temperature for 30 minutes under dark and the absorbance of the blue colour was measured at 660 nm using spectrophotometer. Zero absorbance was adjusted with the blank.

The quantity of proteins in the 100 mg of leaf was then calculated using the standard curve.

Reagent preparation

Reagent A : 2% $Na_2 CO_3$ in 0.1 M NaOH

Reagent B : 0.5% Copper sulphate ($CuSO_4$. $5H_2O$) in 1% potassium sodium tartrate.

Reagent C : Alkaline copper solution which was prepared by mixing reagent A and B in the ratio of 50: 1 at the time of use.

Reagent D : The commercial folin-ciocalteau Reagent was diluted with equal volume of water.

3.5.6 Estimation of osmotic potential

Osmotic potential (O.P.) was estimated by electrical conductivity method as given by Janardhan *et al.* (1975). The 1.0 gm leaf material was homogenized with 20 ml of distilled water with mortar and pestle and was extracted through two layers muslin cloth and final volume was make up to 30 ml with dH_2O. Electrical conductivity (Ec) was measured with direct reading conductivity meter. Fresh weight and dry weight of leaf sample from some plants were also recorded.

Osmotic potential (O.P.) was calculated as

$$\text{O.P. (- bars)} = 0.36 \times \text{Ec} \times \text{D.f.} / 0.987$$

The D.f. was calculated as $$\frac{\text{Fr. wt.} \times \text{Volume made}}{\text{Fr. wt.} - \text{d. wt.}}$$

Where,

D.f. = dilution factor

3.5.7 Photosynthesis (μ mol CO_2 m^{-2} s^{-1})

The rate of Photosynthesis was measured with the help of Infra Rad Gas Analyzer (CID 301, USA). The net exchange of CO_2 between a leaf and the atmosphere was measured directly by inclosing a top most fully expended leaf in the assimilation chamber and the rate was monitored at which the CO_2 concentration changes over a definite time interval. The system automatically calculates the rate of photosynthesis on the basis of preloaded flow and leaf are as per the chamber used. Measurement but taken between 10 to 11 AM in triplicate for all the treatments.

3.5.8 Leaf transportation rate (m mol H_2O m^{-2} 5^{-1})

The leaf transpiration rate was measured directly with the help of Infra Rad Gas Analyzer (IRGA) on the same leaf as described for photosynthesis.

3.5.9 Leaf temperature (^{0}C)

Leaf temperature was measured with the help of IRGA on the same leaf as described for photosynthesis.

3.5.10 Stomatal conductance (m mol m^{-2} 5^{-1})

Stomatal conductance was measured with the help of IRGA on the same leaf as described for photosynthesis.

3.5.11 Growth and yield parameters

3.5.11.1 Number of tillers, spikelets and grains

The total number of tillers/plant, spikelets/plant and grains/ear were counted in each pot and then the average was calculated.

3.5.11.2 Grain yield (g)

After harvest, plants were dried till constant weight was obtained. Then the yield was taken and calculated as per plant basis.

3.5.11.3 1000- seed weight (g)

1000-grains were counted and weight was recorded.

3.6 STATISTICAL ANALYSIS

All observations were taken in triplicates and data were statistically analysed using complete randomized design (CRD).

Standard error of mean (SEm±) and critical difference (CD) were calculated as:

$$\text{SEm}\pm = \sqrt{\frac{2\ \text{MSE}}{r}}$$

$$\text{CD} = \text{SEm} \times t_{\propto}$$

Where,

MSE - Mean square of error

r - Replication

$t_{\propto}$ - Table value of ‘t’ at 5 or 1 % level of significance with error degree of freedom.

4 EXPERIMENTAL RESULTS

The pot experiments conducted during the period of study have following results.

4.1 PHYSIOLOGICAL PARAMETERS

4.1.1 Photosynthesis

The effect of BA on photosynthesis in barley cultivars under non-stress and water conditions has been depicted in Table-1. Results show that at the time of anthesis the photosynthesis was maximum in RD-2052 and minimum in RD-2035. The water stress conditions, reviewed a significant reduction in photosynthesis in all the four cultivars. The photosynthesis of barley increased significantly and linearly with increasing BA concentration under non-stress and water stress conditions. It was maximum in plants treated with 15 ppm BA.

