# STUDIES ON PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ACTION OF BRASSINOLIDE IN MAIZE (*Zea mays* L.) AND ITS EFFECT ON CROP PRODUCTIVITY

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# STUDIES ON PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ACTION OF BRASSINOLIDE IN MAIZE (*Zea mays* L.) AND ITS EFFECT ON CROP PRODUCTIVITY

Thesis submitted in part fulfillment of the requirements for the
degree of **Doctor of Philosophy (Agriculture) in Crop Physiology**
to the Tamil Nadu Agricultural University, Coimbatore

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**2004**

# CERTIFICATE

This is to certify that the thesis entitled "**Studies on physiological, biochemical and molecular action of brassinolide in maize (*Zea mays* L.) and its effect on crop productivity**" submitted in part fulfillment of the requirements for the degree of **Doctor of Philosophy (Agriculture)** in Crop Physiology to the Tamil Nadu Agricultural University, Coimbatore, is a bonafide record of research work carried out by **Mr. K. VENKATESAN** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Place: Coimbatore **Dr. G. PATHMANABHAN**
Date: Chairman

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**K.VENKATESAN**

*DEDICATED TO MY BELOVED SISTER*

# ABSTRACT

## STUDIES ON PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ACTION OF BRASSINOLIDE IN MAIZE (Zea mays L.) AND ITS EFFECT ON CROP PRODUCTIVITY

By

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Year: 2004

Experiments were conducted in the Department of Crop Physiology, TNAU, Coimbatore, to study the physiological, biochemical and molecular action of brassinolide on crop plants. The experiments were conducted in laboratory, greenhouse and farmer's field. The laboratory study consists of identification of optimum concentration of BL for improving the physiological and biochemical characters. Using the best treatment, various stress induction responses were carried out to overcome the stress. In the pot culture study, the combined effect of brassinolide with benzyl adenine and naphthalene acetic acid was studied. In the farmer's field, the effect of brassinolide on yield was studied in 12 crops.

The optimum concentration of BL for increasing the shoot length, root length, DMP, chlorophyll content, soluble protein and NRase activity was identified as 0.10 ppm, where the said parameters values were higher in the maize seedling treated with brassinolide. Brassinolide increased the resistance of maize plant against various abiotic stresses. Pre-treatment of 0.10 ppm brassinolide to maize seedling improved the chlorophyll, soluble protein and reduced the proline content. The enzyme activities of nitrate reductase, peroxidase and superoxide dismuatse were increased by the same treatment under drought, low and high temperature and salinity stresses. The leaf protein profile showed distinct stress protein developed due to drought, low and high temperature and salinity stress.

In the pot culture study, among the growth regulators, brassinolide significantly increased growth and yield. In the interaction effect, BL along with BA and NAA increased the plant height, root length, leaf area and Specific Leaf Weight (SLW). The Specific Leaf Area (SLA) was reduced by combined foliar spraying of the 0.10 ppm BL + 20 ppm BA + 10 ppm NAA.

The growth parameters like Net assimilation rate (NAR), Crop growth rate (CGR), Relative growth rate (RGR) and Total dry matter production (TDMP) were increased by foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA.

The leaf chlorophyll content ‘a’, ‘b’ and total and chlorophyll fluorescence ratio were increased by combined foliar spraying of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA. The biochemical constituents such as soluble protein and nitrate reductase activity were increased while, IAA oxidase activity was decreased in foliar sprying of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA .

The cob length, number of grains per cob, harvest index and yield were increased by foliar spraying of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA. The brassinolide had synergistic effect with NAA rather than BA.

In the on farm trails, the cereal crops responded very well to foliar application of brassinolide. The foliar spraying of 0.20 ppm BL in rice increased the yield and yield attributing parameters. In maize, sorghum and ragi foliar spraying of 0.10 ppm BL increased the number of grains, 1000 seed weight, TDMP and yield.

In pulses, foliar spraying of 0.10 ppm BL at vegetative and flowering stage increased the plant height, LAI, number of pods per plant, pod weight and yield. There was an increase in pod number, pod weight, kernel weight, HI and yield in groundnut due to foliar spraying of 0.20 ppm BL. In sunflower, 0.10 ppm foliar spraying at vegetative and flowering stages increased the number of seed, 100 seed weight and yield. Foliar spraying of 0.10 ppm at vegetative and flowering stages increased the number of sympodia, number of bolls, boll weight, TDMP, HI and yield in cotton.

In fruit crops, foliar spraying of 0.20 ppm BL increased the banana bunch weight, number fingers, fruit weight and yield. In grapes, foliar spraying of 0.10 ppm BL increased bunch weight, yield and fruit quality characters. In tomato, foliar spraying of 0.05 ppm BL increased the number of fruits, fruit weight, TDMP, HI and yield.

It is concluded that, pretreatment of 0.10 ppm brassinolide reduced the effect of drought, low and high temperature and salinity stress by means of increased activities of catalase, peroxidase and SOD and production of stress proteins. This results in the increased activity of NRase, Chlorophyll, soluble protein to overcome the stress responses and improved the shoot length, root length and dry matter. The interaction effect of brassinolide showed that, BL have synergistic effect with NAA in increasing the plant height, LAI, SLW and TDMP due to enhanced activity of NRase, soluble protein and chlorophyll content. Effect of brassinolide on different crops showed that foliar application of 0.20 ppm BL in rice, groundnut and banana, 0.10 ppm BL maize, sorghum, ragi, black gram, green gram, sunflower, cotton and grapes and 0.05 ppm BL tomato increased the yield and yield attributing characters.

# ABSTRACT

## STUDIES ON PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ACTION OF BRASSINOLIDE IN MAIZE (Zea mays L.) AND ITS EFFECT ON CROP PRODUCTIVITY

Student**: K. Venkatesan** Chairman : **Dr. G. Pathmanabhan**

Experiments were conducted at Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, to study the physiological, biochemical and molecular action of brassinolide on crop plants. Brassinolide treated maize seedlings increased the shoot length, root length and dry matter accumulation and the biochemical characters like chlorophyll, enzyme activities. The induction of drought, low and high temperature and salinity stress on enzyme activity was improved by pretreatment of brassinolide at 0.10 ppm and the scavenging enzymes were also improved by which the free radical produced due to stress condition were detoxified. In the pot culture, experiment combined foliar application of 0.10 ppm BL, 20 ppm BA, and 10 ppm NAA increased the plant height, root length, leaf area, chlorophyll, soluble protein, cob length number of grains and yield of maize. The brassinolide and NAA had synergetic effect on the yield and yield characters.

Effect of brassinolide on different crops showed that in rice, groundnut and banana foliar application of 0.20 ppm BL increased the yield and yield attributing characters. Where as in maize, sorghum, ragi, black gram, green gram, sunflower, cotton and grapes 0.10 ppm BL increased the yield and yield characters. In tomato, foliar spraying of 0.05 ppm BL increased the yield and yield attributing characters.

# List of Abbreviations

| | | |
|---|---|---|
| BR | - | Brassinosteroids |
| BA | - | Benzyl adenine |
| BL | - | Brassinolide |
| CAT | - | Catalase |
| FW | - | fresh weight |
| HI | - | Harvest Index |
| Mpa | - | Mega Pascal |
| NRase | - | Nitrate reductase |
| ppm | - | Parts per million |
| SOD | - | Superoxide dismutase |
| SDS | - | Sodium dodecyl sulphate – polyacrylamide gel electrophoresis |
| TEMED | - | N, N, N1, N1, - Tetramethyl ethylene diamine |
| SLW` | - | Specific leaf weight |
| SLA | - | Specific leaf area |
| NAR | - | Net assimilation rate |
| CGR | - | Crop growth rate |
| RGR | - | Relative growth rate |
| TDMP | - | Total dry matter production |
| g | - | Gram |

NAA - Naphthalene acetic acid
ha - hectare
h - Hour
kg - kilogram
mg - milligram
EC - Emulicifying concentrate
PEG - Polyethylene glycol

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## CHAPTER I

# INTRODUCTION

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***A plant hormone is an organic compound synthesized in one part of a plant and translocated to another part, where in at very low concentration it creates physiological response***

**Salisbury and Ross**

Brassinosteroids (BRs) are now considered as a sixth group of phytohormone (Bishop and Yokota, 2001). Exogenous application of brassinosteroids to plants at nanomolar to micromolar concentrations produces a wide spectrum of physiological response (Nakamura *et al*., 2003); it includes promotion of cell elongation and its division, enhancement of treachary element of gravity–induced bending, promotion of ethylene biosynthesis, and enhancement of stress resistance.

Brassinolide (BL) is a steroidal growth substance, which was first isolated from *Brassica napus* L. pollen by Grove *et al* in 1979. Since its discovery, 59 brassinosteroids, (54 unconjugated and 5 conjugated) have been isolated from 58 plant species including 49 angiosperm (12 monocotyledon and 37 dicotyledons), 6 gymnosperms, 1pteridophyte, 1 bryophyte and 1 chlorophyte. It proved the wide distribution of brassinosteroids among plant kingdom. (Bajguz and Tretyn, 2003).

Brassinosteroids are group of naturally occurring polyhydroxy steroidal compounds. Natural brassinosteroids so for identified have common 5α – Cholestan skeleton and their structural variations come from the kind and orientation of functionalities on the skeleton.

Brassinolide, 24 – epibrassinolide and 28 – homobrassinolide are the three biologically active brassinosteroids, being widely used in physiological studies.

Brassinosteroids are present in the plants in extremely at low concentrations. The pollen and immature seeds contain 1 – 100 ng per g fresh weight, while shoot and leaves have still very low content. Brassinosteroids are highly mobile in the plant system. They promote growth, seed germination, rhizogenesis, flowering, senescence, abscission and maturation. Brassinosteroids also confer resistance to plants against various abiotic stresses. Due to multiple effects, brassinosteroids are considered as plants hormones with pliotropic effects.

Brassinosteroids improved the tolerance to low temperature in cucumber (Katsumi, 1991), high temperature in tomato (Mazorra *et al*., 2002), drought in cucumber (Pustovoitova *et al*., 2001) and salinity in rice (Anuradha and Rao, 2003). The role of brassinosteroids in protecting the plants against environmental stresses will be an important research theme and may contribute greatly to the usage of brassinosteroids in different agricultural ecosystem.

Foliar spraying of brassinolide, 28 – homobrassinolide and 24 – epibrassinolide increased the yield of rice, wheat, corn, legumes, groundnut, radish, lettuce, mustard, watermelon, cucumber, tobacco and grapes.

In the field of plant physiology, brassinosteroids play a vital role and provide ample scope in the area of plant hormone research. It also has significance for the plant biotechnology industry. Recent studies have confirmed the significant role of brassinosteroids in overcoming physiological constraints and thereby boosting up crop productivity.

With this background, an investigation was carried out to study the physiological, biochemical and molecular action of brassinolide on the productivity of agriculture and horticulture crops with the following objectives.

- to study the biological activity of brassinolide under *invivo* and *invitro* conditions.
- to elucidate the physiological, biochemical and molecular actions of brassinolide under abiotic stress situation and
- to assess the effect of brassinolide on the productivity of selected agricultural and horticultural crops.

## CHAPTER II

# REVIEW OF LITERATURE

Plant growth is a complex, yet well organized and coordinated process. As early as 1880, Julius Sachs suspected the existence of "chemical messengers" which bring co-ordination of growth among different parts of the plant. Until quite recently, plant growth and development was thought to be regulated only by five groups of hormones, namely auxins, gibberellins, cytokinins, abscisic acid and ethylene. However, there is compelling evidence for considering brassinosteroids, a group of steroidal substances first isolated from the pollen of rape plant (*Brassica napus* L.) as the sixth group of phytohormones (Seeta Ram Rao *et al*., 2002).

Brassinosteroids have unique biological effects on plant growth and development (Sasse, 1997 and 1999). The biosynthetic and metabolic pathways with enzymatic studies and the molecular mode of action of BRs have been investigated (Clouse and Feldmann, 1999; Bishop and Yakota, 2001; Friederichsen and Chory, 2001; Mussig and Altmann, 2001; Schneider, 2002). In addition to their role in plant development, BRs have the ability to protect plants from various environmental stresses, including drought, extreme temperature, heavy metals, herbicidal injury and salinity (Sasse, 1999).

A thorough review of Brassinosteroids and its physiological, biochemical and molecular mechanism and productivity of crops are presented hereunder.

## 2.1. Brassinosteroids

The recognition that certain pollen extracts caused growth promotion, which paved the way for the discovery of BRs in plants. As early as, in 1970, Mitchell *et al*. (1970) screened pollen from nearly sixty species and half of them caused growth of bean seedlings. They also identified, notably the pollen from rape (*B. napus*) produced an unusual response that combined elongation with swelling and curvature (the typical gibberellins response). The growth substance from various pollen sources was name as “brassin” (Yokota, 1999). In, 1974, a collective effort to identify the active factor of brassins was initiated by USDA scientists at the Northern Regional Research Center (NRRC), Peoria, the Eastern Regional Research Center (ERRC), Philadelphia and the Beltsville Agricultural Research Centre (BARC), Maryland. About 227 kg of bee- collected rape (*B. napus*) pollen was processed through a pilot-plant size solvent (2- propanol) extraction procedure at ERRC and partially purified at BARC. Four mg of crystals was obtained at NRRC and was subjected to X-ray crystallographic analysis to determine the structure. This biological active plant growth promoter was named as “brassinolide”(BL) [(22R, 23R, 24S) - 2α, 3α, 22, 23 – tetrahydroxy – 24 – methyl – B – homo – 7 – oxa - 5α - cholestan – 6 - one] and was found to be a steroidal lactone with an empirical formula of $C_{28}$ $H_{48}$ $O_6$ (MW = 480) (Grove et al., 1979). As the first steroidal hormone with growth-promoting nature was obtained from *B.napus*, the name brassinosteroids was given to this new class of substance.

The second BR, termed Castasterone (CS), was isolated in 1982 by Yokota *et al*. (1982) from the insect galls of Chestnut (*Castanea crenata*). Since the discovery of BL, 59 BRs, among them 54 unconjugated and 5 conjugated BRs, have been isolated from 58 plant species including 49 angiosperms (12 monocotyledons and 37 dicotyledons), 6 gymnosperms, 1 pteridophyte (*Equisetum arvense*), 1 bryophyte (*Marchantia polymorpha*) and 1 chlorophyte, the algae (*Hydrodictyon reticulatum*). Thus BRs are widely distributed in plant kingdom, including higher and lower plants (Bajguz and Tretyn, 2003).

BRs were detected in all plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers and grains. Other interesting tissues are insect and crown galls (Fujioka and Sakurai, 1997), for example the galls of *Castanea crenate*, *Distylium racemosum* or *Catharanthus roseus* where BRs had also been found. These plants have higher levels of BRs than the normal tissues. Generally, pollen and immature seeds are especially rich source of BRs, while the concentrations in vegetative tissues are very low compared to those of other plant hormones. In the pollen of *Cupressus arizonica* the concentration of 6 – deoxo TY can be about 6400 – fold greater than BL. (Bajguz and Tretyn, 2003). In general, young growing tissues contain higher levels of BRs than mature tissues (Takatsuo, 1994).

Pollen and immature seeds are the richest sources with range of 1 – 100 ng $g^{-1}$ fresh weight, while shoots and leaves usually have lower amount of 0.01 – 0.1 ng $g^{-1}$ fresh weight (Horan *et al*., 1984). The highest concentration of BR, 6.4 mg of 6 – deoxyo TY per 1 kg pollen, was detected in *Cupressus arizonica* (Griffiths *et al*., 1995; Clouse and Sasse, 1998; Fujioka, 1999).

Among the plant sources investigated, immature seeds of *Phaseolus vulgaris* contain a wide array of BRs, there are 23 free BRs and 2 conjugated (Kim *et al*., 2000; Park *et al*., 2000; Yokota *et al*., 1996). The occurance of BRs in monocotyledons has been demonstrated from four families including twelve plant species (Sakurai and Fujioka, 1993). The presence of BRs in dicotyledons has been demonstrated from three subclasses namely, Apetalae is represented by 6 families including 9 plant species, Chloropetalae is represented by 7 families including 20 plant species and Sympetalae is represented by 7 families including 9 plant species (Bajguz and Tretyn, 2003). The occurrence of BRs in gymnosperms has been reported from six conifers.

Among all naturally occurring BRs, CS and BL are the most important BRs because of their wide distribution as well as their potent biological activity (Kim, 1991; Fujioka, 1999). BRs are derived from the 5-cholestane skeleton and their structural variations come from the type and position of functionality in the A/B rings and the side chain. (Yakota, 1995 and 1997). BRs are a group of naturally occurring and are classified into C27, C28 and C29 steroids (Mandava, 1988; Fujioka, 1999) BR1, BR2, …..BRn designation should be applied only to natural BRs and not to other derived by synthesis and are depending on the alkyl – substitution pattern of the side chain.

With respect to the A – ring, BRs having vicinal hydroxyl groups at C-2$\alpha$ and

C-3$\alpha$ represent a general structural feature of most active BRs, such as BL and CS. Decreasing order by structure activity relationship suggests that the $\alpha$ - oriented hydroxyl group at C-Z is essential for greater biological activity of BRs in plants (Adam, 1994; Bishop *et al*., 1999; Schmidt *et al*., 2000). With respect to the B – ring oxidation stage, BRs are divided into 7-oxalactone, 6-ketone (6-oxo) and 6- deoxo (non-oxidized) type. In general 7- oxalactone BRs have stronger biological activity than 6-ketone types and 6- deoxo types (Bishop *et al*., 1999; Fujioka, 1999). According to the cholestane side chain, BRs are divided into eleven types with different constituents at C-23, C-24 and C-25 (Watanabe *et al*., 2000; Yokota *et al*., 2001).

A detailed understanding of how endogenous BR levels are regulated via synthesis, break down and conjugation is an essential component of a molecular model of BR action. Campestrol was predicted to be the plant sterol progenitor of BL based on side chain structure and the relative biological activities, co-occurrence and molecular structure of teasterone, typhasterol and castasterone suggested that, BL was synthesized from campestrol through these intermediates (Yokota *et al*., 1991). Biosynthesis of BL can be occurred either through early C6 oxidation pathway or through late C6 oxidation pathway. Representatives from both pathways co-occur in many plants, so both could be widespread in the plant kingdom (Fujioka and Sakurai, 1997). The 6 – oxo type BRs are assumed to be biosynthetic precursors of the corresponding 7 – oxolactone compounds especially CS for BL (Morishita *et al*. 1983).

The general synthetic scheme for BR involves modification of the side chain to introduce the desired substituents and introduction of a glycol group at C 2 and C 3 of ring A and the oxygen functions in ring B. The side chain of BL and its analogues has been synthesized using two approaches, both involving readily available sterols as the starting materials (Mandava, 1988). Both the methods require the introduction of vicinal glycol group at C –22 and C –23 to define the stereochemistry. Once the desired side chain is built, both methods introduce the desired substituents in rings A and B.

## 2.2.Brassinosteroids biosynthetic mutants

The role of brassinosteroids in plant growth and development was unclear until the recent cloning of brassinosteroids biosynthetic mutants, *det* 2, *cpd,* and *dwf* 1. The best characterized of these was *det* 2, a de-etiolated *Arabidopsis* mutant that was originally proposed to be a negative regulator of photomophogensis since it had shown characteristics of light grown plants even when grown in the dark (Chory *et al*., 1991). The DET 2 protein is a reductase and functions in the reduction of campesterol to campestanol, one of the initial steps of brassinosteroids biosynthesis. Evidence that DET 2 encodes a biosynthetic enzyme comes from the fact the BRs, but not other growth regulators, rescued the *det* 2 phenotype (Clouse and Sasse, 1998).

The *Arabidopsis* mutant *cpd* (constitutive photomorphogenesis and dwarfism) shows a very similar phenotype to the *det* 2 mutants. Application of brassinosteroids restores cell elongation of hypocotyls, leaves and petioles in both the dark and light, rescues de-etiolating in the dark and alleviates male sterility (Szekeres *et al*., 1996). This supports view that brassinosteroids have several important physiological roles in plants.

The *Arabidopsis dwf* 1 and its alleles *dim* 1 and *cbb* 1 are rescued to wild type by BR treatment (Kauschmann et al., 1996). In the dark-grown *dim* mutant, the de-etiolating phenotype (such as open cotyledons and primary leaves) is observed, but no expression of chlorophyll a/b binding protein, chaleone synthase or Rubisco occurs (Takahashi, *et al*., 1995).