At 10 days after anthesis, the photosynthesis was maximum in RD-2052 and minimum in RD-2035. The water stress caused significant reduction in photosynthesis. The cultivars and BA significantly reduced photosynthesis under water stress conditions. Under both non-stress and water stress conditions it was maximum in plants treated with 15 ppm BA.

At 20 and 30 days after anthesis, the photosynthesis was maximum in RD-2052 and minimum in RD-2035 cultivars. Water stress conditions decreased photosynthesis significantly whereas BA application linearly increased the photosynthesis. The photosynthesis was maximum in plants treated with 15 ppm BA under both non-stress and water stress conditions.

4.1.2 TRANSPIRATION RATE

Table 2 shows the effect of BA on transpiration rate in barley cultivars under non-stress and water stress conditions. The results show that at the time of anthesis transpiration rate was maximum in RD-2052 and minimum in RD-2035. The transpiration of barley was increased significantly and linearly with BA concentration under water stress conditions. It was highest in plant treated with 15 ppm BA under both water stress and non-stress conditions.

At 10 days after anthesis, the transpiration rate was maximum in RD-2052 and minimum in RD-2035. The water stress caused significant reduction in transpiration rate. Although increase in transpiration with BA was non-significant but it was maximum in plants treated with 10 ppm BA under both non-stress and water stress conditions.

At 20 and 30 days after anthesis, the maximum transpiration rate was recorded in RD-2052 and minimum in RD-2035. The transpiration rate was maximum in plants treated with 15 ppm BA under both non-stress and water stress conditions.

4.1.3 Stomatal conductance

Table-3 shows the effect of BA on stomatal conductance in barley cultivars under non-stress and water stress conditions. The results show that at the time of anthesis the stomatal conductance was maximum in RD-2052 and minimum in RD-2035. The water stress caused significant decrease in stomatal conductance in all the four cultivars. The stomatal conductance of barley was increased non-significantly and linearly with BA concentration under non-stress and water stress conditions. It was highest in plants treated with 15 ppm BA under both water stress and non-stress conditions.

At 10 days after anthesis, the stomatal conductance was maximum in RD-2052 followed by RD-387, RD-2552 and RD-2035, respectively. The water stress caused significant reduction in stomatal conductance in all the four cultivars. It was maximum in plants treated with 15 ppm BA under both non-stress and water stress conditions.

At 20 and 30 days after anthesis, the stomatal conductance was maximum in RD-2052 and minimum in RD-2035. Water stress conditions caused significant reduction in stomatal conductance. Stomatal conductance increased linearly and significantly with BA. Under both water stress and non-stress conditions, it was maximum in plants treated with 15 ppm BA.

4.1.4 Leaf temperature

It is apparent from the data in Table-4 that cultivars differed significantly with regards to leaf temperature. Results show that at the time of anthesis leaf temperature was maximum in RD-2035 and minimum in RD-2052. The water stress caused significant increase in leaf temperature. The leaf temperature of barley decreased non-significantly and linearly with BA under water stress conditions. It was minimum in plants treated with 15 ppm BA.

At 10 days after anthesis, the leaf temperature was maximum in RD-2035 and RD-2552 and minimum in RD-2052. The water stress caused significant increased in leaf temperature. Under both water stress and non-stress conditions, it was minimum in plants treated with 15 ppm BA.

At 20 and 30 days after anthesis, leaf temperature was maximum in RD-2035 and minimum in RD-2052. Water stress caused significant increased in leaf temperature. The leaf temperature decreased with increasing BA concentration. Under both non-stress and water stress conditions, it was minimum in plants treated with 15 ppm BA.