It is suggested that dwarfism of plants is caused by impaired gibberellin biosynthesis and /or signaling. However, not all short-stature mutants can be fully explained in terms of gibberellines perception. Based on the idea that mutants in brassinosteroids biosynthesis will have a dwarf phenotype, the dwarf pea mutants *lka* and *lkb* were selected as potential brassinosteroids mutants (Nomura *et al*., 1997). These dwarf mutants have a normal gibberellin status and are not responsive to gibberellins. Application of brassinolide, castasterone, typhasterol, 3-dehydroteasterone and teasterone markedly elongates the cell length, completely rescuing the short phenotype. Thus brassinosteroids are important plant hormones and have significant effect on plant growth and development.

## 2.3. Molecular mechanisms of BR action

### 2.3.1. Mode of action of brassinosteroids

A gene expressed in elongating soybean, BRU 1 (‘brassinosteroids upregulated’), has been isolated; it is activated post-transcriptionally within 2 hours by brassinosteroids, but other plant hormones never activate BRU 1 (Zurek and Clouse, 1994). The sequence of BRU 1 is identical with various genes encoding xyloglucan endotransglycosylase (XET) (Clouse, 1996). An increase in BRU 1 transcript levels correlates with increases in stem elongation in response to brassinosteroids and also with brassinosteroids-mediated increases in plastic extensibility of the cell wall, suggesting that brassinosteroids may promote elongation by altering the mechanical properties of cell wall. Moreover, the increased concentrations of applied BR during early stages of elongation lead to a linear increase in extractable xyloglucan endotransglycosylase activity in the epicotyls (Oh *et al*., 1998).

### 2.3.2. Gene regulation by Brassinosteroids

The first cloning and characterization of gene regulation primarily by brassinosteroids was accomplished in the soybean apical epicotyl elongation system (Zurek *et al*., 1994). Brassinosteroids promotes elongation independently of auxin in this system and differential colony hybridization was employed to identify the gene BRU 1 that is regulated specifically by brassinosteroids. Transcriptional regulation of gene expression by BRs has been demonstrated with the TCH4 gene of *Arabidopsis* (Xu *et al*., 1995). In addition to transcriptional regulation, post transcriptional regulation of gene expression by steroid hormones has also been observed (Nielsen and Shapiro, 1990) and BRU 1 gene of soybean is an example of such post transcriptional gene regulation by a plant steroid (Zurk and Clouse, 1994).

### 2.3.3. Brassinosteroids Signal transudation

Hormones in genitive mutants play an important role in unrevealing signal transudation pathways in plants. A BR-insensitive mutant has been identified in *Arabidopsis* (Clouse *et al*, 1996), which has the ability to elongate roots in the presence of inhibitory concentrations of BR with respect to wild type. The genetic analysis of mutants, which are insensitive to BR, revealed the BRI 1 *Arabidopsis* gene, which encoded the leucine rich repeat receptor - like kinase (LRR-RLK). (Sasse, 1997; Li and Chory, 1997; He *et al*., 2000; Oh *et al*., 2000; Bishop and Yokota, 2001 and Wang *et al*., 2001). This serine / threonine kinase belongs to the wide class of LRR – RLKs and the BRI 1 localization in the cell plasma membrane.

The BRI 1 is a high molecular weight protein of 1196 amino acid residues in length. Its structure is typical for LRR-RLKs. The cytoplasm domain is functionally active and evidently serves for signal transduction after the interaction between the N-terminal domain and phytohormone (Romanov, 2002). Direct assays of BL binding confirmed the role of BRI 1 as the receptor (Wang *et al*., 2001). The number as BL- binding sites and the strength as the response to this phytohormone were correlated with the amount of BRI 1 protein. Brassinolide – binding activity was co-precipitated with BRI 1 and depended on the intactness of its extra cellular domain.

The treatment of *Arabidopsis* seedlings with BL results in the BRI 1 auto phosphorylation (Oh *et al*., 2000; Bishop and Yokota, 2001) indicating the capacity of protein to transmit a signal across the plasma membrane. BRI 1‘s kinase domain displays serine/threonine kinase activity in vitro, and BL treatment in plants induces BRI 1 by auto phosphorylation (He *et al*., 2000). Thus, all available evidence strongly implicate BRI 1 as a critical component of the BR – receptor complex, which likely transduces the BR signal to down stream targets through its kinase domain.

## 2.4. Effect of Brassinosteroids on cell division

The promotion of growth by BR was due to both cell division and cell elongation. Clouse and Zurek (1991) reported that 24-epibrassinolide increased cell division in cultured parenchymatous cells of *Helianthus tuberosus*. In Chinese cabbage protoplasts, 24 – BL when applied with 2,4-D and kinetin, promoted cell division and enhanced cluster and colony formation (Nakajima *et al*., 1996). Oh and Clouse, (1998) observed similar enhancement in the rate of cell division in the leaf protoplasts of *Petunia hybrida* by the use of brassinolide. Restoration of leaf size after administration of BL to the mutant’s *det* 2 and *dwf* 1 of *Arabidopsis* could not be accounted so solely by expansion, supporting a role of BRs in cell division (Nakaya *et al*., 2002). 24-epibrassinolide can substitute for cytokinin in the culture of *Arabidopsis* callus and suspension cells (Sasse, 2003).

## 2.5. Effects of Brassinosteroids on cell expansion.

Whatever the molecular signals, expansion is contingent upon transport of ions, uncharged osmotica, and water across the cell and vacuolar membranes, and upon a plastic cell wall. The microscopic examination of cell files in various plant organs shows that dwarf BR mutant cells are shorter than the corresponding wild–type cells, confirming the role of BRs in elongation (Clouse *et al*., 1996). Seedlings of another *Arabidopsis* mutant, *det* 3 develop as light – grown plants in the dark and have reduced ability to respond to BR. The gene was shown to encode subunit C of the vacuolar H+ - ATPase, which has a role in the control of elongation and also in meristem activity. The mutant has an alternative method of assembly of this ATPase, and regulation of its activity by different signals could occur through different configurations of the protein complex, with a BR signal transduced via the DET 3 protein (Schumacher *et al*.1999).

BR also affects membrane permeability and selectivity in the presence of toxic metals, and the metal ions can be ranked in the order in which BR treatment ameliorated their effects reported by Khripach *et al*., 1999 and 2000. Uozo *et al*. (2000), suggested that BRs are essential for gibberellins sensitivity and that there is crosswalk between BR and gibberellin signaling. The role of membrane-bound endo-1, 4-β -D-glucanases (Molhoj *et al*., 2002), Xyloglucan endotransglucosylation and endo hydrolysis (Doblin *et al*., 2002) in the construction and modification of the cell wall illustrate that further elucidation effects of BRs in the complex relationship and information flow between microtubules, the plasma membrane, and the cell wall in the process of expansion will be very valuable.

In the mesophyll suspension cultures of *Zinnia* permitted the study of expansion only and confirmed at the cellular level that 24-epibrassionlide promoted elongation without significant radial expansion, in contrast to the effect of gibberellins. Unlike the effects of light, the effects of hormones began only after 48hr. (Lee *et al*., 2000). The asymmetric expansion occurring in rice lamina inclination in response to BL involves a calcium – dependent protein kinase (Yang and Komatsu, 2001), which may also hint at species differences, where as Sharma *et al*. (2001), in a study of mitogen-activated protein kinase and calcium – dependent protein kinase in BR signaling in rice, suggested, like Hu *et al*. (2000), that there are other BR receptor besides BRI 1 in plants. Hong *et al*., (2002) considered that the lack of BRs might affect determination of sites for cell division. Turk *et al*. (2003) reported that, in *Arabidopsis*, pea, and tomato the promotion of cell expansion and regulation of photomorphogensis are among the most important roles of BR.

Yamamoto *et al*., (1997) suggested that BRs are most important in the later stages of vascular development. In *Zinnia*, endogenous BRs were shown to increase markedly before stage III of the differentiation process, where secondary wall formation and cell death occur. Yamamoto *et al*., (2001) propose a signaling pathway where by extracellular BRs are detected by a surface receptor like BRI 1, and a signal cascade follows and the final stage of differentiation is then initiated and the processes of lysis of the vacuole and modification of the treachery element follow (Kuriyama and Fukuda, 2002).

## 2.6. Physiological role of Brassinosteroids

Brassinosteroids are a novel group of plant hormones that regulate cell elongation, cell expansion, cell division, reproductive and vascular development, membrane polarization and protein pumping, retard leaf abscission and enhance resistance to stress (Adam, 1994; Creelman and Mullet, 1997). Sasse (1997) suggested that BRs are probably ubiquitous in the plant kingdom. Ubiquity in the plant kingdom in considered as one of the per- requisites to consider a substance as a plant hormone.

BRs are present in the plants in extremely low concentrations and are found physiologically active at that concentration. BL is active at 0.01-µg concentrations in bean second – internode test and at 0.0005 µg concentration in rice lamina inclination assay. BRs are highly mobile in the plant system. Exogenously applied BL to roots of intact young tomato and radish plants affected the hypocotyls and petioles (Takatsuto *et al*., 1983). Gregory and Mandava (1982) reported that treatment of BR on the bases of mung bean hypocotyls caused elongation of epicotyls. These studies clearly indicated the mobility of BRs in the plant systems.

Two bioassay, which are highly specific and sensitive to BRs, were developed. They are bean second internode test and rice lamina inclination test. The bean second internode test was developed during the isolation of BL from the pollen of rape (Groove *et al*., 1979) Mandava and Thompson (1983) reported that, when BL in lanolin paste was treated in the cuttings of the second internode from seedlings of *Phaseolus vulgaris*, it showed elongation, curvature, swelling or splitting. The effect depended on the amount of BL. BL caused elongation, curvature and swelling at lower concentration of 0.01 μg. The rice lamina inclination test originally developed as an auxin bioassay (Maede, 1965) was found to be highly responsive to BRs. From etiolated seedlings of rice, segments consisting of the second leaf lamina, lamina joint and sheath were excised and floated on distilled water containing BRs. Bending of the lamina joint was observed, which was proportional to the concentration of the compounds applied.

## 2.7. Effect of Brassinosteroids on germination

As early as 1949, several plant growth regulators were tested and they acted as germination regulators. Now has been well established that BRs also promote seed germination (Sasse, 1997). Sasse *et al*., (1995) observed in *Eucalyptus camaldulensis* with 24 – EBL seed treatment resulted in substantial improvement in the percentage of seed germination. The application of BL caused enhancement in seed germination of *Lepidium sativa* (Jones- Held *et al*., 1996). Vardhini and Rao (1996) reported that BL, 24 – EBL and 28 – HBL promoted the germination of groundnut seeds.

The ability of BRs to promote seed germination was also observed in the case of rice (Yamaguchi *et al*., 1987; Dong *et al*., 1989), wheat (Sairam *et al*., 1996), broomrape (Takeuchi, 1995), groundnut (Vardhini and Rao, 1998) and tobacco (Leubner- Metzger, 2001). In *Arabidopsis*, Steber and Mc Court (2001) found 24 – EBL and BL treatment increase the seed germination in mutants whose gibberellines biosynthesis is severely inhibited. Hayat *et al*. (2001) reported that soaking of the wheat seeds in 3 μM of HBR for longer duration produced the healthiest seedlings than water soaked seeds.

### 2.8. Effect of brassinosteroids on growth Parameters

### 2.8.1. Plant height

Plant height is one of the morphological parameters influenced by the application of growth regulators. Gregory (1981) reported that brassins increase the growth of barley seedlings. Cerana *et al*., (1983) obtained similar result in Azuki bean. Clouse *et al*., (1992), reported that $10^{-7}$M BL increased the epicotyl length of soybean than the control. Guan and Roddick (1988) observed that shoot length in intact seedling was promoted by epibrassinolide, but a doubling in length required treatment with 1µM Roddick and Ikekawa (1992) reported that the monocotyledon and dicotyledonous seedlings showed increased shoot development when 24-epibrassinolide was given at seedling stage. Daniel *et al*. (1984) studied that the effect of BR on soybean and reported that enhanced growth of seedling due to increased cell wall extensibility and cell wall loosening. Vardhini and Rao, (1996), reported that all the three BRs, BL, 24-epibrassinolide and 28-homobrassinolide promoted the shoot length.

Lini (2001) reported that BL at 0.5 ppm increased the plant height in cotton. In rice, application of BL to seedlings increased the shoot length significantly (Fujii and Saka, 2001). Amzallag (2001) observed similar increase in the shoot length and shoot fresh weight by the application of 1.0-10nm 24-epibrassinolide. Ramesh *et al*. (2001) reported BL at low concentration increased the plant height. Vardhini and Rao (2001) observed that 28-HBL increased the shoot length in tomato. Nagasubramaniam (2003) reported that in baby corn application of BL increased the plant height than the other treatments and control.

### 2.8.2. Root Length

Root development is an important aspect of plant development and it has more response to growth substances. Yopp *et al*. (1981) reported promotion of root elongation in intact cress seedlings with BR concentrations of 0.01 and 0.1m. Romani *et al*. (1983); Cerana *et al*. (1984) reported that BRs are capable of promoting root growth. Guan and Roddick (1988), observed that in mung bean cuttings, 0.1µm 24-

epibrassinolide consistently inhibited adventitious root formation and elongation in both hypocotyl and epicotyl. Allevi *et al*., (1988) reported the incubation of BR would not affect the growth of the maize root. Rooting ability of rice seedlings was significantly increased with foliar application of BRs (Wang and Cheng, 1993).

Roddick and Ikekawa, (1992), observed, in maize that root development tended to be inhibited only by the highest concentration of EBL. Roddick (1994) reported that at higher concentration BRs inhibited the growth and the inhibitory activity occurred in the order BL > 24EBL > 22,23,24-trisepibrassinolide > 28-HBL. In groundnut, foliar application of 28-HBL significantly increased the root growth and fresh weight (Vardhini and Rao, 1998). BRs promoted root growth of maize and sugar beet (Davidtchuck, 1999). Sujatha, (2001) reported that application BR increased the root length in green gram. Vardhini and Rao, (2001) observed increased root growth in tomato by the application of HBL and EBL. Hayat and Ahmad (2003) observed that soaking with HBL decreased the root length at higher concentration in the *Lens culinaris* seedlings. Brassinosteroids were required for lateral root development in Arabidpsis and BR synergistically with auxin to promote lateral root formation (Fang Bao, *et al*., 2004).

Guan and Roddick (1988) observed that at lower concentration of 1µM no inhibition of root, but at higher concentration of 10µm had major effect on root was observed in tomato. Hunter (2001) reported that EBL, retarded root growth in soybean.

### 2.8.3. Leaf Area and Leaf Area Index

Number of leaves, leaf area and leaf area index (LAI) plays an important role in determining the dry matter production of a crop and subsequently the yield. Gregory, (1981) observed that treating seeds with brassin accelerated the leaf growth. Krizek and Mandava (1983) observed that there is reduction in the total leaf area and even a greater reduction in area of the trifoliate leaves when the bean plant was treated with BR. Braun and Wild (1984) reported that foliar application of BR increased the dry and fresh weight of the leaves of wheat and mustard plants. The foliar application of epibrassinolide promoted leaf area in tobacco (Ikekawa and Zhao, 1991). In watermelon, epibrassinolide enhanced the leaf area and growth of the plants (Wang *et al*., 1994). Foliar application of synthetic BR, DAA-6 increased leaf length and width in tobacco (Diz *et al*., 1995).

Foliar spray of BL showed improvement in LAI of rice (Maibangsa *et al*., 1999). Bindu (2000) observed that application of BR markedly increased LAI in groundnut. Hayat *et al*. (2001) reported that, the seedlings of wheat raised from the grains per-treated with HBR possessed significantly higher leaf number, fresh and dry weight as compared to control. Sujatha (2001) observed in green gram, foliar spraying of 0.1 ppm BR at35 and 45 DAS effectively increased the leaf area and an optimum LAI could be achieved for maximum productivity. Lini (2001) observed similar effect in cotton when BR 0.5 ppm sprayed at flowering and boll development stages. Nagasubramaniam, (2003) reported that BR showed positive effect in improving number of leaves per plant and also increased the leaf and leaf area index in baby corn.

### 2.8.4. Specific leaf weight (SLW) and Specific leaf Area (SLA)

Specific leaf weight and Specific leaf Area are the measure of leaf thickness and has high correlation with dry matter production. Foliar application of BR increased the thickness of the 3rd leaf of wheat by increasing the leaf dry weight (Braun and Wild, 1984). Krizek and Mandava (1983) recorded significant increase in specific leaf weight of the primary leaves and the first trifoliate leaf of bean plant. Lini (2001) recorded increased SLA by leaf foliar application of BR. Sujatha, (2001) reported that in green gram application 0.1 ppm BR showed a significant increase in SLW than other treatments and reverse effect in the case of SLA. Nagasubramaniam (2003) in baby corn, inferred that application of BR increased the SLA and SLW and in later stage BR performed well by reducing the SLA comparatively by increasing leaf weight.

### 2.8.5. Crop growth rate (CGR)

Crop production is determined by the CGR as a function of light interception by the leaf area of a crop (Whigham, 1983). HBR application increased the growth rate by 19 per cent in jack pine seedlings (Rajasekaran and Blake, 1998). Umadevi (1998) and Bindu (2000) reported that BL application increased CGR in sesamum and groundnut. Lini (2001) also observed similar findings in cotton. In green gram, Sujatha (2001) reported maximum CGR with the foliar spraying of BR. (Nagasubramaniam (2003) observed increased CGR by the application of BR in baby corn.

### 2.8.6. Relative Growth rate (RGR)

Pandey et al., (1981) observed a positive relationship between RGR and biomass production in cowpea. Bindu (2000) reported that foliar application BR in groundnut had higher relative growth rate than the control. In cotton, BR gave similar result as noticed by Lini (2001). In green gram, Sujatha (2001) reported maximum RGR with the foliar spraying of 0.1 ppm BR. Nagasubramaniam (2003) observed increased RGR by the application of BR in baby corn.

### 2.8.7. Total dry matter production

Photosynthesis is the basic physiological processes determining total dry matter production (TDMP). This is because the carbon contributes more than 80 percent of the TDMP fixed during the photosynthesis. Crop should produce optimum dry matter for good yield. Total dry matter production has a direct relationship with crop productivity. Sairam, (1994) reported that foliar application of 0.05 ppm HBR increased the biomass production in wheat. EBL application in watermelon was found to increase the TDMP of the plant (Wang *et al*., 1994).

Pipattanawong *et al*. (1996) reported that BR treatment increased TDMP in day neutral strawberries. Vardhini and Rao (1998) observed that BL treatment increased the vegetative growth and total biomass production in groundnut. Maibangsa *et al*. (2000), applied BR 0.5 ppm on rice crop and they observed that there was an increased TDMP than the control. Hayat *et al*. (2000) reported that spraying 28-HBL increased the total dry matter in mustard plant. Wheat grain treatment with 3µm 28-HBL for 8 or 12hrs showed significant increase in dry weight per plant (Hayat *et al*., 2001). Lini (2001) showed that application BR 0.5 ppm on cotton increased the TDMP. Nagasubramaniam (2003) reported that application BR on baby corm improved the TDMP.

## 2.9. Biochemical characters

### 2.9.1. Chlorophyll

The photosynthetic pigment namely chlorophyll plays an important role in photosynthesis (Kadam *et al*., 1988). The amount of chlorophyll content in leaves was correlated with the crop growth rate in plants (Sestak, 1971). BL application caused an increase in chlorophyll content in bean plants (Krizek and Mandava, 1983). Improvement in greening of etiolated leaves of maize was reported with BR application (He *et al*., 1991). BR induced increase in chlorophyll content in plants could be due to increase in enzyme protein (Kulaeva *et al*., 1991).

HBL applied either as seed treatment or foliar spray at 0.05 ppm resulted in increased chlorophyll content in irrigated wheat (Sairam, 1994). Foliar application of HBL (0.1 and 0.5 µg/ ml) increased the chlorophyll a, b and total chlorophyll in green gram (Bhatia and Kaur, 1997) and in mung bean leaves at three different stages of growth. . Thangaraj *et al*., (1998) found out BR 0.1 ppm foliar spray at panicle initiation and flowering stages increased the chlorophyll content in rice. Padmapriya (2000) reported that application of 0.5 ppm BL on Chrysanthemum increased the chlorophyll. a, b and total chlorophyll significantly in all cultivars. Bindu (2000) observed that foliar spray of

BR increased the chlorophyll a and b in groundnut Hayat *et al*. (2000) observed that foliar spraying of 0.5 ppm BR increased the chlorophyll a, b and total chlorophyll in rice.

BR application increased that chlorophyll ‘a’ and ‘b’ content in BL sprayed cotton plants (Lini, 2001). Foliar application 0.1 ppm BR increased the chlorophyll content to the maximum when compared to other treatments in green gram (Sujatha, 2001). Sivakumar *et al*. (2001) and (2002) reported that BR foliar spray showed higher chlorophyll content than control in pearl millet. Prakash *et al*. (2003) reported that foliar spray of BR 1mg per litre on 25 and 35 DAS was the best among the treatment when compared to control for chlorophyll. Nagasubramaniam (2003) obtained similar results in baby corn by foliar application of BR. Senthil *et al*., (2003) reported that BR increased the chlorophyll content in soybean.

### 2.9.2. Chlorophyll fluorescence

Chlorophyll fluorescence is an important tool in basic and applied plant physiology. The loss of excess energy absorbed by the chlorophyll molecules in a number of ways such as light, heat and reemission which is known as fluorescence. When a leaf is replaced from dark and brightly illuminated, fluorescence rises rapidly from a low level (Fo) via an intermediate level (I) to a peak level (Fm) and then decreases gradually through several intermediate maximum to a level close to a Fo level (T). The deference between maximum fluorescence signal (Fm) and the low level (5) is called variable component of fluorescence (Fv)

The Chlorophyll fluorescence is the ratio of variable fluorescence to maximum fluorescence. Fv/Fm is a useful measurement, which has been proportional to quantum yield and showed a high degree of photosynthesis. Chlorophyll fluorescence ratio $F_{735}$ / $F_{700}$ was linearly proportional to the chlorophyll content in beach, elm and wild vine (Gitelson *et al*., 1999). Chlorophyll fluorescence depends to a greater extent on pigment content and the absorption of leaves (Dahn *et al*., 1992). In green leaves, about 90% of the emitted chlorophyll fluorescence at 658nm in reabsorbed by the chlorophyll of the leaf (Gitelson *et al*., 1999). The highest chlorophyll fluorescence in baby corn was registered by BL foliar spray than the control (Nagasubramaniam, 2003).

### 2.9.3. Soluble Protein

Soluble protein is an indicator of photosynthetic rate in terms of the RuBP carboxylase activity (Evans et.al, 1975). RuBPcase is an important leaf protein contributing for 50% of total soluble protein in the leaf extract (Makino *et al*., 1983). These soluble protein content acts as an index for photosynthetic efficiency being a measure of RuBP case activity.

Brassinosteroids promoted fraction – 1-protein (F-I-P) synthesis and caused greater amounts of soluble protein (Braun and wild, 1984). BR induced increase in photosynthesis in irrigated and stressed plants due to increase in enzyme protein (Kulaeva *et al*., 1991). HBL application at 0.1 ppm increased the soluble protein content in irrigated wheat plants (Sairam, 1994). Seed treatment with BL increased the protein synthesis in yellow lupin (Mironenko *et al*., 1996). Vardhini and Rao (1998) reported that foliar application of BL and 24EBL at 0.5µM to 3.0µM concentration increased soluble protein contents in groundnut. Thangaraj *et al*. (1998) reported that soluble protein or RuBPcase content was enhanced in rice by the application of BR 0.1 ppm at panicle initiation and flowering Stage.

Bindu (2000) reported that BR treated groundnut plants indicated enhanced soluble protein content and the Photosynthetic efficiency owing to higher RuBPcase activity. Maibangsa, (2000) noted that BR foliar application increased the soluble protein in rice. Lini (2001) reported that BR application increased the soluble protein content in cotton significantly than the unsprayed control. Soluble protein content was enhanced by the application of 0.1 ppm BR at 35 and 45 DAS in green gram was observed by Sujatha (2001). Sivakumar *et al*., (2001) and (2002) recorded increased soluble protein content in pearl millet by foliar application of BR. Prakash *et al*. (2003) reported in groundnut by the application of 1mg/ litre on 35th DAS, increased the soluble protein content. Nagasubramaniam, (2003), observed that foliar spray of BR on baby corn improved the soluble protein content. Senthil *et al*. (2003) reported that BR application increased the soluble protein in Soybean.

### 2.9.4. Nitrate reductase activity (NRase)

Nitrate reductase is a substrate inducible enzyme, which plays a key role in nitrogen metabolism in crops and thereby associated with plant growth and development. NRase catalyses the conversion of nitrate to nitrite and was reported as the rate limiting step in nitrate to nitrite and also as the rate-limiting step in nitrate transformation into proteins. The amount of nitrate reduced showed actual nitrogen accumulation (Harper and Hageman, 1972). Mai *et al*. (1989) reported that NRase activity was increased in rice on application of BR. Shen *et al*. (1990) observed that seed treatment with BR would not reduce the effect of NRase activity in maize under moisture stress. Singh *et al*. (1993) found that application of BR enhanced NRase activity in *Cicer arietinum.*

Increased NRase activity was observed in wheat crop with the application of BL (Sairam, 1994). BR treatment increased the nitrate reductase activity in sesame (Umadevi, 1998). NRase activity was improved in rice with foliar spray of BL (Maibangsa *et al*., 1999). Bindu (2000) observed that 0.5 ppm BR treated plant recorded highest NRase activity in groundnut. Seed treatment of wheat seeds with 3 μM BL for 8 or 12 hrs enhanced the activity NRase than the control (Hayat *et al*., 2001). BR application (0.1 ppm) recorded increased NRase activity in pearl millet (Sivakumar *et al*., 2001). Sujatha (2001) in green gram noticed enhanced NRase activity by foliar spray of 0.1 ppm BR. Lini (2001) noted significant increase of NRase activity in cotton, when BR at 0.5 ppm at 30 and 45 DAS was given as foliar application. Nagasubramaniam (2003) found enhanced NRase activity in baby corn due the application of BR at 25th and 45th DAS. Senthil *et al*. (2003) showed that application of BR in soybean improved the activity of NRase enzyme than the control. Hayat and Ahmed (2003) reported that soaking *Lens culinaris* seeds with 28 – HBL improved the NRase activity than control.

### 2.9.5. IAA oxidase activity

IAA oxidase activity determines the auxin levels and thereby has an influence on apical dominance. Galsten and Dalberg (1954) measured IAA oxidase activity and the growth response of 7-8 days old etiolated pea seedlings and they found that IAA oxidase activity was low in region of high auxin content and high in region of low auxin content. Han *et al*. (1988) showed that foliar application of BR increased IAA synthesis in tobacco. Eun *et al*. (1989) observed that BL- treated segments of squash contain higher level of IAA than water treated. Sakurai and Fujioka (1993) reported that BR has negative effect on IAA oxidase activity. Endogenous auxin content was increased with application of BR at 30 mg/litre in broad bean (Helmy *et al*., 1997). Lowest IAA oxidase enzyme activity was reported in sesame with the application of BR  (Umadevi, 1998).

Bindu (2000) observed an increased level of IAA due to reduced activity of IAA oxidase by application of BR in groundnut. Foliar spray of BR (0.1 ppm) showed negative effect on IAA oxidase in pearl millet (Sivakumar *et al*., 2001). Foliar spraying of 0.1 ppm BR reduced the IAA oxidase activity; there by it increased the unoxidised auxin in the green gram (Sujatha, 2001) Lini (2001) reported that BR treatments decreased the activity of the enzyme in cotton.

Karnachuk *et al*. (2002) observed that *A. thaliana* seedlings grown in darkness contained similar amount of free and bound IAA. The treatment of these seedlings with EBL increased the content of free IAA by several times. Nagasubramaniam (2003) reported that BR recorded least IAA oxidase activity indicating suppression on IAA destruction.

## 2.10. Effect of Brassinolide on Flowering

Suge (1986) reported that in *Luffa cylindrica*, BR was found to induce sex modification in the staminate inflorescence. Ikekawa and Zhao (1991) who reported that BL treatment increased the number of flowers in wheat. Wang *et al*, (1994) reported that spraying of EBL 0.1mg/ litre increased flower number in watermelon. Foliar application of BR resulted in increasing the number of flowers in strawberry (Pipatamawong *et al*., 1996). Maibangsa *et al*. (2000) reported that, in rice, BR spray recorded highest percentage of fertile spikelet and number of spikelets per panicle.

Bindu (2000) reported that BR stimulated synchronous flowers production during the early phase, hence affording a longer filling period for the developing pods of groundnut which ensure higher yield. Ramesh *et al.* (2001) reported that BR 0.75 ppm foliar spray increased the number of flowers per plant in China aster. Lini (2001) reported that BR application increased the number for flowers per plant in cotton. Fertility co-efficient was also increased by BR treatment in both hybrid Savitha and variety MCU 5. Sujatha (2001) reported that in green gram 0.1 ppm BR increased the number of flowers per plant.

## 2.11. Yield and Yield Parameters.

After the discovery of BRs in plant systems, studies was initiated to explore the possibilities of using these new substances for improving the yield of economically useful plants. Foliar spraying of BRs substantially increased the yield and yield parameters of cereals, pulses, oil seeds, fiber crops and horticultural crops like fruits, vegetables and flowers crops. In china, 28- HBL has been registered as a plant growth regulator for tobacco, sugarcane, rapeseed and tea for improving the yield and yield parameter (Seeta Ram Rao *et al*., 2002). In Russia, 24-EBL has been registered as a regulator, for potato, tomato, cucumber, pepper and barley for improving the yield and yield parameter (Khripach et al., 1997).

### 2.11.1.Cereals

#### 2.11.1.2. Rice

Fuzzi *et al*. (1991) reported that BR treatment increased the gratin weight and percentage of ripened grain in rice. Thangaraj *et al*. (1998) conducted an experiment in rice during thaladi season and found that BR 0.1 ppm as foliar spray at panicle initiation and flowering stage increased the yield and the increase in the yield was 33.7 percent over control. Thirthalingappa *et al*. (1999) studied the effect GA and HBR on rice hybrid seed production. The foliar spray of 0.1 ppm HBR+ 60 ppm GA increased the number of tillers per plant, flag leaf angle, productive length, number of spikelets, seed set percent and 100 seed weight.

Jin and Chen (1999) reported that application of natural BL at tillering and ear emergence increased rice yield significantly. Maibangsa et al. (2000) in their pot culture experiment, with rice variety CO 45, spraying 0.5 ppm of BR at panicle initiation and flowering stages increased the number of productive tillers, numbers of spikelet per panicle, filled spikelet percentage, 1000 groin weight, grain yield and Harvest index compared to control.

#### 2.11.1.2. Wheat

Sairam (1994) reported that HBL was applied either as a seed treatment or foliar spray in two contrasting wheat varieties, generally 0.05 ppm either as a seed treatment or foliar spray was more effective than the 0.01 ppm treatment. The HBL increased the numbers ears per plant, number of grains per ear, 1000 grain weight, grain yield and harvest index. Sairam *et al*. (1996), HBL treatment increased the seed yield was associated with increase in ear number per plant opined that grain number per ear, 1000 grain weight and HI. Seed treatment of wheat with 24-EBL increased the crop yield (Nilovskaya *et al*., 2001).

#### 2.11.1.3. Pearl Millet

Ravichandran and Pathmanabhan (2000) studied the influence of pre-sowing seed hardening on yield and yield parameters of pearl millet under rain fed conditions and reported that treating the seed with BL 0.1 ppm solution for 6hrs increased the ear head length, number of ear head per plant, 1000 grain weight, Stover yield, biological yield, grain yield and Harvest index over the other treatments and control. Sivakumar *et al*., (2001) studied the effect of growth regulators on grain yield in pearl millet. Among the treatments BR (0.1 ppm) foliar spray during 30th and 50th DAS increased the grain yield over the control.

#### 2.11.1.4. Baby Corn.

Nagasubramaniam (2003), in baby corn spraying of BR 0.1 ppm on 25 and 45 DAS increased the length and diameter of the cob, fresh weight of the cob and corn. BR also increased the harvest index.

### 2.11.2. Pulses

Sujatha (2001) reported that in green gram application of BR 0.1 ppm on 35 and 45 DAS significantly increased the number of pods per plant, number of cluster per plant, 100 seed weight, pod yield and Harvest index.

Bhatia and Jatinder Kaur (1997) reported that effect of HBL and Humicil on yield of mungbean. They observed the foliar application of HBL 0.1 and 0.5 µg/ml recorded more number of pods per plant, increased pod length, more number of seeds per pod, 100 seed weight and seed yield of the crop in comparison to the control

Nakaseko and Yoshida (1989) observed that foliar spraying of BL increased the number of pods per plant and 1000 seed weight in soybean and *vigna angularis*.

### 2.11.3. Oil seeds

#### 2.11.3.1. Groundnut

Vardhini and Rao (1998), studied the effect of 28-HBL on yield of groundnut and they inferred that application of HBL on 30th, 45th, and 60th DAS improved the pods per plants, weight of the pods and it significantly increased the yield over the control.

### 2.11.3.2. Sesamum

Uma Devi (1998) reported that application of BL increased yield and HI in sesamum.

### 2.11.3.3. Mustard

Hayat *et al*. (2000) observed that HBL treated mustard plants were healthier than those treated with water. HBL increased the number of pods per plant, number of seeds per pod, 100 seed weight and seed yield significantly.

Hayat *et al*. (2001a) studied the effect of Phytohormones in mustard plants. They observed that, foliar application of $10^{-8}$ M of HBR on 30 days old plant, increased the number of pods per plant, number of seeds per pod, 100 seed weight and seed yield over the control and other treatments.

### 2.11.4. Cotton

Ramraj *et al*. (1997) observed that foliar application of 28-HBL increased seed cotton yield compared to control. Lini (2001) reported that foliar spray of BL significantly enhanced the yield and yield components in cotton. The number of sympodia, number of bolls per plant, boll weight and harvest index were highest in the treatment BR 0.5 ppm sprayed on 45th and 70th DAS. Seed cotton yield was also significantly increased by BR application in both hybrid and variety.

### 2.11.5. Horticulture crops

BRs are used in many horticulture crops such as fruits, vegetables, flowers and spices etc, which resulted in increased yield and yield attributing characters.

### 2.11.5.1. Banana

Ganesan and Raman (2004) reported that, in banana spraying of BL at 100ml per acre at bunches with developing fingers and on the bunches after removal of flower recorded highest bunch weight in Nendran and Lally poovan varieties.

### 2.11.5.2. Grapes

Xu *et al*. (1994) reported that application of EBL increased the fruit yield in grapes. Watanabe *et al*. (1997) studied the potential of BR compound (TS 303) as a growth regulator. They sprayed TS 303 in the 12years old vines of grapes cv. Kyoho, 7day before flowering. TS 303 promoted fruit set in grapes and also improved the rooting of grape cuttings.

### 2.11.5.3. Navel Orange

Sugiyama and Kuraishi (1990) treated the flowers of navel orange with 0.1 and 0.01 ppm BL at anthesis. Fruit set was higher in BR treated trees than the control. Total yield / tree was highest in 0.1 ppm than the control.

### 2.11.5.4. Peach and Pear

Zhang – Gechang (1999) sprayed BL on 6 years old peach and pear trees 1 – 5 days after flower drop, 10- 15 days later and 25 – 30 days after second spray increased the peach yield and pear yield.

### 2.11.5.5. Litchi

Zhang- Gecheng (1999) sprayed 0.15% BL on anthesis in litchi increased the yield over the control.

### 2.11.5.6. Tomato

Vardhini and Rao (2001), Studied the effect of BL, 28-HBL and 24-EBL on the growth and yield of tomato. All the three BRs stimulated the yield. The BRs significantly increased the number of fruit per plant and total fruit weight per plant over the control.

Nunez *et al*. (1995) studied the effect of Biobrass – 6 on tomato yield. They sprayed Biobrass – 6 at 0.1 to 10 ppm after planting or at beginning of flowering. The increased fruit number and fresh fruit weight was observed in 0.1 ppm Biobrass –6 sprayed at beginning of flowering.

### 2.11.5.7. Potato

Ramraj *et al*. (1997) sprayed 28-HBL on potato as foliar spray with 0.25 on 0.5 mg/lit at 25th and 35th days after emergence and this foliar application of HBL gave significant increase in the yield over control.

### 2.11.5.8. Onion

Nunez *et al*. (1998), in onion used two BRs analogues namely Biobras-6 and Biobras-16 to increase the yield. Spraying at 25th and 50th days after planting gave best results with 0.1 mg Biobras – 16 applied on 50DAP. It gave 20% increased yield over control.

### 2.11.5.9. Watermelon

Wang Yu gin (1994) observed that spraying with 0.1 ppm EBL at flowering increased the fruit yield by 20% over the control.

### 2.11.5.10. *Lens culinaris*

Hayat and Ahmad (2003) reported that soaking of the seeds with 28-HBR increased the pod number per plant, 1000 seed weight and seed yield significantly over the control.

### 2.11.5.11. Cut roses

He Sheng Gen *et al*. (1997) showed that cut flowers of Chinese roses treated with EBR at 0.01 ppm prolonged vase life and also increased the fresh weight.

### 2.11.5.12. Chrysanthemum

Padma priya (2000) reported that in chrysanthemum application of BR at 0.2 ppm resulted in the highest diameter and increased in number of flower in the cultivar Indira.

### 2.11.5.13. China Aster

Ramesh *et al*. (2001) studied the effect of growth regulators on China Aster and they found that application of BR at 0.75 ppm increased the number of flowers per plant and yield of flower over the control.

### 2.11.5.14. Fenugreek

Farahat (2002) reported that BR at 50. ppm and iron at 100 ppm resulted in the highest values for number of pods per plant, number of seeds per plant, pod length, 1000-seeds weight, seed yield and harvest index.

## 2.12. Interaction of BR with plant hormones

BRs elicit pronounced effects on stem elongation and plant morphogenesis. They have growth- promoting effects similar to those of other plant hormones.

### 2.12.1. Interaction of BR with Auxin

Tanimoto and Masuda (1971) demonstrated that the epidermis is necessary for IAA- induced elongation of segments in pea stems and they proposed that the epidermis was the target tissue in IAA- induced elongation. In a short – term experiment with BR, growth stimulation in Azuki bean epicotyls segments were associated with an increase of acid secretion and by an early hyperpolarization of the transmembrane electrical potential (Cerena *et al*., 1983). The mode of action of BR was probably different from that of IAA, although the action of BR may be mediated in part by endogenous IAA. Cohan and Meudt (1983) emphasized that the action of BR affects neither the synthesis nor the breakdown of IAA in *Phaseolus vulgaris*. In the same experiment, IAA also induced similar response of growth, acid secretion, and PD. The effect of BR and IAA on growth, acid secretion and PD of the Azuki bean epicotyls were clearly additive, even at optimal concentrations of the compounds, thus suggesting a difference in their primary mode of action. Romani *et al*. (1983) observed that in apical root segment of maize, EBL induces a significant stimulation of growth, associated with an increase of acid secretion.

Katsumi (1985) studied the interaction of HBL with IAA and $GA_3$ in the elongation of hypocotyls sections of light grown cucumber seedlings. HBL acted synergistically with IAA and not with GA3, showed only an additive effect. Sequential treatment of section with HBL and then with IAA also resulted in synergistic enhancement of auxin- induced elongation, but when order was reversed, HBL was inactive. They concluded that BR enhances auxin action and poses growth-promoting activity, which is independent of that of $GA_3$. Mandava (1988) showed that in much bioassay system, it has been demonstrated that action of BR is quite similar to that of auxin and BR after showed synergism with auxin.  In persimmons fruit application of IBA and BR decreased the fruit drop (Suzuki *et al*., 1988).

Eun *et al*. (1989) reported that endogenous levels of IAA in BR – treatment segments of squash hypocotyls increased throughout incubation, while levels of IAA in untreated segments decreased, suggesting that BR stimulates the elongation of hypocotyls by sustaining that of endogenous IAA. If BR were directly or indirectly to change the level of endogenous IAA, the final resultant elongation would be more directly controlled by IAA than by BR and could, therefore, be explained by the action of IAA.