4.1.5 Relative water content

Table 5 shows the effect of BA on relative water content in barley cultivars under non-stress and water stress conditions. The results show that at the time of anthesis, the relative water content was maximum in RD-2052 and minimum in RD-2035. The water stress caused significant decrease in relative water content of barley. Relative water content increased significantly and linearly with BA concentration under both non-stress and water stress conditions. It was highest in plants treated with 10 ppm BA.

At 10 days after anthesis, the relative water content was also maximum in RD-2052 and minimum in RD-2035. The water stress caused significant reduction in relative water content. It was maximum in plants treated with 15 ppm BA under both non-stress and water stress conditions.

At 20 days after anthesis, the maximum relative water content was recorded in RD-2052 and minimum in RD 2035 while at 30 days after anthesis it was recorded maximum in RD-387. It was highest in plants treated with 15 ppm BA.

4.1.6 Total chlorophyll content

The effect of BA on total chlorophyll content in barley cultivars under non-stress and water stress conditions has been depicted in Table-6. Results show that at the time of anthesis total chlorophyll was maximum in RD-2052 and minimum in RD-2035. The water stress conditions, review a significant reduction in total chlorophyll in all the four cultivars. The total chlorophyll content of barley increased significantly and linearly with increasing BA concentration under both non-stress and water stress conditions. It was maximum in plants treated with 15 ppm BA.

At 10 days after anthesis, the total chlorophyll was also maximum in RD-2052 and minimum in RD-2035. The water stress caused reduction in total chlorophyll content in all the four cultivars. The BA increased total chlorophyll content under both non-stress and water stress conditions non-significantly. It was maximum in plants treated with 15 ppm BA.

At 20 and 30 days after anthesis, the total chlorophyll content was maximum in RD-2052 and minimum in RD-2035. Water stress conditions significantly decreased total chlorophyll content. The total chlorophyll content was maximum in plants treated with 15 ppm BA under both non-stress and water stress conditions.

4.1.7 Protein content

It is apparent from the data Table-7 that cultivars differed significantly with regards to protein content. Results show that at the time of anthesis, the protein content was maximum in RD-2052 and RD-387 and minimum in RD-2035. The water stress conditions, review a significant reduction in protein content in all the four cultivars. The protein content of barley increased non-significantly and linearly with increasing BA concentration under non-stress and water stress conditions. It was maximum in plants treated with 15 ppm BA.

At 10 days after anthesis, the protein content was maximum in RD-2052 and minimum in RD-2552 and RD-2035. The water stress caused significant reduction in protein content. The cultivars and BA significantly reduced protein content under water stress conditions. Under both non-stress and water stress conditions, it was maximum in plants treated with 10 ppm BA.

At 20 and 30 days after anthesis, the protein content was maximum in RD-2052 and minimum in RD-2035 and RD-2552. Water stress conditions decreased protein content significantly in all the four cultivars. The maximum protein content was recorded in plants treated with 10 ppm BA under both non-stress and water stress conditions.

4.1.8 Osmotic potential

Table-8 shows the effect of BA on osmotic potential in barley cultivars under non-stress and water stress conditions. The results show that at the time of anthesis, the osmotic potential was maximum in RD-2035 and minimum in RD-2052. The water stress conditions, review a significant reduction in osmotic potential in all the four cultivars. The osmotic potential of barley decreased significantly and linearly with increasing BA concentration under both water stress and non-stress conditions. It was minimum in plants treated with 15 ppm BA.

At 10 days after anthesis, the osmotic potential was maximum in RD-2035 and minimum in RD-2052. The water stress caused reduction in osmotic potential. Irrespective of cultivars, BA decreased osmotic potential under both non-stress and water stress conditions significantly. It was minimum in plants treated with 15 ppm BA.

At 20 and 30 days after anthesis, the osmotic potential was maximum in RD-2035 and minimum in RD-2052. Water stress conditions reviewed a significant reduction in osmotic potential. The osmotic potential decreased significantly and linearly with BA, it was minimum in plants treated with 15 ppm BA under both non-stress and water stress conditions.