Iwahori *et al*., (1990) studied the retardation of abscission of citrus leaf and fruit- let by BL, and they reported that BR was as effective, as or more effective than IAA in preventing leaf abscission, suggesting that BR is far more effective than IAA. Katsumi (1991) also reported that the effect of BR was unique and different from that of auxin. Clouse *et al*. (1992) found that the BR induced elongation seemed to differ from auxin- induced elongation in terms of effect of gene expression in elongating soybean epicotyls, implying that BR induces elongation by a mechanism different from that of IAA. Tominaga *et al*. (1994) observed that BR stimulated elongation of etiolated squash hypocotyl segments with outer tissue removed, while IAA has no effect on peeled segments. Heping Cao and Shankun Chen (1995) reported that BR and IAA had synergistic effect in rice lamina joint inclination.

### 2.12.2. BR interaction with GA

Gregory and Mandava (1982) reported that there is no synergistic between the exogenously applied $GA_3$ and BR, but the two growth promoters act in an additive manner at concentration of $10^{-8}$ M to $10^{-9}$ M Takeuchi *et al*. (1995) reported that *Orobanche minor* seeds treated with BR and $GA_3$ increased the rate of germination. $GA_3$ was more effective than BL. BL and $GA_3$ showed an additive relationship in increasing the seed germination rate. $GA_3$ and BL moderately promoted the conditioning process and that these two compounds acted synergistically.

### 2.12.3. BR interaction with Kinetin

Mandava *et al*. (1981) reported that BR was compared with $GA_3$ and kinetin, the cucumber cotyledon assay was sensitive to cytokinins in the dark, to both cytokinins and gibberellins in continuous light, and not sensitive to auxin at low concentration. In Chinese cabbage protoplasts, 24 – BL when applied with 2,4-D and kinetin, promoted cell division and enhanced cluster and colony formation (Nakajima *et al*., 1996).

### 2.13. Brassinosteroids and plant stress tolerance

BRs increased the resistance of plants against various biotic and abiotic stresses. The effect of BRs on plant tolerance to desiccation and over heating was first to be studied. BR treated plants were resistant to high and low temperature, drought and salt stresses. Chandrasehker (2004) reported under flooding condition BL increased the number of fruits per plant, fruit set percent and yield of chilies.

#### 2.13.1. Drought stress

Kulaeva *et al*. (1991) reported that application of BRs resulted in increased tolerance to drought stress in sugar beet. Sairam (1994) studied the effect of HBL on metabolic activity and grain yield of wheat under normal and water stress condition. He reported that HBL as foliar spray at 30 days increased the leaf relative water content and transpiration and decreased diffusion resistance in water stressed plant. It also had positive effect on nitrate reductase and gultamine synthetase activities, photosynthesis, chlorophyll and soluble protein content in stressed, irrigated and revived plants. The beneficial effect was also observed on grain yield and yield-attributing parameters such as grain number per ear, 1000 grain weight, ear number per plant and HI.

Sairam *et al*. (1996) reported that HBL application as seed treatment in wheat resulted in increased germination, α – amylase activity and total soluble protein in 48 hr old seedlings and shoot length in 96 hr old seedlings under induced moisture- stress. Hui-lian *et al*. (1994) reported that under drought condition spraying of sorghum plants with EBL improved the yield. Li *et al*. (1998) studied the effects of brassinolide, uniconazole and methyl jasmonate on the antioxidant system in seedlings of drought resistant (PAN 6043) and drought sensitive (SC 701) cultivars of *Zea mays L*. When seedlings treated with the three regulators were subjected to water stress (–1.0MPa PEG 6000 solution), the activities of superoxide dismutase, and catalase were increased due to drought stress by maintenance of increased antioxidant enzyme activity and antioxidant substance levels. Mazorra and Nunez (2000) stated that BR analogues were able to increase both superoxide dismutase and peroxidase activity and have a possible enhanced tolerance of this treatment to oxidative stress.

Pustovoitova *et al*. (2001) studied the EBL on plant drought resistance in cucumber plants. The plant treated with EBL at concentrations from $10^{-11}$ to $10^{-6}$M improved the resistance to desiccation and overreaction, increased the content of water in their leaves, and accelerated plant growth under water deficiency. One of the plants protective responses induced by the hormonal treatment was the accumulation of osmotically active compounds; free amino acids in particular, which evidently contributed to plant osmoregulation.

. Wu Shao Hua (2001) reported that BR influenced the activities of SOD, POD in strawberry leaves under-water stress. In the initial stage the activity was higher in BR treated plants.

### 2.13.2.Temperature stress

Kulaeva *et al*. (1989) observed that plant tolerance to high temperatures was evidently determined by the increase in the heat – resistance of protein synthesis, which results, in particular, the heat stability of membranes. Brassinosteroids – treated tomato and rice plants grew better than control plants under low-temperature stress (Kamuro and Takatsuto, 1991). The tolerance in plants to high temperature due to application of BRs was associated with induction of de novo polypeptide (heat shock protein) synthesis in wheat leaves (Kulaeva et *al*., 1991). Brassinosteroids improved the tolerance of maize (He *et al*., 1991) and cucumber (Katsumi, 1991) seedlings against low-temperature stress. BR increased tolerance to high temperature in wheat leaves (Kulaeva *et al*., 1991).

In rice, Wang and Zeng (1993) reported that 24-EBL increased the resistance against chilling stress (1-5˚C) and the tolerance was associated with increased ATP, Proline levels and SOD activity, this indicating BR involvement in membrane and osmoregulation. Wilen *et al*. (1995) demonstrated that EBL markedly enhanced viability of brome grass cells following exposure of cell suspension cultures to high temperature stress. Krishna *et al*. (1997) showed that EBL enhanced surviving of swede seedlings treated with a very high temperature.

Sangeeta Dhaubhadel *et al*. (1999) showed that EBR had a profound and reproducible effect on the survival rate of *Brassica napus* and tomato seedlings after exposure to a lethal heat treatment. A mild heat treatment prior to the lethal heat stress should be given for survival. This phenomenon was refereed to as acquired thermo- tolerance, but in EBR treatment precondition was not requested for enhanced survival of EBR – treated. There was considerable evidence that heat shock proteins (HSPs) play a vital role in acquired thermo-tolerance. They reported that all four classes of HSPs were observed to accumulate to higher level in EBR – treated *B. rapus* seedlings than in untreated seedlings after heat stress. Increased stress resistance of EBR – treated seedlings maybe due, at least in part, to the increased accumulation of the various HSPs in these seedlings. They speculated that the higher accumulation of HSPs in EBR-treated seedlings was more likely to be the cause for, rather than the effect of the increased thermo-tolerance of these seedlings.

Fujii and Saka (2001) reported that seed treatment with 2 $X10^{-8}$ M BR promoted the early growth of rice seedlings under low temperature. Foliar spraying of BR on rice seedlings at the 4th leaf stage increased the plant height and root growth. Thus gave BR an increased the cell expansion at a low temperature.

Sam *et al*. (2001) observed that high temperature stress in tomato caused disorganization of chloroplast and mitochondrial internal membrane system. This was more marked in the cells treated with BB6.

Asao *et al*. (2002) reported that TNZ 303 (mixture of jasmonic acid derivative and brassinosteroids derivative) seed treatment decreased the occurrence of deformed leaves and mitigated the suppression of vegetative growth of seedlings whose apices were treated with 4˚C water.

Mazorra *et al*. (2002) studied the influence of BRs on antioxidant enzymes activity in tomato under different temperatures. EBR increased SOD activity in leaves at both temperatures at (25˚C and 40˚C). The increased SOD activity after EBR treatment at 25˚C suggested that EBR – promoted activation of SOD might decrease the possible toxic concentration of $O_2$ radicals. In the presence of EBR, catalase activity was enhanced. This increase in catalase activity might be important in eliminating $H_2O_2$ excess. The EBR treated leaves were also stimulated peroxidase activity at 40˚C probably indicating an interaction between BRs and heat shock protein.

### 2.13.3. Salinity stress

Salinity is major environmental stress affecting the crop productivity. Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concerned.

Vardhini and Rao (1997) reported that BRs (BL, 24-EBL and 28-HBL) on salinity-induced inhibition of seedling growth of groundnut was reversed the growth inhibitory effects of salinity stress. They also promoted the growth in terms of fresh and dry weight.

Anuradha and Rao (2001) reported, in rice supplementation of the saline solution with 24-EBL and 28-HBL treated rice seed considerably reduced the inhibition of seed germination and seedling growth. This enhanced growth of seedlings by BRs was also associated with increased levels of soluble protein.

Anuradha and Rao (2002) reported that increased seed germination by 3.0µM BR in rice under supplemented saline condition. The protein synthesis was alleviated and further activated by BL as reflected in higher contents in seedlings from BL supplemented saline treatment. The proline level was increased when the rice seedlings were subjected to salinity stress and supplementing NaCl with BRs further enhanced the proline contents. Here the proline played an important role in osmoregulation and increased tolerance.

In rice, plants germinated from BRs – treated seeds had a greater fresh weight than the control under saline condition. There was also considerable increase in the dry weight. Seed application of BRs considerably restored the pigment level grown in saline medium. The chlorophyll a, b and total chlorophyll was increased under the BR-treated seeds grown under saline environment. (Anuradha and Rao (2003).

The activity of the NRase plays a pivotal role in the supply of nitrogen and the growth and productivity of plants. Anuradha and Rao (2003) reported that high salt concentration inhibits NRase activity but with the ability of BRs given as a seed treatment reduced the impact a salt stress on the NRase and increased the activity. Abraham *et al*. (2003) reported that BL fails to stimulate proline dehydrogenase enzymes that catalyze the rate – limiting steps of proline degradation under the cell stress condition, in the shoots of *Arabidpis*.

# CHAPTER III

## MATERIALS AND METHODS

The present investigation was carried out to find out the optimum concentration of brassinolide, which causes a significant impact on the growth and yield improvement of crop and also to study the interaction effect with other plant growth regulators. An attempt was also made to find out the optimum concentration, for overcoming the induced stress like drought, cold, temperature and salinity. The investigation was programmed into a series of experiments comprising of laboratory, greenhouse / pot culture and field. A brief presentation of the materials used and methodologies followed for different experiments and field trials in the present research are given here under.

### 3. Experimental materials

The maize hybrid COH 3 released from TNAU, Coimbatore was utilized in this study. The brassinolide was purchased from M/s Godrej. Agrovet Ltd., Mumbai, in the name of Double (Concentration 1000 ppm EC).

### 3.1. Laboratory experiment

The objective was to find out the optimum concentration of brassinolide which would alleviate the stresses like drought, temperature (high and low), and salinity and also increase the yield of the crops.

### 3.1.1. Effect of Brassinolide on seedling

Preliminary experiment was conducted to find out the optimum concentration of BL required for inducing maximum growth in maize seedling. Maize seeds were surface sterilized with 0.1per cent mercuric chloride and washed thoroughly with distilled water. Twenty-five seeds were put in Petridishes provided with Whatman No. 1 filter paper, allowed for germination and two days old seedlings were transferred to various concentrations of BL for 8 hours. After 8 hours of treatment, seedlings were transferred to Petridishes containing water and allowed to grow and on7th day the observations were recorded.

**Treatments Details**

**$T_1$** : Control
**$T_2$** : 0.01 ppm BL
**$T_3$** : 0.05 ppm BL
**$T_4$** : 0.10 ppm BL
**$T_5$** : 0.20 ppm BL
**$T_6$** : 0.30 ppm BL
**$T_7$** : 0.40 ppm BL
**$T_8$** : 0.50 ppm BL

**Replications: Three**
**Design : CRD**

### 3.1.2. Influence of BL in overcoming drought stress effect on seedlings

Maize seeds were surface sterilized with 0.1 per cent mercuric chloride and washed thoroughly with distilled water. Twenty-five seeds were put in Petridishes provided with Whatman No. 1 filter paper, allowed for germination and two days old seedlings were transferred to Petridishes containing different concentrations of BL for 8hours. The seedlings were transferred to -1.0MPa PEG 6000 solution and kept for 16 hours. The seedlings were then transferred to Petridishes with distilled water for recovery. The observations were recorded on7th day after germination.

**Treatments Details**

**$T_1$** : Control + PEG 6000 → recovery

**$T_2$** : 0.01 ppm BL + PEG 6000 → recovery

**$T_3$** : 0.05 ppm BL + PEG 6000 → recovery

**$T_4$** : 0.10 ppm BL + PEG 6000 → recovery

**$T_5$** : 0.20 ppm BL + PEG 6000 → recovery

**Replications: Four**

**Design : CRD**

PEG 6000 at (-1.0 MPa) for 16 hours

### 3.1.3. Influence of BL in overcoming Low Temperature stress on seedlings

Maize seeds of 25 numbers after surface sterilization with 0.1% mercuric chloride and washed thoroughly with distilled water, were put in Petridishes provided with Whatman No. 1 filter paper, allowed for germination and two days old seedlings were transferred to Petriplates containing different concentration of BL for 8hours. The seedlings were then transferred to Petridishes containing distilled water and subjected to chilling stress at 4° C for four hours. After low temperature treatment seedlings were transferred to Petridishes containing water for recovery. Observations were recorded on 7th day after germination.

**Treatments Details**

**$T_1$** : Control + Chilling stress → recovery

**$T_2$** : 0.01 ppm BL + Chilling stress → recovery

**$T_3$** : 0.05 ppm BL + Chilling stress → recovery

**$T_4$** : 0.10 ppm BL + Chilling stress → recovery

**$T_5$** : 0.20 ppm BL + Chilling stress → recovery

**Replications: Four**
**Design : CRD**

Chilling stress at 4° C for four hours

### 3.1.4. Influence of BL in overcoming High Temperature stress on seedlings

Maize seeds of 25 numbers after surface sterilization with 0.1% mercuric chloride and washed thoroughly with distilled water, were put in Petridishes provided with Whatman No. 1 filter paper, allowed for germination. Then two days old seedlings were transferred as follows.

**Treatments Details**

**$T_1$** : Absolute control

**$T_2$** : Untreated → Lethal stress → recovery

**$T_3$** : Temperature induction → Lethal stress → recovery

**$T_4$** : 0.05 ppm BL + Temperature induction → Lethal stress → recovery

**$T_5$** : 0.10 ppm BL + Temperature induction → Lethal stress → recovery

**$T_6$** : Temperature induction → 0.05 ppm BL + Lethal stress → recovery

**$T_7$** : Temperature induction → 0.10 ppm BL + Lethal stress → recovery

**Replications: Three**

**Design : CRD**

Induction temperature →Temperature stress at 35°C for 4 hours

Lethal stress →Temperature stress at 50°C for 2 hours

Recovery → 48 hours at 25°C

### 3.1.5. Influence of BL on salinity tolerance stress in seedlings

Maize seeds were surface sterilized with 0.1% mercuric chloride and washed thoroughly with distilled water. Twenty-five seeds were placed in Petridishes provided with Whatman No. 1 filter paper allowed for germination. Then two days old seedlings were transferred as follows.

**Treatments Details**

$T_1$ : Absolute control

$T_2$ : Untreated → Lethal stress → recovery

$T_3$ : Induction → Lethal stress → recovery

$T_4$ : 0.05 ppm BL + Induction → Lethal stress → recovery

$T_5$ : 0.10 ppm BL + Induction → Lethal stress → recovery

$T_6$ : Induction → 0.05 ppm BL + Lethal stress → recovery

$T_7$ : Induction → 0.10 ppm BL + Lethal stress → recovery

**Replications: Three**

**Design : CRD**

Induction → NaCl 200 mM for 8 hours

Lethal stress → NaCl 400 mM for 16 hours

Recovery → 48 hours

## 3.1.6. Observation

### 3.1.6.1. Morphological characters

### 3.1.6.1.1.Shoot length

Five seedlings were taken and the shoot length was measured from the collar region to the youngest fully opened leaf and expressed as cm.

### 3.1.6.1.2. Root length

Five seedlings were taken and the root length was measured from the collar region to the root tip and expressed in cm.

### 3.1.6.1.3. Seedling dry weight

The entire seedling was dried at 80°C in a hot air oven for 24 hr and the dry weight was expressed in grams expressed in mg plant$^{-1}$.

### 3.1.6.2. Biochemical characters

### 3.1.6.2.1. Chlorophyll content

Chlorophyll ‘a’, Chlorophyll ‘b’ and total chlorophyll contents were estimated in the fully expanded leaf at 7th day (Yoshida *et al.,* 1976) and expressed in mg g$^{-1}$ fresh weight

### 3.1.6.2.2. Soluble protein

Soluble protein content was estimated from leaf samples following the method of Lowry *et al*. (1951) and expressed as mg g$^{-1}$ fresh weight.

### 3.1.6.2.3.Proline

The proline content was estimated from the fully opened leaf following the method of Bates *et al*. (1973) and expressed in µmol g$^{-1}$FW.

### 3.1.6.2.4. Nitrate reductase activity

The fully expanded leaf was used for estimating the nitrate reductase (NRase) activity. The method described by Nicholas *et al.,* (1976) was used to estimate NRase activity and the activity was expressed as µmoles $NO^{-}_{2}$ produced g$^{-1}$ h$^{-1}$ fresh weight.

### 3.1.6.2.5. IAA oxidase activity

IAA oxidase activity was estimated in the leaf by following the method proposed by Parthasarathy *et al*. (1970) using Gorden Weber reagent. The enzyme activity was expressed as μg unoxidised auxin $g^{-1}$ $hr^{-1}$.

### 3.1.6.2.6. Free radical scavenging enzymes

### 3.1.6.2.6.1. Catalase

Catalase activity was determined following the method of Luck (1974). One gram sample was extracted in 0.067 M phosphate buffer (pH 7.0). A known volume of extract was added to the cuvette containing 3 ml hydrogen peroxide plus phosphate buffer. The time taken for percent change in absorbance (Δt) at 240 nm was recorded for calculating the enzyme activity and expressed as enzyme units $g^{-1}$ $min^{-1}$ tissue. All the operations were carried out at 0-5°C.

### 3.1.6.2.6.2. Peroxidase

Peroxidase activity was determined by adopting the procedure of Malik and Singh (1980). One gram sample was extracted in 0.1M phosphate buffer (pH 7.0) A known volume of extract was added to experimental cuvette containing 3 ml phosphate buffer and 0.05 ml guaiacol reagent and 0.03 ml hydrogen peroxide solution was added quickly to the cuvette and increase in absorbance at 436 nm was recorded. The **Δt** in minutes was used to calculate the enzyme activity. The enzyme activity was expressed as enzyme units $litre^{-1}$.

### 3.1.6.2.6.3. Superoxide dismutase (SOD)

SOD activity was determined by using the salt nitroblue tetrazolium (NBT) as described by Beau Champ and Fridovich (1971) and expressed as enzyme units $mg^{-1}$ protein $min^{-1}$. The reagents used in enzyme assay are mentioned here under.

**Reagents**

| | Stock | Working solution | Total volume |
|---|---|---|---|
| 1. | Riboflavin 200 $\mu$M (100x) 3.8 mg/50ml | 2 $\mu$M | 30 $\mu l$ |
| 2. | Buffer 250 mM (5x)<br>$KH_2PO_4$ 3.402g/100ml<br>$K_2HPO_4$ 4.354g/100ml | 50mM | 600 $\mu l$ |
| 3. | Methionine 130 mM (10x) 0.9698 g/50ml | 13MM | 300 $\mu l$ |
| 4. | EDTA 10mM (100x) 0.3722 mg/5ml | 0.1mM | 30 $\mu l$ |
| 5. | NBT 7500 $\mu$M (100x) (4.08 mg/5ml) | 75 $\mu$M | 30 $\mu l$ |
| 6. | Enzyme extract in Phosphate buffer (pH 7.8) | - | 50 $\mu l$ |
| 7. | Distilled water | | |
| | i. for sample | 2.04 | 1.96 |
| | ii. Blank (NBT- enzyme extract) | - | - |
| | iii. Reference blank (Enzyme) | - | 2.01 |

The absorbance was measured at 560 nm calculations.