4.1.9 Membrane injury

It is apparent from the data Table-9 that cultivars differed significantly with regards to membrane injury. Results show that at the time of anthesis, membrane injury was maximum in RD-2552 and minimum in RD-2052. The water stress conditions caused significant increased in membrane injury. The 5 ppm BA increased the membrane injury but the difference was non-significant. The minimum membrane injury was recorded with 15 ppm BA.

At 10 days after anthesis, the membrane injury was maximum in RD-2552 and minimum in RD-2052. The water stress conditions caused increase the membrane injury in all the four cultivars. BA decreased the membrane injury under both non-stress and water stress conditions and it was minimum in plants treated with 15 ppm BA.

At 20 and 30 days after anthesis, the membrane injury was maximum in RD-2552 and minimum in RD-2052. Water stress conditions caused significant enhancement in membrane injury. The membrane injury decreased significantly with BA application under both non-stress and water stress conditions. It was minimum in plants treated with 15 ppm BA.

4.2 YIELD AND YIELD ATTRIBUTES

4.2.1 Total number of tillers per plant

Table-10 shows the effect of BA on total number of tillers per plant in barley cultivars under both non-stress and water stress conditions. The results show that maximum number of tillers was recorded in RD-2052 and minimum in RD-2035. The water stress caused significant decrease in total number of tillers per plant in all the four cultivars. The total number of tillers per plants increased significantly and linearly with BA concentration under both non-stress and water stress conditions. It was maximum in plant treated with 15 ppm BA.

4.2.2 Total number of spikelets per ear

Table 10 show the effect of BA on total number of spikelets per ear in barley cultivars under both non-stress and water stress conditions. The results show that maximum number of spikelets was recorded in RD-2052 and minimum in RD-2035. The water stress cause significant reduction in total number of spikelets per ear in all the four cultivars. The total number of spikelets per ear increased significantly and linearly with BA concentration under both non-stress and water stress conditions. It was maximum in plants treated with 10 ppm BA.

4.2.3 Number of grains per ear

Table-11 shows the effect of BA on total number of grains per ear in barley cultivars under both non-stress and water stress conditions. The results show that water stress caused significant decreased in total number of grains per ear in all the four cultivars. It was maximum in RD-2052 and minimum in RD-2035. The water stress significantly reduced the grain number in all the four cultivars. The total number of grains per ear increased significantly and linearly with BA concentration under both non-stress and water stress conditions. It was maximum in plants treated with 10 ppm BA under both non-stress and water stress conditions.

4.2.4 GRAIN YIELD PER PLANT

Table-11 shows the effect of BA on grain yield per plant in barley cultivars under both non-stress and water stress conditions. The results show that

BA treatments caused significant increase in grain yield per plant. The water stress caused significant reduction in grain yield per plant. It was maximum in RD-2052 and minimum in RD-2035. The grain yield per plant increased significantly and linearly with BA concentration under both non-stress and water stress conditions. It was maximum in plants treated with 10 ppm BA under both non-stress and water stress conditions.

4.2.5 1000-grains weight

It is apparent from the Table-11 that different cultivars differed significantly with regards to 1000-grains weight. It was maximum in RD-2052 and minimum in RD-2035. The water stress caused significant reduction in 1000-grains weight. The 1000-grains weight increased significantly and linearly with BA concentration under water stress conditions. It was maximum in plants treated with 10 ppm BA under both non-stress and water stress conditions.

4.2.6 CORRELATION ANALYSIS

The correlation analysis indicated that most of the parameters have positive correlation with grain yield of barley (Table 12 & 13).

Photosynthesis, transpiration, stomatal conductance, relative water content, protein content showed positive and highly significant correlations with grain yield in all the four stages. Leaf temperature and membrane injury showed negative and highly significant correlations with grain yield in all the four stages (Table 12).

All growth and yield parameters showed positive correlations with grain yield. Correlations of total number of tillers, number of spikelets, number of grains and 1000- seed weight were highly significant with grain yield (Table 13).

5 DISCUSSION

The results of the experiment entitled “Benzyladenine induced modulation of photosynthesis and productivity in barley under water stress conditions” have been presented in preceding chapters. During the course of result presentation, many significant variations in the criterion used for treated evaluation were observed due to effect of different treatments. In this chapter only significant variations have been discussed and compared with findings of other workers.

In context of barley the physiological studies have been very limited mainly on account of the major concentration of research on physiology of wheat (Asana *et al.* 1987).

Drought affects almost every plant processes but photosynthesis is the first affected process in C_3 and C_4 plants (Donaldson, 1996; Pena-Valdivia, 1994). Water stress conditions decrease photosynthetic rate significantly are reported by (Shangguan *et al.* 2000; Reddy, 2000; Siddique *et al.* 2000; Iqbal and Wright, 1998 and Janacek, 1997). Nyachiro *et al.* (2001) reported that water stress decreased photosynthesis efficiency in the range of 44.5 to 55.7 per cent and caused a decrease in total chlorophyll content in wheat. Our results also suggest that water stress reduced photosynthetic rate significantly while BA enhanced it under both non-stress and water stress conditions (Table 1). The results show that drought tolerant cultivar RD-2052 shows higher photosynthesis rate than other cultivars at 0, 10, 20 and 30 days. The reduction in photosynthesis on account of water stress in RD-2052 was lower as compared to the other cultivars such as RD-387, RD-2552 and RD-2035. BA spray tilts the balance towards increased photosynthesis in the over all water photosynthesis syndrome (Kudoyarove *et al.* 1993). The application of synthetic cytokinin helped not only in mitigating the effect of stress through maintaining a high CK/ABA ratio but also increased crop productivity per se via enhanced uptake of CO_2 by stomata (Livne and Vaadia, 1972). Water stress affects photosynthesis directly by affecting various biochemical processes involved in photosynthesis and indirectly by reducing the intake of CO_2 through stomatal closure in response to water stress (Gupta, 1975). The BA application has been found to increase photosynthesis and RUBISCO activity in wheat (Yonghua *et al.* (1997) and maize (Dong *et al.* 1995) under water stress conditions. The BA also increased the content of CK and lowered the level of ABA in the leaves and spikes of wheat as a result, photosynthesis activity in leaves increased leading to an increase in growth and yield (Elagina and Yakushkina, 1997).

Crops and varieties are found to differ with respect to their transpiration and stomatal behaviour under moisture stress conditions. Yadav *et al.* (1994) observed increased transpiration rate in wheat genotypes contrasting in drought tolerance in responses to BA treatment. The BA increased transpiration rate under both non-stress and water stress conditions (Table 2). The results indicate that drought tolerant cultivar RD-2052 showed higher response to this parameter on account of water stress. The increase in transpiration rate was elicited through enhanced effect of BA on stomatal opening. Gorny (2001) studied the effect of water stress on photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency in barley and found that water stress reduced these parameters. Higher rates of transpiration in barley than that of wheat can attribute to higher number of awns in barley (Johnson *et al.* 1974). Day *et al.* (1981) studied the effect of drought on transpiration flux density which was markedly non liner with low water potential and was similar for irrigated and unirrigated conditions. Livne and Vaadia (1965); Meidner (1967) and Gupta *et al.* (2001) reported stimulation of transpiration in barley leaves under water deficient conditions. Biddington and Thomas (1978) observed enhanced transpiration in excised oat leaves under the influence of different cytokinin treatments. Enhancement of stomatal opening and consequent increase in transpiration is a known response of cytokinin particularly when water supply is adequate (Livne and Vaadia, 1972; Purohit, 1993; Milborrow, 1981).