Calculations:

Absorbance value-reference blank = x

Absorbance value –sample = y

$$Z\%\ \text{inhibition} = \frac{x - y}{x} \times 100$$

$$50 \text{ percent inhibition} = \frac{Z}{50} = \text{A unit}$$

*i.e.*, 50$\mu l$ enzyme extract yielded A unit

$$1000\mu l \textit{ yields} = \frac{A}{50} \times 1000$$

$$\frac{B}{\text{Protein value}} = \text{SOD assay value (enzyme units mg}^{-1}\text{ protein)}$$

### 3.1.7. Molecular studies

#### 3.1.7.1. Poly acrylamide - sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) of proteins

SDS- PAGE analysis was performed to identity the protein profile in maize subjected to different stress condition. The method of Laemmli (1970) with slight modifications as per the need of present study given by Sadasivam and Manickam (2000).

A known quantity of leaf and root samples were homogenized in Tris – HCl (pH 7.0) buffer in prechilled pestle and mortar and homogenate centrifuged at 10,000 rpm for 10 min and supernatant was utilized for analyzing for analyzing the protein through electrophoresis.

**Materials**

**Stock acrylamide solution**

| | |
|---|---|
| Acrylamide | 30g |
| Bisacrylamide | 0.8g |
| Water | 100 ml |

**Separating gel buffer (pH 8.8)**

| | |
|---|---|
| 1.875 m Tris - HCI | 22.7 |
| Water | 100 ml |

**Stacking gel buffer (pH 6.8)**

| | |
|---|---|
| 0.6 M Tris - HCI | 7.26 g |
| Water to | 100 ml |

**Polymerising agents**

| | |
|---|---|
| APS | 0.5g/20ml,freshly prepared |

| | |
|---|---|
| TEMED | Fresh from refrigerator |

### Electrode buffer (pH 8.2-8.4)

| | |
|---|---|
| 0.05 M Tris | 12g |
| 0.192 M glycine | 28.8g |
| 0.1% SDS | 2g |
| Water to | 2L |

### Sample loading buffer (5 X conc)

| | |
|---|---|
| Tris HCI buffer (pH 6.8) | 5ml |
| SDS | 0.5g |
| Sucrose | 5g |
| Mercaptoethanol | 0.25 ml |
| Bromophenol blue (0.5% W/V) | 1ml |
| Water | 10ml |

### SDS 10% solution (Stored at room temperature)

### Protein stain solution

| | |
|---|---|
| Coomassie brilliant blue R 250 | 0.1g |
| Methanol | 40ml |
| Acetic acid | 10ml |

| | |
|---|---|
| Water | 50ml |
| **Destainer** | As above without the dye |

## Procedure

Thoroughly cleared glass plates and spacers were assembled properly and were clamped in an upright position on a gel casting unit with 2 percent agar melted and poured bottom to seal the chamber leak proof between glass plates. 10-15 percent poly acrylamide gels were used to get better resolution of protein.

| **Separating gel** | **15%** | **10%** |
|---|---|---|
| Stock acrylamide | 20 ml | 13.3 ml |
| Tris – HCl (pH 8.8) | 8 ml | 8.0 ml |
| Water | 11.4 ml | 18.1 ml |
| Degassed for 3 to 5 min | | |
| APS (5%) | 0.2 ml | 0.2 ml |
| SDS (10%) | 0.4 ml | 0.4 ml |
| TEMED | 20 µL | 20 /u L |

| **Stacking gel** | **4%** |
|---|---|
| Stock acrylamide | 1.35 ml |
| Tris – HCl (pH 6.8) | 1.00 ml |

| | |
|---|---|
| Water | 7.50 ml |
| Degassed for 3 to 5 min | |
| APS (5%) | 50 µL |
| SDS (10%) | 0.1 µL |
| TEMED | 10 µL |

The gel solution was poured in the chamber space between the glass plates carefully leaving 4 cm from top. Allowed to polymerize for 30– 60 minutes. A layer of distilled water was added on top the gel to get a smooth layer on top.

After removing the distilled water from the top of gel, stacking gel (4%) was added, comb of desired lane number or wells was placed and kept for polymerization (30-60minutes)

After polymerization the comb was removed and the gel plate was removed from the casting unit and placed in the electrophoresis apparatus. Concentration of the protein sample was adjusted to 5-200 µg in a volume of 25-50 µl, by mixing the sample-loading buffer, boiled for 2-3 minutes. The sample was cooled and carefully injected into each well with a micro syringe. Then the apparatus was filled with electrode buffer in both tanks, cathode connected at top and anode at bottom; and the DC power pack was switched on to provide a constant current of 15 mA initially till the dye front crossed the stacking gel. Then current supply was increased to 30 mA until bromophenol blue reached bottom of gel. The gel was removed from plates and immersed in staining solution overnight. After proper staining the gel was transferred to destaine with gentle shaking and changing the destined frequently till appropriate visibility of the proper bands in gel. The gel was photographed.

### 3.2. Greenhouse experiment

Experiments were carried out in pots in the glasshouse to study and understand the interaction effect of brassinolide with benzyl adenine and naphthalene acetic acid on the physiological, biochemical and yield characters.

The treatments, which performed well in the laboratory experiments, were utilized for studying in the pot culture experiments conducted in the glasshouse of Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore.

#### 3.2.1. Experimental details

Pot mixture was prepared by mixing thoroughly two parts of soil and one part of well decomposed farmyard manure and sand and filled in pots of size 27 x 18 cm, which can hold 10 kg volume of pot culture mixture. The maize seeds were sown and all the agronomical cultural practices were followed.

*Treatment details*

**$T_1$:** Control
**$T_2$:** 0.05 ppm BL
**$T_3$:** 0.10 ppm BL
**$T_4$:** 10 ppm BA
**$T_5$:** 20 ppm BA
**$T_6$:** 10 ppm NAA
**$T_7$:** $T_2 + T_4$
**$T_8$:** $T_3 + T_4$

**$T_9$:** $T_2+T_5$

**$T_{10}$:** $T_3+T_5$

**$T_{11}$:** $T_2+T_6$

**$T_{12}$:** $T_3+T_6$

**$T_{13}$:** $T_2+T_4+T_6$

**$T_{14}$:** $T_2+T_5+T_6$

**$T_{15}$:** $T_3+T_4+T_6$

**$T_{16}$:** $T_3+T_5+T_6$

BL: Brassinolide

BA: Benzyl Adenine

NAA: Naphthalene Acetic Acid

**Particulars**

Design : Completely Randomized Design

Replications : Three

Crop : Maize

Hybrid : COH 3

Treatments : 16

### 3.2.2. Soil characteristics

**Table.1. Physical and chemical properties of the soil used for pot culture experiment**

| **Particulars** | **Pot culture experiment** |
|---|---|
| **Physical properties** | |
| Field capacity (%) | 22.00 |
| pH | 7.4 |
| EC ($dSm^{-1}$) | 0.2 |
| **Chemical composition (kg ha$^{-1}$)** | |
| Available nitrogen | 235 |
| Available phosphorus | 15 |
| Available potassium | 419 |

### 3.2.3.Observations

### 3.2.3.1. Morphological parameters

### 3.2.3.1.1.Plant height

The plant height was measured in the three plants from the base of the shoot to the longest leaf tip and the mean values were worked out and expressed in cm.

### 3.2.3.1.2.Root length

Root length was determined in three plants and their mean length from the base of the root to the root tip was measured and expressed in cm.

### 3.2.3.2. Growth attributes

### 3.2.3.2.1. Leaf Area

Leaf area was measured by using Leaf Area Meter (Licor Model 3100) and expressed as $cm^2$ $plant^{-1}$.

### 3.2.3.2.2. Specific Leaf Weight

Specific leaf weight was calculated by using the formula of Pearce ***et al.*** (1968) and expressed in g $cm^{-2}$.

$$\text{SLW} = \frac{\text{Leaf dry weight per plant}}{\text{Leaf area per plant}}$$

### 3.2.3.2.3. Specific Leaf Area

Employing the formula of Kvet ***et al.*** (1971), SLA was calculated by using leaf area and leaf dry weight and expressed in $cm^2$ $g^{-1}$.

$$\text{SLA} = \frac{\text{Leaf area}}{\text{Leaf dry weight per plant}}$$

### 3.2.3.2.4. Net Assimilation Rate (NAR)

The method proposed by Gregory ***et al.*** (1917), subsequently modified by Williams (1946) was employed for calculating the NAR on leaf dry weight basis and the values were expressed in g $cm^{-1}$ $day^{-1}$.

$$\textbf{NAR} = \frac{\log_e L_2 - \log_e L1}{L_2 - L_1} \times \frac{W_2 - W_1}{t_2 - t_1}$$

Where,

$W_1$ and $W_2$ = Dry weight of whole plant at $t_1$ and $t_2$ respectively.

$L_1$ and $L_2$ = Leaf dry weight at time $t_1$ and $t_2$ respectively

$t_1$ and $t_2$ = Time in days

### 3.2.3.2.5. Crop Growth Rate

The crop growth rate was estimated by using the formula of Watson (1958) and expressed in g $m^{-2}$ $day^{-1}$.

$$\text{CGR} = \frac{W_2 - W_1}{\rho\,(t_2 - t_1)}$$

Where,

$W_1$ and $W_2$ = Whole plant dry weight at time $t_1$ and $t_2$ respectively.

$t_2$ and $t_1$ = Time in days

$\rho$ = Ground area occupied by plant ($m^2$)

### 3.2.3.2.6. Relative Growth Rate

The relative growth rate was calculated by using the formula suggested by Williams (1946) and expressed in g $g^{-1}$ $day^{-1}$.

$$\text{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where,

$W_1$ and $W_2$ = Whole plant dry weight at $t_1$ and $t_2$ respectively.

$t_1$ and $t_2$ = Time in days

### 3.2.3.2.7. Total dry matter production

Plant samples were first shade dried, then oven dried at 80°C for 24 hrs. The dry weight of whole plant including the seed weight was taken and expressed in g $\text{plant}^{-1}$.

### 3.2.3.3. Chlorophyll

The procedure described in **3.1.6.2.1.**, was adopted to record the chlorophyll content.

### 3.2.3.4. Chlorophyll fluorescence

The chlorophyll fluorescence was measured using the instrument, Plant Efficiency Analyzer (Hansatech, U.K.) (PEA), as described by Krause and Weis, (1984).

Key fluorescence parameters *viz.*, $F_o$, (initial fluorescence), $F_v$ (variable fluorescence), $F_m$ (maximal fluorescence) and the ratio of $F_v/F_m$ were measured automatically from the instrument.

$$\frac{F_v}{F_m} = \frac{\text{Variable fluorescence}}{\text{Maximum fluorescence}}$$

$F_v/F_m$ is a useful ratio that depicts the proportion of quantum yield relation to a high degree of photosynthesis.

### 3.2.3.5. Biochemical parameters

#### 3.2.3.5.1. Leaf soluble protein

The procedure described in **3.1.6.2.2.,**was adopted to record the soluble protein content.

#### 3.2.3.5.2. Nitrate reductase activity

The procedure described in **3.1.6.2.4.,** was adopted to record the NRase activity.

#### 3.2.3.5.3. IAA oxidase activity

The procedure described in **3.1.6.2.5.**was adopted to record the IAA oxidase activity.

### 3.2.3.6. Yield and yield parameter

#### 3.2.3.6.1. Cob length

The cob from each treatment was taken and the length was measured and expressed in cm.

#### 3.2.3.6.2. Number of grains per cob

From each treatment the number of grains were counted and the average was worked out.

#### 3.2.3.6.3. Yield

The grain yield per plant of each treatment was recorded and expressed in g plant$^{-1}$.

#### 3.2.3.6.4. Harvest Index

Harvest index was calculated from the dry weight of grain and total dry weight of plant at harvest.

$$HI = \frac{\text{Economical yield}}{\text{Biological yield}}$$

## 3.3. On Farm Trial

On farm trials were carried out in the farmers field to study the effect of brassinolide on yield and yield parameters of different agricultural and horticultural crops.

The treatments, which performed well in the laboratory experiments, were utilized for studying the yield parameters under on farm trails.

### 3.3.1. Experimental details

| **Crop** | **Variety (or) Hybrids** |
|---|---|
| **Agricultural crops** | |
| 1. Rice | W.ponni |
| 2. Maize | COH 3 |
| 3.Sorghum | COH 4 |
| 4.Ragi | CO 13 |
| 5.Blackgram | KM 2 |
| 6.Greengram | CO 6 |
| 7.Groundnut | TMV 9 |
| 8. Sun flower | Morden |
| 9.Cotton | MCU 7 |

**Horticultural crops**

| | |
|---|---|
| 1. Banana | Robusta |
| 2. Grapes | Bangalore blue |
| 3. Tomato | PKM 1 |

### *Treatment details*

**$T_1$:** Control

**$T_2$:** 0.01 ppm BL

**$T_3$:** 0.05 ppm BL

**$T_4$:** 0.10 ppm BA

**$T_5$:** 0.20 ppm BA

**Particulars**

Design : Randomized Block Design

Replications : Four

Treatment : Five

Stages of foliar spray : Vegetative and Flowering

### 3.3.2. Observation

#### 3.3.2.1. Rice

The observation recorded were plant height, LAI at flowering stage, number of tillers, number of productive tillers, number of grains per panicle, 1000 grain weight, grain yield, HI, BC ratio.

#### 3.3.2.2. Maize

The recorded observations were plant height, LAI, cob length, number of grains per cob, 100 seed weight, grain yield, stover yield, HI. BC ratio was also computed.

#### 3.3.2.3. Sorghum

The observations were recorded on growth and yield parameters namely, plant height, LAI, 1000 seed weight, grain yield, stover yield, HI and BC ratio.

#### 3.3.2.4. Ragi

The recorded observations were Plant height, LAI, number of tillers, number of panicle per plant, seed weight per panicle, grain yield, TDMP, HI, BC ratio.

#### 3.3.2.5. Black gram

The recorded observations were Plant height, LAI, number of pods per plant, pod weight, yield, TDMP, HI and BC ratio.

#### 3.3.2.6. Green gram

The observations on growth and yield attributing characters were recorded and they were plant height, LAI, number of pods per plant, pod weight, yield, TDMP, HI and BC ratio.

### 3.3.2.7. Groundnut

The recorded observations were plant height, LAI at flowering, number of pegs per plant, number of pods per plant, pod weight, kernel weight, yield, TDMP, HI and BC ratio.

### 3.3.2.8. Sunflower

The recorded observations were plant height, LAI at flowering, capitulam diameter, percent grain filling, 100 seed weight, yield, TDMP, HI and BC ratio at harvest.

### 3.3.2.9. Cotton

The recorded observations were plant height, LAI, number of sympodia per plant, number of bolls per plant, percent boll set, boll weight, kapas yield, TDMP, HI, and BC ratio.

### 3.3.2.10.Banana

Observation was recorded on Pseudostem height, LAI, bunch weight, number of fingers per bunch, finger weight, yield, TDMP, and BC ratio.

### 3.3.2.11.Grapes

The recorded observations were number of bunch per vein, bunch, 100 berry weight, yield, TSS, titrable acidity and BC ratio.

### 3.3.2.12. Tomato

The recorded observations were plant height, LAI at flowering, number of fruits per plant, percent fruit set, fruit weight, yield, TDMP, HI, and BC ratio.

### 3.3.3. Benefit cost ratio

The BC ratio gives the return per rupee invested and was calculated using the formula

$$\text{BCR} = \frac{\text{Gross return (Rs.)}}{\text{Cost of cultivation (Rs.)}}$$

### 3.4. Statistical analysis

Statistical analyses of various data recorded from the different experiments were made employing methods appropriate to the design adopted for the specific experiment. The statistical analytical procedure elaborated by Panse and Sukhatme (1961) was made use to interpret the data suitably.

## CHAPTER IV

# EXPERIMENTAL RESULTS

The present investigation was to find out the optimum concentration of brassinolide, which helpful to increase the growth and yield and also to study the interaction effect with other plant growth regulators. To find out the optimum concentration, which will over come the induced stress like drought, cold, temperature and salinity. To achieve the objectives, a series of experiments in laboratory, greenhouse and field were carried out. The results of various experiments were statistically analyzed and the data are presented in appropriate tables with suitable figures.

## 4.1. Laboratory experiment

### 4.1.1. Effect of Brassinolide on Maize seedling (Table: 2)

### 4.1.1.1. Morphological Characters

### 4.1.1.1.1. Shoot length (cm)

The BL treatments on maize seeding significantly increased the shoot length. The shoot length was ranged from 13.4 to 18.3. The highest shoot length was recorded in 0.10 ppm BL (18.3), which was on par with 0.20 ppm BL (17.4). The treatments 0.30 ppm BL (16.8), 0.40 ppm BL (16.3) and 0.05 ppm BL (16.2) were on per to each other. The lowest shoot length was recorded in control (13.4), which was on per with 0.01 ppm BL (13.6).

### 4.1.1.1.2. Root length (cm)

Treatment of brassinolide significantly influenced the root length of the maize seedlings. The root length was ranged from 12.1 to 13.9. The highest root length was recorded by 0.10 ppm BL (13.9) that was on par with 0.30 ppm BL (13.8), 0.20 ppm (13.5) and 0.40 ppm BL (13.3). The treatment 0.50 ppm BL (12.9) was on par with 0.05 ppm BL (12.7). The lowest was recorded in 0.01 ppm, which was on par with control (12.5).

### 4.1.1.2. Biochemical Characters (Fig. 1)

### 4.1.1.2.1. Chlorophyll Content (mg $g^{-1}$)

#### 4.1.1.2.1.1.Chlorophyll ‘a’

Brassinolide application significantly increased the chlorophyll ‘a’content in maize seedling. The chlorophyll ‘a’ was ranged from 0.245 to 0.364. When the seeding was treated with 0.10 ppm BL, it recorded the highest chlorophyll content of 0.364 followed by 0.20 ppm BL (0.347), which was on par with 0.30 ppm BL (0.339), 0.40 ppm BL (0.339) and 0.50 ppm BL (0.347). The lowest chlorophyll ‘a’ content was recorded in control (0.245).

#### 4.1.1.2.1.2.Chlorophyll ‘b’

Significant difference was noticed in increasing the chlorophyll ‘b’ by application of brassinolide and it ranged from 0.173 to 0.295. Maximum chlorophyll ‘b’ was recorded in 0.10 ppm BL (0.295) followed by 0.20 ppm BL (0.276), 0.30 ppm BL (0.275) and 0.40 ppm (0.273). The lowest chlorophyll b content was recorded in control (0.173).

#### 4.1.1.2.1.3.Total Chlorophyll

The total Chlorophyll ranged from 0.418 to 0.659 by the application of BL to the maize seeding and treatments showed significant difference. The maximum total chlorophyll was recorded in 0.10 ppm (0.659) followed by 0.20 ppm BL (0.623), which was on par with 0.30 ppm BL (0.614) and 0.40 ppm BL (0.611). The treatment 0.05 ppm BL (0.447) and 0.01 ppm BL (0.437) were on par and the control (0.418) recorded the lowest total chlorophyll.

#### 4.1.1.2.2. Soluble Protein (mg $g^{-1}$)

A significant result was noticed for the soluble protein by treatment of different concentration of brassinolide to maize seedlings. The Soluble protein ranged from 4.76 to 6.62. The maximum soluble protein was recorded in the treatment 0.10 ppm BL (6.62) that was on par with 0.20 ppm BL (6.40). The treatment 0.01 ppm BL (6.11) was on par with 0.30 ppm BL (5.95), 0.40 ppm BL (6.13) and 0.50 ppm BL (6.15). The lowest was recorded in control (4.76).

#### 4.1.1.2.3. Nitrate reductase activity (µmole $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$)

Nitrate reductase activity was ranged from 3.42 to 4.65 and which was highly significant with the different treatments of brassinolide. The maximum NRase activity was observed in 0.10 ppm BL (4.65) followed by 0.20 ppm (4.34), which was on par with 0.30 ppm (4.23). The treatment 0.40 ppm (4.00) was on par with 0.50 ppm (4.19). The lowest NRase activity was recorded in control (3.42), which was on par with 0.01 ppm BL (3.48).

#### 4.1.1.2.4. IAA oxidase activity (µg unoxidised auxin $g^{-1}$ $hr^{-1}$)

Application of different concentration of brassinolide decreased the IAA oxidase activity. The IAA oxidase activity ranged from 54.3 to 58.9. The lowest activity was observed in 0.10 ppm BL (58.9) that was on par with 0.20 ppm BL (58.3). The treatment 0.05 ppm BL (56.5) was on par with 0.30 ppm BL (57.8). The highest activity was recorded in control (54.3).