Stomatal conductance was also found to be enhanced by BA treatment while water stress reduced it at all the stages (Table 3). Results shows that stomatal conductance was higher in RD-2052 and RD-387 compared to RD-

2035 and RD-2552 under both non-stress and water stress conditions. The stomatal regulations is the basis of drought avoidance through water saving and water spending strategies (Jat *et al.* 1991 and Lal, 1991). Closure of stomata on accounts of water stress is well documented response (Hsiao, 1973; Farquhar and Sharkey, 1982; and Singh *et al.* 1993). The BA enhanced stomatal conductance by promoting stomatal opening. The role of cytokinin in stomatal opening is well documented and polyamines are known to mobilize K^+ and target KAT-1 like inward K^+ channel in guard cells and modulate stomatal movement (Liv *et al.* 2000). The BA restored transpiration to some extent under water limiting conditions in pot cultivar when water deficit in leaf were avoided, stomata continued to open and the stomatal resistance decreased (Raschke and Drake, 1970; Schulze *et al.* 1972). Enhancement of stomatal opening and consequent increase in transpiration is a known response of cytokinin particularly when water supply is adequate (Purohit, 1993; Milborrow, 1981). Curiously, cytokinin activity decreased under water stress (Warrier *et al.* (1987) and application of CK favoured stomatal opening (Itai and Benzioni, 1974). Kumar and Sankhala (1983) also supported maximum stomatal opening during rainy season and minimum during summer. Stomatal behaviour, which influenced the water status of the plants tissues during a period of water deficit is of importance in determining growth of the plants during drought (Bates and Hall, 1981). Yadav and Singh (1981, 1984 and 1985) reported lower stomatal conductance in barley under non-irrigated conditions.

Leaf temperature is an important physio-biological parameter which have been implicated in maintenance of optimum foliage temperature, rate of transpiration, evaluation of stress and identification of stress tolerance (Singh and Kanemasu, 1983; Idso *et al.* 1981; O‘Toole, 1984; Chopra and Kalra, 1988). The present investigation indicates the significant role of BA in leaf temperature in barley under water stress (Table 4). Drought tolerant cultivar RD-2052 shows lower leaf temperature under water stress conditions. This table also showed BA decreased leaf temperature under both non-stress and water stress conditions. The increase in leaf temperature on accounts of water stress in RD-2052 was lower as compared to the RD-387, RD-2035 and RD-2552. Miller *et al.* (1971) and Carlson *et al.* (1972) showed in barley that temperature and water saturation deficit of leaves were significantly correlated. The elevated leaf temperature indicates foliar warming on account of stomatal closure (Bora and Kumar, 1990). The relationship of transpiration with leaf temperature is also not unique and depends on interaction of complex physical processes like radiation, conduction and convection which in term are influenced by crop geometry, leaf position, leaf angle and leaf morphology (Gates, 1964 and Gupta *et al.* 2001).

Under water stress conditions, the relative water content decreased in all the four cultivars but its reduction in tolerant cultivars was lesser than the susceptible cultivars (Kumar *et al.* 1994; Ritchie *et al.* 1990; Sairam *et al.* 1990 and Deshmukh *et al.* 1991). Plant growth regulators act as modulators of plant responses to water stress. Application of polystilmulin K (Polymer form of Benzyladenine) on wheat increased leaf relative water content. (Grigoryuk *et al.* 1990). The present investigation indicates the significant role of BA in increasing relative water content in barley under both non-stress and water stress conditions (Table 5). Drought tolerant cultivars RD-2052 showed higher relative water content under both non-stress and water stress conditions. The Table also shows that BA increased RWC under both non-stress and water stress conditions. The reduction in relative water content on account of water stress in RD-2052 was lower compared to the drought sensitive cultivars RD-387, RD-2035 and salt tolerant cultivar RD-2552. Day *et al.* (1978) observed that barley may be more sensitive to drought at specific stages of its development or it may be affected by shortage at water of any time of its growing stage. RWC has been used as a reproducible index of plant water status (Mccaig and Ramagosa, 1991). The increase in relative water content as a results of BA treatment may be due to their enhanced effect in water absorption. Kumar and Purohit (1997) reported that cytokinin increased water absorption of crop plants.

Gupta *et al.* (2000) reported that exogenous cytokinin application reduced chlorophyll degradation in wheat cultivars under drought stress. Dong and Yu (1984) also observed delayed in chlorophyll degradation by cytokinin treatment. Elagina and Yakushkina (1997) also supported the finding that exogenous application of cytokinin increased chlorophyll stability index in drought sensitive and drought tolerant cultivars. Our results are also indicate similar trend. Treatment with BA increased chlorophyll content under both non-stress and water stress conditions. Drought tolerant genotype RD-2052 showed higher chlorophyll content than RD-387, RD-2552 and RD-2035 under water stress conditions (Table 6). The results also indicated that BA increased chlorophyll content under both non-stress and water stress conditions. Goswami and Srivastava (1988) reported delayed loss of chlorophyll, decreased protease activity and enhanced soluble protein content in leaves of sunflower by BA. Rani *et al.* (1988) reported that delaying of senescence in *Vigna radiata* by foliar application of 25 mg^{-1} l BA at the pre-anthesis stage should be viewed in terms of enhancement in growth contributing factors rather than the delay in senescence. The foliar spray of BA enhanced chlorophyll content while water stress reduced that.

BA exhibited some restoration of protein content under water stress in pot culture. Osmoproctants like glycine, betaine may act as a protein stabilizer. It was also suggested that water stress had interfered with protein synthesis or has induced proteolysis (Naylor, 1972). The protein content in all of the cultivars was found lower under water stress than non-stress conditions. This lower protein content may be the result of an increased activity of protein degrading enzyme protease under water stress. In barley increased activity of protease was found as a result of water stress (Kubis and Krzywanski, 1991). The present investigation also indicates the significant role of BA in increasing protein content in barley under water stress (Table 7). Drought tolerant cultivar RD-2052 showed higher protein content under water stress conditions. This table also showed that BA increased protein content under both non-stress and water stress conditions. The reduction in protein content on account of water stress in RD-2052 was lower as compared to the RD-387, RD-2552 and RD-2035. Leibovitch and Smith (1994) and Krawczyk *et al.* (1996) in barley observed that protein content was increase due to cycocel in stress conditions.

Blum *et al.* (1999) concluded that higher osmotic adjustment cultivar tends to yield better than low osmotic adjustment cultivar under drought stress. Salma and Awadalla (1989) also reported on increase in osmotic pressure (decrease in osmotic potential) in cotton when sprayed with 10 ppm kinetin. The present investigation is supporting the previous findings. Treatment with BA decreased osmotic potential under both non-stress and water stress conditions. Drought sensitive cultivar RD-2035 showed higher osmotic potential than RD-387, RD-2552 and RD-2052 (Table 8). The results also indicated that BA decreased osmotic potential under both non-stress and water stress conditions. The reduction in leaf osmotic potential on account of water stress may also lead to osmotic adjustment which can be inferred on the basis of the maintenance of positive turgor (Lange *et al.* 1976; Hanson and Hitz, 1982; Morgan, 1984). Yadav *et al.* (1997) also reported that BA partially alleviated the adverse effects of water stress on *Cicer arientinum* by simulating the accumulation of metabolites in the leaves. Khan *et al.* (1999) reported that osmotic adjustment by accumulation of metabolites and maintenance of turgor by decreasing osmotic potential at lower leaf water potential are the mechanisms for drought tolerance in wheat cultivars.

Cell membrane injury in RD-2052 and RD-387 was lower as compared to RD-2035 and RD-2552 under both non-stress and water stress conditions (Table 9). Results also indicated that BA decreased membrane injury under both non-stress and water stress conditions. Gupta *et al.* (2000) reported that exogenous cytokinin application decreased membrane injury in wheat cultivars under drought stress. The BA increased the activities of susperoxide dismutase, catalase and peroxidas in cold stressed seedling of paddy (Xuefeng *et al.* 1998), reduced lipid peroxidation in primary leaves of *Vigna mung* seedsling (Abbas and Singh, 1997). Navari-Izzo *et al.* (1993) and Nyachiro *et al.* (2001) also found that electrolyte leakage (EL) enhanced under water stress conditions. Singh *et al.* (1992) suggested that membrane stability can be used as a criterion for screening drought tolerant genotypes. It can be inferred that cytokinin can reduced membrane injury under water stress.