### 4.1.2. Influence of BL in overcoming drought stress effect on maize seedlings (Table: 3 and 4) (Plate:1)

### 4.1.2.1. Morphological characters

#### 4.1.2.1.1. Shoot length (cm)

The influence of brassinolide on drought induction showed significant difference in shoot length of seedlings. The shoot length ranged from 8.3 to 12.4. The drought stress reduced the plant height in all the treatments but by the induction of BL, the plant height was recovered. The maximum plant height recovered in 0.01 ppm BL (12.4) on par with 0.20 ppm (11.5). The treatment 0.05 ppm BL (10.8) and 0.01 ppm BL (10.5) were on par with each other. In the control it recorded the lowest shoot length of 8.3.

#### 4.1.2.1.2. Root length (cm)

The application brassinolide had significant influence on the root length when it was given thought stress. The root length was ranged from 6.5 to 10.8. The maximum root length was recorded in 0.10 ppm BL (10.8) that was on par with 0.20 ppm BL (10.2), 0.40 ppm BL (9.6) and 0.50 ppm BL (9.5). The control recorded the lowest recovery of 6.5 for the root length.

#### 4.1.2.1.3. Seedling dry weight (g)

The drought had pronounced effect on seedling dry weight. By the application of brassinolide the drought effect was reduced in the seedling dry weight. The influence of brassinolide showed significant different for various treatment in seedling dry weight. The seedling dry weight ranged from 0.103 to 0.145. Maximum seedling dry weight was produced by 0.10 ppm BL (0.145), which was on par with 0.20 ppm BL (0.140) and lowest was recorded in control (0.103).

### 4.1.2.2. Biochemical Characters

#### 4.1.2.2.1. Chlorophyll content (mg g-1)

#### 4.1.2.2.1.1.Chlorophyll ‘a’

Significant recovery of chlorophyll ‘a’ was noticed in drought stressed seedling by brassinolide. The chlorophyll ‘a’ was ranged from 0.131 to 0.227. The maximum chlorophyll ‘a’ was recorded in 0.1 ppm BL (0.227) was on par with 0.20 ppm BL (0.216). The lowest was recorded in the control (0.131).

#### 4.1.2.2.1.2.Chlorophyll ‘b’

Brassinolide application showed significant recovery for chlorophyll ‘b’ in drought stressed seedling. The chlorophyll ‘b’ content was ranged from 0.106 to 0.164. The maximum chlorophyll ‘b’ was noticed in 0.10 ppm BL (0.164), which was on par with 0.20ppn BL (0.160) and 0.05 ppm BL (0.155) and 0.01 ppm BL (0.150) were on par to each other. The lowest chlorophyll ‘b’ was recorded in control (0.106).

#### 4.1.2.2.1.3.Total Chlorophyll (Fig. 2)

The brassinolide application significantly recovered the total chlorophyll in drought stressed seedlings. The total chlorophyll content was ranged from 0.237 to 0.391. The total chlorophyll was recorded in 0.10 ppm BL (0.391) was on par with 0.20 ppm BL (0.376). The lowest total chlorophyll content was recorded in control (0.237).

#### 4.1.2.2.2. Soluble protein (mg $g^{-1}$) (Fig.2)

A significant result was obtained by application of brassinolide for drought stress seedlings. The soluble protein ranged from 2.26 to 3.67. As the concentration of brassinolide increases the soluble protein was also increased in recovered seedlings. The highest soluble protein content was recorded in 0.10 ppm BL (3.67) on par with 0.20 ppm BL (3.55). The lowest soluble protein was record in the control (2.26).

#### 4.1.2.2.3. Proline (µmole $g^{-1}$ FW)

Significant difference was noticed for the proline accumulation by treating BL under drought stress. The proline ranged from 24.12 to 59.42. The maximum proline accumulation was noticed in control (59.42). The lowest accumulation of proline was in 0.1 ppm BL (24.12), which was on par with 0.20 ppm BL.

#### 4.1.2.2.4. Nitrate reductase activity (µmole $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$) (Fig.3)

The NRase activity in the stressed plant was very low in control (2.04) than the brassinolide treated stress plant. The NRase activity showed significant difference among the treatments. Among the treatments tried, the treatment 0.10 ppm (3.28) recorded the maximum NRase activity, which was on par to 0.20 ppm BL (3.14), and was on par with 0.05 ppm BL (3.10). The NRase activity was ranged from 2.04 to 3.28.

### 4.1.2.2.5. IAA oxidase activity (µg unoxidized auxin $g^{-1}$ $hr^{-1}$)

A significant result was obtained for IAA oxidase activity in the brassinolide applied stressed plant. The IAA oxidase activity was ranged from 62.5 to 76.0. The lowest activity was recorded in 0.1 ppm BL (76.0), which was on par with 0.05 ppm BL (73.6) and 0.20 ppm BL (70.3).

### 4.1.2.2.5. Free radical scavenging enzymes (Fig.3)

### 4.1.2.2.5.1.Catalase activity (enzymes units x $10^4$ $g^{-1}$ $min^{-1}$)

Catalase enzyme would scavenge the free radical that was produced by the drought stress. The catalase activity was significantly increased in BL pretreated seedlings during drought stress. The activity ranged from 1.52 to 2.69. The maximum catalase activity was recorded in 0.10 ppm BL (2.69), which were on par to 0.05 ppm BL (2.57) and 0.20 ppm BL (2.55). The lowest catalase activity was recorded in control (1.52).

### 4.1.2.2.5.2. Peroxidase activity (enzymes units $1^{-1}$ $h^{-1}$)

The enzymes peroxidase would scavenge the free radical, which were produced during the drought stress. The peroxidase activity was ranged from 16.2 to 38.3. The activity was highly significant to different treatments tried. The maximum activity was recorded in 0.10

ppm BL (38.3), which was on par with 0.05 ppm BL (34.7) and 0.20 ppm BL (34.3). The lowest peroxidase activity was recorded in control (16.2).

#### 4.1.2.2.5.3. Super oxide dismutase (enzyme unit mg protein$^{-1}$min$^{-1}$)

The maximum activity of SOD was recorded in 0.10 ppm BL (2.12), which was on par with 0.20 ppm BL (2.06) and 0.05 ppm BL (2.00). The lowest activity was observed in control (1.37). The SOD activity was significant to brassinolide treatment under drought stress. The SOD ranged from 1.37 to 2.12.

### 4.1.3. Influence of BL in overcoming Low Temperature stress on maize seedlings (Table : 5 and 6) (Plate:1)

#### 4.1.3.1. Morphological Characters

#### 4.1.3.1.1. Shoot length (cm)

The application of brassinolide has significant influence on the shoot length when it was given chilling stress. The shoot length was ranged from 6.5 to 10.8. The maximum shoot length recovered in the treatments 0.10 ppm BL (10.8), which was on par with 0.20 ppm BL (10.6) and 0.30 ppm BL (9.8). The lowest shoot length was recorded in control (6.5).

#### 4.1.3.1.2. Root length (cm)

The influence of BL on cold induction showed significant difference in root length of the seedlings. The root length ranged from 4.2 to 9.1. The drought stress reduced the root length but the in the presence of brassinolide the effect of stress was reduced. The maximum root length was observed in 0.10 ppm BL (9.1), which was on par with 0.20 ppm BL (8.7), 0.05 ppm BL (8.2). The lowest root length was recorded in control (4.2).

#### 4.1.3.1.3 Seedling dry weight (g)

Application of brassinolide has significantly reduced the cold stress effect on seedling dry weight. The seedling dry weight was produced in 0.10 ppm BL (0.148), which was on par with 0.20 ppm (0.142), 0.05 ppm BL (0.134). The lowest seedling dry weight was recorded in control (0.095).

### 4.1 3.2. Biochemical characters

#### 4.1.3.2.1. Chlorophyll content (mg $g^{-1}$)

#### 4.1.3.2.1.1.Chlorophyll ‘a’

Significant recovery of chlorophyll ‘a’ was noticed in cold stressed seedling by brassinolide. The chlorophyll ‘a’ was ranged from 0.117 to 0.208. The chlorophyll ‘a’ content was recorded in 0.10 ppm BL (208) 0.20 ppm BL (0.198) 0.05 ppm BL (0.195). The lowest chlorophyll ‘a’ was recorded in control (0.117).

#### 4.1.3.2.1.2.Chlorophyll ‘b’

The brassinolide application significantly recovered the chlorophyll ‘b’ in cold stressed seedlings. The chlorophyll ‘b’ content was ranged from 0.076 to 0.146. The maximum chlorophyll ‘b’ was recovered in the treatment 0.10 ppm BL (0.146) which was on par with 0.20 ppm BL (0.136) and also with 0.05 ppm BL (0.132). The treatment 0.05 ppm BL was on par with 0.1 ppm BL (0.126). The lowest was recorded in control (0.076).

#### 4.1.3.2.1.3.Total chlorophyll (Fig.4)

Significant recovery of total chlorophyll was recorded in cold stressed seedling by brassinolide treatments. The total chlorophyll ranged from 0.193 to 0.354. The highest total chlorophyll content was recorded in 0.10 ppm BL (0.354), which was on par with 0.20 ppm BL (0.334) and 0.05 ppm BL (0.327). The lowest total chlorophyll content was recorded in $T_1$ (0.193).

### 4.1.3.2.2. Soluble protein (mg $g^{-1}$) (Fig.4)

The soluble protein in the cold stressed seedling was high in 0.10 ppm BL (3.23) was on par with 0.20 ppm BL (3.01). The treatment 0.20 ppm BL was on par with 0.05 ppm BL (2.96), and 0.01 ppm BL (2.84). The lowest soluble protein content was recorded in control (2.25) Significant difference was noticed in brassinolide treated stressed plant than in the control. The soluble protein was ranged from 2.25 to 3.23.

### 4.1.3.2.3. Proline (µmole $g^{-1}$ FW)

The proline accumulation was higher when the seedling were under stress condition. Treatment of brassinolide significantly reduced the accumulation of proline there by reduced chilling effect. The proline ranged from 21.05 to 52.42. The lesser amount was recorded in 0.10 ppm BL (21.52) that was on par with 0.20 ppm BL (24.32). The maximum proline was recorded in control (52.42)

### 4.1.3.2.4. Nitrate reductase activity (µmole $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$) (Fig.5)

The NRase activity showed significant difference for the brassinolide treated cold stressed plant. The NRase activity was ranged from 1.82 to 2.97. The maximum NRase activity was recorded in 0.10 ppm BL (2.97) was on par with 0.20 ppm BL (2.90) and 0.05 ppm BL (2.88). The lowest NRase activity was recorded in control (1.82).

### 4.1.3.2.5. IAA oxidase activity (µg unoxidised auxin $g^{-1}$ $hr^{-1}$)

Significant difference was obtained for IAA oxidase activity by brassinolide application. The IAA oxidase activity was ranged from 62.6 to 69.5. The lowest activity was recorded by control (69.5) followed by 0.1 ppm BL (67.6), which was on par with 0.01 ppm BL (67.3).

#### 4.1.3.2.6. Free radical Scavenging enzymes (Fig.5)

#### 4.1.3.2.6.1. Catalase activity (enzyme units x $10^4$ $g^{-1}$ $min^{-1}$)

Catalase activity would scavenge the free radical, which were produced by the cold stress. The catalase activity was significantly increased in BL treated seedling under cold stress condition. The catalase activity was ranged from 1.25 to 2.42. The highest catalase activity was recorded in 0.10 ppm BL (2.35), which on par with 0.20 ppm BL (2.38) and 0.05 ppm BL (2.35). The lowest activity was recorded in control.

#### 4.1.3.2.6.2. Peroxidase activity (enzyme units $1^{-1}$ $hr^{-1}$)

The peroxidase activity was significantly increased in cold stressed plant by treatment of brassinolide. The peroxidase activity was ranged from 36.5 to 15.4. The maximum activity was recorded in 0.10 ppm BL (36.5) followed by 0.20 ppm BL (32.3). Which was on par with 0.05 ppm BL (30.8). The lowest peroxidase activity was recorded by control (15.4).

#### 4.1.3.2.6.3. Superoxide Dismutase (enzyme units mg $protein^{-1}$ $min^{-1}$)

The SOD enzyme would scavenge the free radical, which were produced during the cold stress. The SOD was ranged from 2.27 to 1.26. The maximum SOD was recorded in 0.10 ppm BL (2.27) was on par with 0.20 ppm BL (2.23). The lowest SOD activity was recorded in control (1.26).

### 4.1.4. Influence of BL in overcoming High Temperature stress on maize seedlings

**(Table: 7 and 8) (Plate: 1)**

### 4.1.4.1. Morphological Characters

#### 4.1.4.1.1. Shoot length (cm)

The seedling treated with BL significantly recovered the shoot length in temperature induced stressed seedlings. The shoot length was ranged from 4.8 to 12.9. The maximum shoot length was recorded by absolute control (12.9). The treatment $T_5$ (11.3) recorded closer value to the control and which was on par with $T_4$ (10.1) than the treatment $T_3$ (9.5) and $T_6$ (8.8). The lowest was recorded in $T_3$ (4.8) where the temperature induction and lethal stress was given.

#### 4.1.4.1.2. Root length (cm)

The root length was ranged from 3.8 to 11.3 and significant difference was recorded in brassinolide treated seedling under temperature stress. The root length was nearly equal to absolute control in $T_5$ (10.2) and the treatment $T_4$ (8.7), $T_7$ (8.1) and $T_6$ (7.5) were on par to each other. The maximum root length was recorded in control (11.3) and the lowest was recorded in $T_3$ (3.8).

#### 4.1.4.1.3. Seedling dry weight (g)

Significant difference was noticed for seedling dry weight in BL applied maize seedling, which treated with temperature. The seedling dry weight ranged from 0.135 to 0.155. The treatment $T_5$ (0.138) recovered higher dry matter than the other treatments and which was on par with $T_4$ (0.136), $T_7$ (0.134) and $T_6$ (0.123). The maximum dry weight in seedling was recorded by absolute control (0.155) and the lowest recorded by $T_3$ (0.063), which was on par with $T_2$ (0.087).

### 4.1.4.2. Biochemical characters

#### 4.1.4.2.1. Chlorophyll (mg $g^{-1}$)

#### 4.1.4.2.1.1.Chlorophyll ‘a’

The chlorophyll ‘a’ significantly improved by the treatment of brassinolide in temperature stressed seedling. The chlorophyll ‘a’ ranged from 0.092 to 0.175. The treatment $T_5$ (0.217) near value that of the control than the other treatments. The maximum chlorophyll ‘a’ was recorded in absolute control (0.234) and the lowest in $T_3$ (0.053).

#### 4.1.4.2.1.2.Chlorophyll ‘b’

Treating the temperature-induced plant significantly improved the chlorophyll ‘b’ content and the chlorophyll ‘b’ was ranged from 0.042 to 0.175. The highest chlorophyll ‘b’ was recorded in control (0.175), which was on par with $T_5$ (0.156). The treatment $T_5$ was on par with the treatments $T_4$ (0.149) and $T_7$ (0.136). The lowest was recorded in $T_3$ (0.042), which was on par with $T_2$ (0.058).

#### 4.1.4.2.1.3.Total Chlorophyll (Fig. 6)

The total chlorophyll content was ranged from 0.095 to 0.409. The total chlorophyll content was recorded significantly by application of BL, which induced with the temperature. The maximum recovery was recorded in $T_5$ (0.373) followed $T_4$ (0.344). The treatments $T_6$ (0.298) and $T_4$ (0.318) were on par to each other. In absolute control (0.409) maximum total chlorophyll content was recorded and the lowest in $T_3$ (0.095).

#### 4.1.4.2.2. Soluble protein (mg $g^{-1}$) (Fig. 6)

Significant results were obtained by the treating the seedling with BL in temperature induce maize seedling. The soluble protein was ranged from 2.05 to 4.89. The maximum recovery of soluble protein was observed in $T_5$ (4.10) that was on par with $T_4$ (3.82). The treatments $T_7$ (3.36) was on par with $T_6$ (3.25). The highest soluble protein content was recorded in control (4.89) and the lowest was recorded in $T_3$ (2.05), which was on par with $T_2$ (2.29).

### 4.1.4.2.3. Proline (µmole $g^{-1}$ FW)

The free amino acid proline was accumulating high during the temperature stress. The treatment of BL significantly reduced the temperature induction stress by reduced the proline content. The proline was ranged from 11.24 to 57.42. In temperature-stressed plant, the reduction in proline content was observed in $T_5$ (22.12), which was on par with $T_4$ (24.34). The treatment $T_7$ (49.32) was on par with $T_6$ (45.65). The highest proline was recorded in $T_3$ (57.42) that was on par with $T_2$ (55.42) and the lowest proline content was recorded in absolute control (11.24).

### 4.1.4.2.4. Nitrate reductase activity (µmole $NO^{-}_2$ $g^{-1}$ $hr^{-1}$) (Fig.7)

The NRase in temperature-induced plant had lesser activity than the seedling treated with BL. There was significant increase in NRase activity in BL treated seedlings. The NRase ranged from 1.38 to 3.29. The control (3.29) recorded the highest NRase activity, which was on par with $T_5$ (3.01). The treatment $T_4$ (2.93) was on par with $T_7$ (2.78). The lowest was recorded in $T_3$ (1.38), which was on par with $T_2$ (1.52).

### 4.1.4.2.5 IAA oxidase activity by (µg unoxidised auxin $g^{-1}$ $hr^{-1}$)

The IAA oxidase activity was decreased in temperature stressed seedling than the seedling treated with BL. There was a significant difference noticed for IAA oxidase activity. It was ranged from 22.2 to 58.5. The minimum activity in BL treated seedling was recorded in $T_5$ (42.2), which was on par with $T_4$ (40.5). The treatment $T_7$ (35.5) and $T_6$ (33.5) were on par to each other. The lowest activity was noticed in absolute control (58.5) and the maximum in $T_3$ (22.2).

### 4.1.4.2.6. Free radical Scavenging enzyme (Fig.7)

### 4.1.4.2.6.1. Catalase activity (enzyme units x$10^4$ $g^{-1}$ $min^{-1}$)

The catalase activity was significantly increased in BL treated seedling under temperature stress condition. The maximum activity in treated seedling was in $T_5$ (2.95), which was on par with $T_4$ (2.73). The treatment $T_7$ (2.39) was on per with $T_6$ (2.09). The lowest was recorded in $T_3$ (0.98), which was on par with $T_2$ (1.20) and $T_1$ (1.06).

### 4.1.4.2.6.2. Peroxidase activity (enzyme units $1^{-1}$ $hr^{-1}$)

The peroxidase enzyme activity would scavenge the free radical, which were produced during temperature stress. It was significantly differ for the BL treated plants. The peroxidase activity was ranged from 21.30 (52.13), which was on par with $T_4$ (48.23). The lowest was recorded by absolute control (20.54), which was on par with $T_3$ (21.30).

### 4.1.4.2.6.3. Superoxide dismutase (enzyme units mg $protein^{-1}$ $min^{-1}$)

The maximum activity of SOD was recorded in $T_5$ (2.95), which was on par with $T_4$ (2.62) $T_7$ (2.55) and $T_6$ (2.25). The treatments $T_3$ (1.03) recorded the lowest activity of SOD, which was on par with T2 (1.42). SOD activity was significant to the BL applied temperature stressed and recovered seedlings. The SOD activity was ranged from 1.03 to 2.95.

## 4.15. Influence of BL on salinity tolerance stress in maize seedlings (Table: 9 and 10) (Plate:1)

### 4.1.5.1. Morphological Characters

### 4.1.5.1.1. Shoot length (cm)

Treatment of BL significantly increased the shoot length in salinity stressed maize seedlings. The shoot length was ranged from 4.3 to 13.6. The maximum shoot length was recovered by treating BL in by salinity induced seedlings in the treatment $T_4$ (11.4) which was on par with $T_5$ (10.3) and The treatment $T_6$ (8.1) which was on par with $T_7$ (8.9). The maximum shoot length was observed in absolute control (13.6) and lowest was recorded in $T_3$ (4.3).