BA increased growth and yield attributes both under non-stress and water stress conditions (Table 10 & 11). The reduction in growth, yield and yield attributes on account of water stress was alleviated through BA treatment. Both growth and yield parameters which includes number of tillers per plant, number of spikelets per plant, number of grains per ear, grain yield per plant and 1000-grains weight are significantly increased in response to BA treatment. Previous findings indicates that BA promoted growth and increased productivity. BA increased photosynthetic activity leading to an increased in grain mass and productivity in wheat (Elagina and Yakushkina, 1997), increased seed yield in Pigeon pea (Singh and Kakralya, 1992) and increased tiller number, biomass and re-growth after cutting in barley (Christiansen *et al.* 1995).

6 SUMMARY AND CONCLUSION

The study entitled “Benzyladenine induced modulation of photosynthesis and productivity in barley under water stress conditions” was conducted in the cage house at SKN college of Agriculture, Jobner during the *rabi* season of 2002-2003 under pot culture with the following objectives:

1. To observe influence of BA induced modulation of photosynthetic attributes, growth and yield of barley under water stress conditions.
2. To observe effect of BA on productivity of barley under water stress conditions.

In the pot experiment, four barley cultivars namely RD-2052, RD-387, RD-2035 and RD-2552 were grown in ceramic pots under normal condition till anthesis stage. At anthesis, plants were sprayed with Benzyladenine (0, 5, 10 and 15ppm). Immediately after spray, half of the plants were subjected to water stress by withholding irrigation and the non-stressed plants were irrigated as frequently as needed.

In the experiments, zero day after Benzyladenine spray, observation were taken in both water stressed and non-stressed plants in between 10 to 12 AM on the first fully expanded leaf from the top (flag leaf). Observations including relative water content, photosynthesis, stomatal conductance, leaf temperature, transpiration rate, osmotic potential, membrane injury, chlorophyll content and protein content were monitored on the flag leaf at 0, 10, 20 and 30 days after anthesis. Total number of tillers, total number of spikelets per plant was taken at maturity stage. Number of grains, grains yield and 1000-grains weight were recorded after harvest.

BA treatment significantly increased photosynthesis, transpiration, relative water content, stomatal conductance, chlorophyll content and protein content whereas, significant decrease in membrane injury, leaf temperature and osmotic potential was recorded in all four cultivars under both non-stress and water stress conditions. The 10 ppm concentration of BA was effective under both non-stress and water stress conditions. The significant differences were also recorded on four cultivars with respect to these parameters.

The cultivar RD-2052 exhibited maximum rate of photosynthesis, transpiration, stomatal conductance, relative water content, chlorophyll content and protein content compared to other cultivars under both non-stress and water stress conditions. The membrane injury, leaf temperature and osmotic potential was minimum in RD-2052 at all the stages than other cultivars under both non-stress and water stress conditions.

Water stress registered a significant decrease in rate of photosynthesis, transpiration, stomatal conductance, relative water content, chlorophyll content and osmotic potential whereas, membrane injury, leaf temperature increased in all the four cultivars.

All growth, yield and yield attributes were significantly increased on account of BA treatment but water stress significantly reduced these parameter. Significant genotypes variation existed in all the parameters. Number of tillers, number of spikelets, grain yield, number of grains and 1000- seeds weight were highest in RD-2052 under both non-stress and water stress conditions. Benzyladenine increased productivity by reducing the adverse effect of drought stress on growth and yield attributes.

The correlation analysis indicated that photosynthesis, transpiration, stomatal conductance, relative water content and protein content were highly and positively correlated with grain yield whereas, leaf temperature and membrane injury was highly but negatively correlated. All growth and yield parameters were also positively and highly correlated with grain yield.

On the basis of this one year experiment it may be concluded that cultivar RD-2052 has higher yield potential than other cultivars under both non-stress and water stress conditions. Application of BA significantly increased all growth and yield parameters under both conditions. Spray at anthesis stage with BA at 10 ppm was found effective concentration for increasing the growth and yield of all cultivars under both non-stress and water stress conditions.

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