#### 4.1.5.1.2. Root length (cm)

Treatment of brassinolide significantly increased the root length of salinity induced maize seedling. The root length was ranged from 3.1 to 10.5. The root length was nearly equal to absolute control (10.5) in the treatment $T_5$ (9.3) that was on par with $T_4$ (8.9) in salinity-induced seedlings. The treatment $T_7$ (8.1) was on par with $T_6$ (7.1). The minimum root length was recorded in $T_3$ (3.1).

#### 4.1.5.1.3. Seedling dry weight (g)

Application of BL significantly improved the seedling dry weight in salinity-induced seedlings. The seedling dry weight ranged from 0.061 to 0.181. The maximum improvement was achieved by the treatment $T_5$ (0.164) that was on par with $T_4$ (0.149). The treatment $T_7$ (0.121) was on par with $T_6$ (0.109). The maximum dry weight in seedling was recorded by absolute control (0.181) and the lowest recorded by $T_3$ (0.061), which was on par with $T_2$ (0.073).

### 4.1.5. 2. Biochemical characters

#### 4.1.5.2.1. Chlorophyll (mg $g^{-1}$)

##### 4.1.5.2.1.1. Chlorophyll ‘a’

Significant recovery of chlorophyll ‘a’ was noticed in salinity induced seedlings treated with BL. The chlorophyll ‘a’ ranged from 0.057 to 0.226. The maximum chlorophyll ‘a’ was recovered in the treatment $T_5$ (0.202), which was on par with $T_4$ (0.183). The treatment T7 (0.150) was on par with $T_6$ (0.142). The maximum chlorophyll ‘a’ was recorded in absolute control (0.226) and the lowest in $T_3$ (0.057).

#### 4.1.5.2.1.2. Chlorophyll ‘b’

Brassinolide treatment significantly improved the chlorophyll ‘b’ content in salinity induced maize seedlings and the chlorophyll ‘b’ was ranged from 0.060 to 0.153. The maximum chlorophyll ‘b’ was recovered $T_5$ (0.136), which was on par with the treatments $T_4$ (0.128) and $T_7$ (0.123). The lowest was recorded in $T_3$ (0.060).

#### 4.1.5.2.1.3. Total Chlorophyll (Fig.8)

Significant result was obtained for total chlorophyll by pre-treatment of BL to salinity-induced seedlings. The content was ranged from 0.117 to 0.379. The maximum recovery was recorded in $T_5$ (0.338) followed by $T_4$ (0.311). The total chlorophyll was recorded maximum in absolute control (0.379) and the lowest in $T_3$ (0.117).

#### 4.1.5.2.2. Soluble protein (mg $g^{-1}$) (Fig.8)

Pretreatment of BL significantly influenced the soluble protein content on salinity-induced seedling. The soluble protein was ranged from 2.03 to 3.83. The maximum recovery of soluble protein was observed in $T_5$ (3.72) that was on par with $T_6$ (3.35), $T_4$ (3.25) and T7 (3.23). The maximum soluble protein content was recorded in control (3.83) and the minimum was recorded in $T_3$ (2.03).

#### 4.1.5.2.3. Proline (µmole $g^{-1}$ FW)

Proline, the free amino acid was accumulating high during induced salinity stress. The treatment of BL significantly reduced the salinity stress by reduction in the proline content. The proline was ranged from 10.14 to 45.45. The minimum proline content was in $T_5$ (16.86), which was on par with $T_4$ (20.12). The highest proline was recorded in $T_3$ (45.45) that was on par with $T_2$ (41.32) and in absolute control it recorded 11.24.

#### 4.1.5.2.4. Nitrate reductase activity (µmole $NO^-_2$ $g^{-1}$ $hr^{-1}$) (Fig.9)

The NRase activity was ranged from 1.34 to 4.02. The NRase activity was recorded maximum in $T_5$ (3.90) was on par with $T_4$ (3.72). The control (4.02) recorded the highest NRase activity and minimum activity in T3 (1.34). There was significant increased in NRase activity in BL treated seedlings under induced salinity.

#### 4.1.5.2.5. IAA oxidase activity by (µg unoxidised auxin $g^{-1}$ $hr^{-1}$)

The IAA oxidase activity showed significant decrease in salinity stressed seedling than the seedling treated with BL. The minimum activity in treated seedling was recorded in $T_5$ (48.2), followed by $T_4$ (42.7). The lesser activity was noticed in absolute control (57.3) and the highest in $T_3$ (21.4).

#### 4.1.5.2.6. Free radical Scavenging enzyme (Fig.9)

##### 4.1.5.2.6.1.Catalase activity (enzyme units x $10^4$ $g^{-1}$ $min^{-1}$)

The catalase activity was significantly increased in BL treated seedling under salinity stress condition. The activity was ranged from 1.1 to 2.8.The maximum activity was in $T_5$ (2.8), which was on par with $T_4$ (2.7) and $T_7$ (2.6). The lowest was recorded in $T_1$ (1.1), which was on par with $T_3$ (1.2) and $T_2$ (1.3).

#### 4.1.5.2.6.2.Peroxidase activity (enzyme units $1^{-1}$ $hr^{-1}$)

The peroxidase enzyme activity would scavenge the free radical, which were produced during salinity stress. It was significantly differ among the treatment. The peroxidase activity was ranged from 25.36 to 43.15. The maximum activity was recorded in $T_5$ (43.15) followed by $T_4$ (39.63). The lowest was recorded by $T_1$ (25.36).

#### 4.1.5.2.6.3.Superoxide dismutase (enzyme units mg protein $^{-1}$ $min^{-1}$)

SOD activity was significant to the BL applied salinity stressed and recovered seedlings. The SOD activity was ranged from 1.22 to 2.73.The maximum activity of SOD was recorded in $T_5$ (2.73), followed by $T_4$ (2.42) that was on par with $T_7$ (2.15). The treatments $T_1$ (1.67) and $T_2$ (1.44) were on par to each other. The lowest activity of SOD was recorded in $T_3$ (1.22).

## 4.1.6. Effect of brassinolide on Protein profile (Plate: 2a and b)

### 4.1.6.1. Drought stress

The leaf protein profile separated through SDS-PAGE showed clear distinct bands isolated due to the effect of brassinolide on drought implication. There was a clear distinct five bands with the Rm value of 0.51, 0.68, 0.71, 0.83 and 0.95. Among these the treatment $T_4$, the bandwidth Rm value of 0.83 and 0.95 is highly denser and significant compared to other treatments.

#### 4.1.6.2. Low temperature

The protein profile isolated from the leaf pretreatment with BL and subjected to low temperature showed a clear three protein bands with an Rm value of 0.67, 0.80 and 0.93. Here again the treatment $T_4$ clearly showed a thicker band with an Rm value 0.67.

#### 4.1.6.3. High Temperature

The induction response of brassinolide to high temperature stress revealed that a protein profile band, which could be visualized as four with the Rm value 0.54, 0.71, 0.84 and 0.95. Among the treatments the pre-treatment of BL to the seedlings then subjected to induction responses produced a significant thicker protein band.

#### 4.1.6.4. Salinity

The induction response of brassinolide to salinity stress revealed that a protein band which was visualized as four with the Rm value of 6.2, 0.74, 0.81 and 0.88. Among the treatment tried, the pretreatment of BL to the maize seedling then subjected to induction response produce a significant thicker band.

## 4.2. Glasshouse experiment

The brassinolide concentrations 0.05 and 0.10 ppm were used in the pot culture experiment as these two concentrations preformed better in laboratory experiments. To study the effect of BL interaction with benzyl adenine and naphthalene acetic acid, the above concentrations were tried. The observation on various morpho-physiological, biochemical, yield and yield components were studied and subjected to statistical analysis. The results obtained are presented below

### 4.2.1. Morphological characters

#### 4.2.1.1. Plant height (cm) (Table: 11) (Fig.10)

The date on plant height was recorded at 30, 60 and 90 DAS. The plant height showed a gradual increase throughout the growth period and reached the maximum at harvest in all the treatments.

Brassinolide as foliar spray individually increased the plant height compared to other growth regulators. The maximum plant height was recorded in the higher concentrations than the lower concentration of plant growth regulators. The highest was recorded in the foliar application of 0.1 ppm BL (186.3) and the treatment 0.05 ppm BL, 200 ppm BA and 10 ppm NAA were on par with each other. The foliar application of 10 ppm BA recorded the lowest plant height.

In combined application of brassinolide with other plant growth regulators, all the treatments irrespective of their individual concentration increased the plant height, compared to application of plant growth regulators individually. The maximum plant height was observed in higher concentrations of growth regulators. The highest plant height was recorded by 0.10 ppm BL + 10 ppm NAA (191.3) and which was par with 0.05 ppm and 10 ppm NAA. The treatment 0.05 ppm + 20 ppm BA and 0.1 ppm + 20 ppm BA were recorded on par for the plant height.

When all the plant growth regulators were combined and sprayed the plant height increased to the maximum extent. Here also growth regulators at higher concentrations increased the plant height once again. The highest plant height was recorded by the treatments 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (199.2) followed by 0.05 ppm BA + 20 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA significantly increased the plant height. The highest plant height was recorded by the application of 0.1 ppm BL+20 ppm BA + 10 ppm NAA (199.2) compared to other treatments and control (173.7). The percent increase in the plant height due to treatment was 14.5 compared to control.

#### 4.2.1.2. Root length (cm) (Table: 12)

The response of root length to plant growth regulators was differs among the different treatments. Application of growth regulators increased the root length over the crop growth periods and the maximum root length was obtained in the harvesting stage of the crop.

Application brassinolide and other growth regulators individually increased the root length at all the concentration than the control. The highest was obtained in the treatment 0.1 ppm BL and 20 ppm BA (54.0) followed by 0.05 ppm BL (52.0) and 10 ppm NAA was on par with 10 ppm BA.

In combined application BL with other growth regulators there was an increased in the root length irrespective of their concentrations when applied individually. The maximum plant height was recorded in 0.10 ppm BL + 20 ppm BA (60.0) was on par with 0.05 ppm BL + 20 ppm BA.

The root length was increased when all the growth regulators were combined and sprayed. At the higher concentration of growth regulators, the root length obtained maximum possible. Foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (67.5) increased the root length followed by 0.10 ppm BA +10 ppm NAA.

The results on the root length for the foliar application of growth regulators increased the root length to its maximum and the highest was recorded by foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (67.5) than the other treatment and control.

### 4.2.2. Growth attributes

#### 4.2.2.1. Leaf Area (cm$^2$ plant $^{-1}$) (Table: 13)

The leaf area was increased from 30DAS, reaching its maximum at 60DAS and there after it declined in all the treatments and control.  The influence of the growth regulators on leaf area was increased at all the concentrations on 60DAS.

Application of brassinolide and other growth regulators individually increased the leaf area at 60DAS.  The maximum leaf area was obtained in the 0.10 ppm BL (3353) foliar spraying and which was on par with the foliar spraying of 0.05 ppm and 10 ppm NAA.  10 ppm BA recorded the less leaf area among the plant growth regulators applied.

When BL was combined with either BA or NAA and foliar sprayed, the leaf attained its maximum area irrespective of the concentrations of growth regulators on 60DAS.  Foliar application of BL 0.1 ppm + 10 ppm NAA (3410) recorded the maximum leaf area followed by 0.05 ppm BL+ 10 ppm NAA.  The treatment 0.05 ppm BL + 10 ppm BA, 0.10 ppm BL + 10 ppm BA and 0.10 ppm BL + 20 ppm BA (3366) were on par with each other.

Application of all the growth regulators in combined sprayed, recorded the maximum leaf area at 60DAS.  In this, irrespective of the individual concentrations, all the combined treatments increased the leaf area.  The maximum leaf area was recorded in foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (3495) followed by 0.05 ppm BL + 20 ppm BA + 10 ppm NAA.

The leaf area recorded a significant difference between the different treatments and control.  The maximum leaf area was obtained in the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (3495) at 60DAS and the increased leaf area due to this treatment was 16.8 percent compared to control.

### 4.2.2.2. Specific leaf weight (mg cm$^{-2}$) (Table: 14) (Fig.11)

Specific leaf weight (SLW) was computed from the values of leaf dry weight and leaf area. SLW progressively increased up to 30DAS then it declined up to 60DAS and a slight in crease was observed on 90DAS. Application of growth regulators individually in combined had increased the SLW.

When the growth regulators were applied individually, brassinolide recorded the highest SLW in all crop growth stage. The highest SLW was recorded in the foliar application of 0.10 ppm BL (7.20), which was on par with 10 ppm NAA. The foliar application of 0.05 ppm BL and 20 ppm BA were on par to each other.

In the combined application of BL with either BA or NAA, the SLW was increased at 30DAS. The foliar application BL at 0.10 ppm+10 ppm NAA (7.80) recorded the maximum SLW and it was on par with 0.05 ppm BL + 10 ppm NAA. The treatment 0.10 ppm BL + 20 ppm BA and 0.05 ppm BL + 20 ppm BA were on par with each other in SLW.

When the BL, BA and NAA were applied combined, it recorded the highest SLW at 30 DAS and a decrease at 60 DAS and slightly increased at 90DAS. The highest SLW was recorded by foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (8.80) at 30DAS followed by 0.05 ppm BL + 20 ppm BA + 20 ppm NAA.

The interaction effect of BL with BA and NAA, showed a significant difference between the treatments and the control for SLW. The highest SLW was obtained by the foliar application of 0.10 ppm BL + 20 ppm BA + 20 ppm NAA (8.80) than the other treatments and control (6.30) at 30DAS.

#### 4.2.2.3. Specific leaf area ($cm^2$ $g^{-1}$) (Table: 15) (Fig.12)

The specific leaf area (SLA) showed inverse relationship with specific leaf weight. Significant difference was observed between the different treatments and control.  The SLA showed a gradual increase through out the crop growth period and it reached maximum at harvest in all the treatments.

When BL was sprayed individually it decreased the SLA compared to other growth regulators were applied individually.  The minimum SLA was recorded in the higher concentration of plant growth regulators.  The minimum SLA was recorded by 0.10 ppm BL, which was on par with 0.05 ppm BL at 30DAS.

When BL was combined with BA or NAA, there was a decreased in the SLA irrespective of the concentration than when they were applied individually.  The minimum SLA was noticed in higher concentrations of growth regulators.  The lowest SLW was recorded by 0.10 ppm BL + 10 ppm NAA (225) and which was on per with 0.05 ppm BL + 10 ppm NAA.  The treatment 0.10 ppm BL + 20 ppm BA (282) and 0.05 ppm + 10 ppm BA were on par to each other and the treatment.10 ppm BL + 10 ppm BA (293) and 0.05 ppm BL + 10 ppm BA were on par.

When all the plant growth regulators were combined and sprayed, the SLW was decreased to minimum.  Here also growth regulators at higher concentrations had more influence in reducing the SLA.  The lowest SLA was recorded by the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (235) followed by 0.05 ppm + 20 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA significantly decreased the SLA. The lowest SLA was recorded by the application of 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (235) compared to other treatments and control (308).  The percent decrease in the SLA due to this treatment was 22.7 percent over the control.

### 4.2.2.4 Net Assimilation Rate (mg cm$^{-2}$ day$^{-1}$) (Table: 16)

The Net Assimilation rate (NAR) showed significant difference among the growth regulators sprayed. The NAR was increased upto 30DAS and than it decreased there after during the crop growth period in all the treatments and control. The range for the NAR was between 2.51 to 0.72.

When the growth regulators were applied individually, brassinolide showed higher NAR than the other growth regulators. With reference to the concentrations of the growth regulators, at higher concentration they recorded the maximum NAR. Foliar application of 0.10 ppm BL (2.23) recorded the highest NAR at 30DAS followed by 0.05 ppm BL. The lowest NAR was recorded in 10 ppm BA (2.01), which was on par with control, and 0.20 ppm BA.

In combined application of brassinolide either with BA or NAA, all the treatments irrespective of their individual concentrations increased the NAR, compared to application of plant growth regulators individually. The maximum NAR was obtained in higher concentrations of growth regulators. The highest NAR was recorded by 0.10 ppm BL + 10 ppm NAA (2.35). The treatment 0.10 ppm BL + 20 ppm BA and 0.05 ppm + 20 ppm BA were on par with each other.

When BL, BA and NAA were combined and sprayed it increased the NAR to the maximum. The growth regulators at higher concentration increased the NAR once again at all the growth stages. The highest NAR was recorded by 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (2.51) which was on par with 0.05 ppm BL + 20 ppm BA + 10 ppm NAA 0.05 ppm BL +10 ppm BA + 10 ppm NAA and 0.10 ppm BL +10 ppm BA + 10 ppm NAA.

The highest NAR was recorded in foliar spraying of 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (2.51) than the other treatments and control (2.01). The increased NAR on this treatment was 24.8 per cent over the control.

### 4.2.2.5. Crop Growth Rate (gm$^{-2}$ day$^{-1}$) (Table: 17)

The Crop Growth Rate (CGR) was increased upto 30DAS and it was declined upto 60DAS and they're after it increased upto 90DAS and it obtained the maximum extent. The same pattern of Crop Growth Rate was followed for all the treatments and control.

Application of BL and other growth regulators individually increased the CGR in all crop growth period. The BL as foliar spray individually applied increased the CGR than other growth regulators. The highest CGR was recorded in the foliar application of 0.10 ppm BL (38.57) at 90DAS and it was followed by 10 ppm NAA.

In combined application of BL either with BA or NAA the NAR attained maximum irrespective of the concentration of the growth regulators on 90DAS. Application of 0.1 ppm BL + 10 ppm NAA (41.02) recorded the highest NAR and it was followed by 0.05 ppm BL + 10 ppm NAA.

Application of all the growth regulators by combined spray recorded the maximum CGR at 90DAS followed by 30DAS. Irrespective of the individual concentrations, all the combined treatments increased the NAR. The highest NAR was recorded by 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (43.13) which was on par with 0.05 ppm BL + 20 ppm BA + 10 ppm NAA and 0.10 ppm BL + 10 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA significantly increased the NAR in all the treatments at different growth stages. The highest NAR was recorded by the treatments 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (43.13) than other treatments and control (31.08) at 90DAS. The increase in NAR was 38.7 percent over the control.

### 4.2.2.6. Relative Growth Rate (g $g^{-1}$ $day^{-1}$) (Table: 18)

The Relation Growth Rate (RGR) showed a gradual decrease from 30 to 60DAS and there after it steadily decline towards the harvest. The highest RGR was recorded at 30 DAS followed by 60 DAS and 90 DAS. The effect of plant growth regulators showed significant difference between the treatments and control.

When the growth regulators were applied individually brassinolide showed maximum RGR than the others. The RGR was increased by the application of higher concentrations of growth regulators individually than in their lower concentrations. Foliar application of 0.10 ppm BL (0.148) increased the RGR at 30DAS. The treatment 0.05 ppm BL (0.143) 10 ppm NAA and 20 ppm BA were on par with each other.

In combination of BL with either BA or NAA, the RGR was increased in all the combination of treatments at all the stages of the crop growth period. The maximum RGR was noticed in the higher concentrations treatments. The highest RGR was recorded by the treatment 0.10 ppm BL + 10 ppm NAA (0.155). The treatment 0.05 ppm BL + 10 ppm NAA (0.151) was on par with 0.10 ppm BL + 20 ppm BA and the treatment 0.10 ppm BL + 10 ppm BA (0.136) and 0.05 ppm + 10 ppm BA were on par with each other.

When all the plant growth regulators were combined and sprayed, the RGR was increased to its maximum extent. Here also growth regulators at higher concentrations had more influence in increasing the RGR. The highest RGR was recorded by the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (0.167) at 30DAS followed by 0.05 ppm BL + 20 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA showed significant difference between the treatments over the control for RGR. The highest RGR was recorded by 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (0.167) than other treatments and control (0.133) at 30DAS. The percent increase of RGR over the control was 25.6.

#### 4.2.2.7.Total Dry matter production (g plant$^{-1}$) (Table: 19) (Fig.13)

Dry matter is an important criterion as it determines the source – sink relationship. The data on total dry matter production (TDMP) was recorded at 30, 60 and 90DAS. The TDMP showed a steep increase throughout the growth period and recoded the maximum dry matter accumulation at harvest in all the treatments.

Application of BL, BA and NAA individually increased the accumulation of dry matter. When brassinolide was foliar sprayed individually increased the TDMP compared to the other growth regulators. The maximum accumulation of dry matter was noticed in the foliar application of 0.10 ppm BL (150.55) which was on par with the following treatments 0.05 ppm BL, 10 ppm NAA and 20 ppm BA.

In combined foliar spray of BL either with BA and NAA, all the treatments irrespective of their individual concentrations increased the accumulation of total dry matter compared to application of plant growth regulators individually. The maximum accumulation was obtained by foliar spraying 0.10 ppm BL + 10 ppm NAA (153.25) which was on par with 0.05 ppm BL + 20 ppm BA.

When all the plant growth regulators are combined and foliar sprayed, the accumulation of total dry matter was increased and attained the maximum extent. The plant growth regulators at higher concentrations increased the accumulation of TDMP once again. More accumulation of TDMP was recorded in 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (165.36) followed by 0.10 ppm + 10 ppm BA + 10 ppm NAA which was on par with 0.05 ppm + 20 ppm BA + 10 ppm NAA.

The highest accumulation of TDMP was recorded in foliar spraying of 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (165.36) than the other treatment and control (140.62). The TDMP showed a significant difference among the treatments and the increased percent of TDMP was 17.6 than the control.

### 4.2.3. Chlorophyll content

#### 4.2.3.1. Chlorophyll 'a' (mg $g^{-1}$) (Table: 20)

The maximum chlorophyll a content was recorded at 60DAS followed by 90DAS and 30DAS in the active photosynthetic leaves. Significant difference was exhibited between the treatment and control in the crop growth period. The chlorophyll content was increased upto 60DAS and it was decline there after, till harvest.

Application of brassinolide BA and NAA individually increased the chlorophyll a content at all the concentrations than the control. The highest chlorophyll a content was recorded in the treatment 0.10 ppm BL (0.505) followed by 0.05 ppm BL, which was on par with 10 ppm NAA. The treatments 20 ppm BA (0.434) and 10 ppm BA were on par to each other for chlorophyll a content.

In combined application of BL either with BA or NAA, the chlorophyll a content attained maximum, irrespective of concentration at 60DAS. Application of 0.10 ppm BL + 10 ppm NAA (1.123) recorded the highest followed by 0.05 ppm BL + 10 ppm NAA which was on par with 0.10 ppm BP + 20 ppm BA.

When all the plant growth regulators were combined and sprayed, the chlorophyll a content was increased to the maximum extent. Here also the growth regulators at higher concentrations increased the chlorophyll a content. The highest chlorophyll a was recorded by the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (1.385) which was on par with the 0.05 ppm + 20 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA significantly increased the chlorophyll a content. The highest chlorophyll a content was recorded by the application of 0.1 ppm BL + 20 ppm + 10 ppm NAA (1.385) compared to other treatments and control (0.807).

### 4.2.3.2. Chlorophyll ‘b’ (mg $g^{-1}$) (Table: 21)

The response of chlorophyll b to plant growth regulators was highly significant among the treatments. The maximum chlorophyll b content was recorded at 60DAS. Application of plant growth regulators increased the chlorophyll b content from 30DAS to 60DAS and than there is a steady decrease in the chlorophyll b content till harvest.

Application of BL, BA and NAA individually increased the chlorophyll content at all concentrations than the control and the maximum was obtained at 60DAS. The highest chlorophyll b content was in 0.10 ppm BL (0.386) followed by 10 ppm NAA, which was on par to each other.

When BL was combined with either BA or NAA, all the treatments showed an increase in chlorophyll b content irrespective of the concentrations. The maximum chlorophyll b content was recorded in 0.10 ppm BL + 10 ppm NAA. (0.842) and was followed by 0.05 ppm + 20 ppm BA.

When brassinolide, BA and NAA were combined and sprayed it increased the chlorophyll b content to the maximum. The growth regulators at higher concentrations increased chlorophyll b content at all the growth stage. The highest chlorophyll b content was recorded by 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (1.021) followed by 0.05 ppm BL + 20 ppm BA +10 ppm NAA (0.962) which was on par with 0.1 ppm BL + 10 ppm BA + 10 ppm NAA.

The highest chlorophyll b content was recorded in foliar application of 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (1.021) than other treatments and control (0.3437) at 60DAS.

### 4.2.3.3. Total Chlorophyll (mg $g^{-1}$) (Table: 22) (Fig. 14)

Total chlorophyll content showed significant difference between the treatments and control. The highest total chlorophyll content was recorded at 60DAS in all the treatments. The total chlorophyll content was increased from 30DAS to 60DAS and then there was decrease in the content

Application of BL and other growth regulators individually increased total chlorophyll content in all treatments. At higher concentrations, the total chlorophyll content was recorded the highest. The highest total chlorophyll content was recorded by 0.1 ppm BL (1.667) followed by 10 ppm NAA which were on par with each other.

When BL was combined with BA or NAA, there was increased in total chlorophyll content in all the treatments than when they are applied individually. The maximum total chlorophyll content was recorded by 0.01 ppm BL + 10 ppm NAA (1.965). The treatment 0.05 ppm BL + 20 ppm BA and 0.10 ppm BL + 10 ppm BA were on par with each other.

When all the plant growth regulators were combined and sprayed the total chlorophyll content increased to the maximum extent at 60DAS. The highest total chlorophyll content was recorded in the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (2.406) followed by 0.05 ppm BL + 20 ppm BA + 10 ppm NAA.

The highest total chlorophyll content was recorded by application of 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (2.406) compared to other treatments and control (1.244). And total chlorophyll showed significant difference among the treatments.

### 4.2.3.4. Chlorophyll fluorescence (Fv/Fm) (Table: 23)

The date on chlorophyll fluorescence was measured at 30, 60 and 90 DAS. The ratio of chlorophyll fluorescence (Fv/Fm) was calculated by variable fluorescence (Fv) to the maximum fluorescence (Fm). The ratio of Fv/Fm value was increased from 30DAS to 60DAS and there after the ratio was decreased. Application of BL, BA and NAA had significant effect on the chlorophyll fluorescence ratio.

When brassinolide and BA and NAA were sprayed individually they increased the Fv/Fm ratio irrespective of the concentrations at all stage of the crop growth. The maximum ratio was obtained on foliar spraying of 0.10 ppm BL (0.795). The treatment 10 ppm NAA (0.787) was recorded on par with the treatment 0.05 ppm BL.

In combined application of BL with either BA or NAA, all the treatments irrespective of the concentration increased the ratio, compared to application of plant growth regulators individually. The maximum Fv/Fm ratio was recorded in higher concentrations of the growth regulators. The highest was recorded by 0.10 ppm BL + 10 ppm NAA (0.815) followed by 0.05 ppm BL + 10 ppm NAA.

When all the growth regulators were combined and sprayed the Fv/Fm ratio increased to the maximum extent at 60DAS. The maximum ratio was recorded by the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (0.846) followed by 0.05 ppm BL + 20 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA significantly increased the chlorophyll fluorescence ratio. The maximum ration was observed by the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (0.846) than other treatments and control (0.765) at 60DAS. The percent increase of chlorophyll fluorescence was 10.6 over the control.

### 4.2.4. Biochemical characters.

#### 4.2.4.1. Leaf Soluble Protein (mg $g^{-1}$) (Table: 24) (Fig.15)

The leaf soluble protein increased steadily from 30DAS to 60DAS and declined there after upto 90DAS. The maximum soluble protein was recorded at 60DAS in all the treatments and control.

Application of BL, BA and NAA individually increased the soluble protein at 60DAS. The highest soluble protein was obtained in the 0.10 ppm BL (14.59) foliar application and which was followed by 10 ppm NAA (14.32 and was on par with 0.05 ppm BL. The lowest soluble protein was recorded by the foliar application 10 ppm BA at 60DAS.

Foliar application of BL along with either BA or NAA, the leaf soluble protein recorded maximum at higher concentrations than lower concentrations on 60DAS. 0.10 ppm BL + 10 ppm NAA (15.12) as foliar spray increased the soluble protein on 60DAS which was on par with the treatment 0.05 ppm BL + 10 ppm NAA. The treatment 0.05 ppm BL + 10 ppm NAA recorded on par with 0.10 ppm BL + 20 ppm BA.

In combined foliar application of BL, BA and NAA, the highest leaf soluble protein was recorded on 60DAS irrespective of the concentrations of the plant growth regulators. The highest soluble protein was recorded in foliar application of 0.1 ppm BL + 20 ppm BA + 10 ppm NAA (16.25) followed by 0.05 ppm BL + 20 ppm BA + 10 ppm NAA.

The interaction effect BL with BA and NAA showed significant difference between the treatments for soluble protein. The highest leaf soluble protein was recorded by the foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (16.25) than other treatments and control (13.58). The percent increase of soluble protein in the best treatment was 19.7 than the control.

### 4.2.4.2. Nitrate reductase activity (µ moles $No_2^-$ $g^{-1}$ $hr^{-1}$) (Table: 25) (Fig.16)

The activity of nitrate reductase (NRase) plays an important role in nitrogen assimilation. The higher enzyme activity was recorded at 60DAS. The NRase activity increased from 30DAS to 60DAS and after that there was a decrease in the enzyme activity. Application of growth regulators significantly increased NRase activity, whether it applied individually or in combined.

Application of BL, BA and NAA individually increased the soluble protein in all treatments. The highest NRase activity was recorded in 0.1 ppm BL (7.20), which was on par with 10 ppm NAA foliar spraying. 10 ppm BA recorded the less activity.

When BL was combined with either BA or NAA and foliar sprayed, the NRase activity was increased on 60DAS, irrespective of concentrations of the growth regulators. 0.10 ppm BL + 10 ppm NAA (7.40) recorded the highest NRase activity which was on par with 0.05 ppm + 10 ppm NAA, 0.10 ppm BL + 20 ppm BA, 0.05 ppm BL + 20 ppm BA and 0.10 ppm BL + 10 ppm BA.

Application of all growth regulators in combined, recorded the maximum NRase activity at 60DAS. In this, irrespective of individual concentrations all the combined treatments increased the NRase activity. The highest activity was recorded by the treatment 010 ppm BL + 20 ppm BA + 10 ppm NAA (7.80) which was on par with other combined treatments of all growth regulators.

Interaction effect of BL with BA and NAA showed a significant difference among the treatment in NRase activity. The highest NRase activity was recorded in the foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (7.80) than other treatments. The percent increased activity of NRase was 20.9 over the control.

#### 4.2.4.3. IAA oxidase activity (µg of unoxidised auxin $g^{-1}$ $hr^{-1}$) (Table: 26) (Fig.17)

Auxin plays an important role in the apical dominance in maize crop. But the activity of IAA oxidase will reduce the growth of the plant and was indirectly measured by the unoxidised auxin present in plants. The IAA oxidase showed a significant difference among the different treatments. The unoxidised auxin was higher in the initial stages of plant growth and later on it will decreases. The same trend was followed in all the treatments. The highest IAA oxidase activity was observed at 90DAS.

**Foliar application of BL, BA and NAA individually decreased the IAA oxidase activity at 30 DAS and increased at 90 DAS. The maximum IAA oxidase was noticed in the control. The highest IAA oxidase activity was recorded in 10 ppm BA (87.9)**

**followed by 20 ppm BA (90.4). The treatment 0.05 ppm BL and 0.10 ppm BL were on par with each other for IAA oxidase. The lowest IAA oxidase activity was noticed in 10 ppm NAA (104.6).**

**When BL was combined with either BA or NAA and foliar sprayed, IAA oxidase activity was higher in the treatment 0.05 ppm BL + 10 ppm NAA (85.0) and the lowest activity was recorded in the treatment 0.1 ppm BL + 10 ppm NAA (103.2) at 90 DAS. The activity of enzyme was increased by lower concentrations than the higher concentrations of the growth regulators.**

**When BL was combined with BA and NAA applied foliar, recorded the highest IAA oxidase activity at lower concentration than the higher concentration. The highest activity was recorded in foliar spraying of 0.05 ppm BL + 10 ppm BA + 10 ppm NAA (105.4). The other combined treatment of BL with BA and NAA showed on par.**

**The combine effect of BL with BA and NAA showed a significant difference among the treatments for IAA oxidase activity. The lower activity was observed in the treatment 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (108.5).**

### 4.2.5. Yield and Yield components (Table: 27)

#### 4.2.5.1. Cob length (cm)

The length of cob had significant role in deciding the sink size. The length of cob showed significant difference among the treatment. The cob length was ranged from 10.4 to 14.4.

Foliar application of BL, BA and NAA individually increased the cob length. The highest cob length was recorded in 0.10 ppm BL (11.9) which was on par with 0.05 ppm BL and 10 ppm NAA. The lowest was recorded in 10 ppm BA (10.6), which was on par with 20 ppm BA and control.

Foliar application of BL along with either BA or NAA increased the cob length at higher concentrations than the lower concentrations. Application of 0.10 ppm BL +10 ppm NAA (12.0) recorded the highest cob length which was on par with 0.05 ppm BL + 10 ppm NAA, 0.10 ppm BL + 20 ppm BA and 0.05 ppm BL + 20 ppmBA.

When all the growth regulators were combined and sprayed, cob length was reached its maximum. The highest cob length was recorded in foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (14.4) it was followed by 010 ppm BL + 10 ppm BA + 10 ppm NAA.  This treatment showed on par with 0.05 ppm BL + 20 ppm +10 ppm NAA.

The interaction effect of BL with BA and NAA was significantly increasing the cob length. The highest cob length was recorded in 0.10 ppm BL + 20 ppm BA + 10 ppm NAA than the other treatments and control (10.4).

#### 4.2.5.2. Number of grains per cob

The number of grains per cob was significantly differing from treatments and control. Number of grains per cob ranged from 262 to 315. All the treatments increased the number of grains per cob.

Application of BL and other growth regulators individually, showed an increased in grain number per cob irrespective of the concentrations. The more number of grains per cob was recorded in 0.10 ppm BL (282) that was on par with 10 ppm NAA and 10 ppm NAA was on par with 0.05 ppm BL.

When BL combined with either BA or NAA and foliar sprayed, number of grains per cob was increased in all the treatments. The highest was recorded in 0.10 ppm BL + 10 ppm NAA (293) followed by 0.05 ppm BL + 10 ppm NAA that was on par with 0.10 ppm BL + 20 ppm BA.

Application of BL, in combination with BA or NAA, increased the number of grains per cob in all treatments. More number of grains per cob was recorded in 0.10 ppm BL +20 ppm BA +10 ppm NAA (315) followed by 0.05 ppm BL +20 ppm BA + 10 ppm NAA.

The interaction effect of BL with BA and NAA significantly increased number of grains per cob. Number of grains was more in 0.10 ppm BL + 20 ppm BA + 10 ppm NAA (315) than other treatments and control (262). The percent increased number of grains per cob was 20.2 over control.

#### 4.2.5.3. Yield (g plant $^{-1}$) (Fig.18)

Grain yield was highly significant in all the treatments and control. The grain yield was ranged from 79.5 to 61.8.

Foliar application of BL, BA and NAA individually increased grain yield. The maximum grain was recorded by 0.10 ppm BL (65.3), it was on par with 10 ppm NAA, 0.05 ppm BL, 20 ppm BA and 10 ppm BA.

When BL was combined with either BA or NAA, grain yield was increased in all concentrations of growth regulators compared to control. The grain yield was obtained in the treatment 0.10 ppm BL + 10 ppm NAA (68.4) which was on par to 0.05 ppm BL + 10 ppm NAA. There was decreased in grain yield when BA was combined with BL.

In combined application of BL with BA and NAA, all the treatments recorded more yield irrespective of concentration. The maximum grain yield was recorded in 0.10 ppm BL + 20 ppm BA +10 NAA (79.5). The treatment 0.10 ppm BL + 20 ppm BA +10 ppm NAA and 0.05 ppm BL +10 ppm BA +10 ppm NAA were on par to each other.

The interaction effect of BL, BA and NAA, grain yield was recorded maximum in 0.10 ppm BL + 20 ppm BA and 10 ppm NAA (79.5) than other treatments and control (61.8). The increased percent of grain yield per plant was 28.6 over the control.

### 4.2.5.4. Harvest index (Fig.18)

The HI showed significant difference among the treatments. HI was highest in all the treatments than the control irrespective of their concentrations.

When BL, BA and NAA were individually sprayed, they recorded the highest HI than control. The highest HI was recorded in 0.10 ppm (0.436), which was on par with 10 ppm NAA, 10 ppm BA and 20 ppm BA.

In combined application of BL with BA and NAA, HI index highest in 0.10 ppm BL + 10 ppm NAA. The treatment BA along with BL at both concentrations showed lesser HI than they were applied individually.

When all growth regulators were combined and sprayed HI was increased in all the treatments. The highest was recorded in 0.10 ppm BL + 20 ppm BA +10 ppm NAA (0.498) followed by 0.05 ppm BL + 20 ppm BA +10 ppm NAA.

Interaction effect of growth regulators showed that 0.10 ppm BL +20 ppm BA +10 ppmNAA (0.498) recorded the highest HI than other treatments and control (0.421) The percent increased of HI in the best treatment was 18.3 over control.

## 4.3. On Farm Trial

The brassinolide concentration 0.01, 0.05, 0.10 and 0.20 ppm were tried for the on farm experiments in different agricultural and horticultural crops. These treatments were preformed well in the laboratory experiment. The observation on various morphological, yield and yield parameters were recorded and subjected to statistical analysis. The results obtained are presented in this section.

### 4.3.1. Rice (Table: 28) (Plate: 3)

### 4.3.1.1. Morphological Characters.

**4.3.1.1.**1. Plant height (cm)

Foliar application of brassinolide showed significant difference between the treatments. The plant height was ranged from 66.5 to 77.4. As the concentration increases the plant height also increased. Foliar application of 0.20 ppm BL (77.4) increased the plant height and which was on par with 0.10 ppm BL than control (66.5). The percent increase of plant height for the best treatment was 16.4 than the control.

#### 4.3.1.1.2. Leaf Area Index

The leaf area index (LAI) was calculated during the flowering stage of the crop. Application of brassinolide increased the LAI and increasing the concentration increased it. The highest LAI was recorded by the treatments 0.20 ppm BL followed by 0.10 ppm BL (4.75), which was on par with 0.05 ppm BL. In control the index was 4.34. Application of BL had significant inference on the LAI for different concentrations.

#### 4.3.1.2. Yield and yield parameters

##### 4.3.1.2.1. Number of tillers per plant

Foliar spraying of brassinolide significantly increased the number of tillers per plant. More number of tillers was recorded in the treatment 0.20 ppm BL, Which was followed by 0.01 ppm BL (14.6), 0.05 ppm BL (14.1), 0.01 ppm BL (13.1) and control (12.6).

##### 4.3.1.2.2. Number of productive tillers

The number of productive tillers had important role in increasing the yield. Application of brassinolide showed significant difference among the treatments. More number of productive tillers were produced by the foliar spraying of 0.20 ppm BL (9.4), which was on par with 0.10 ppm BL (9.2). The treatment 0.01 ppm BL and 0.05 ppm BL were on par to each other and in the control 8.3 productive tillers were produced.

##### 4.3.1.2.3. Number of grains par panicle

Number of grains par panicle showed significant difference among the concentrations of brassinolide. The highest number of grains was recorded by the foliar application of 0.20 ppm BL (221) that was on par with 0.10 ppm BL (209), 0.05 ppm BL and (206) 0.1 ppm BL (189). The control recorded 124 grains per panicle.

#### 4.3.1.2.4. 1000 grain weight (g)

1000 grains weight is important factor in decoding the source capacity. The highest 1000 grains weight was recorded by foliar application of 0.20 ppm BL (14.8), which was on par with 0.10 ppm BL (14.6). And the treatment 0.05 ppm BL and 0.01 ppm BL were on par with each other. The lowest 1000grain weight was recorded by control (14.2).

#### 4.3.1.2.5. Grain yield (q ha$^{-1}$) (Fig. 19)

The grain yield showed significant difference among the treatments. The highest was recorded by foliar application of 0.20 ppm BL (40.11), which was par with 0.10 ppm BL, than control (35.60). The percent increase of grain yield in the best treatment was 12.4 than the control.

#### 4.3.1.2.6. Harvest index

The harvest index showed significant difference among the treatments and control. The highest was recorded by 0.20 ppm BL (0.528), which was on par with 0.10 ppm BL (0.521) and 0.05 ppm BL (38.74) than control (0.479).

#### 4.3.1.2.7. Benefit cost ratio

The highest benefit cost ratio was obtained by foliar application of 0.20 ppm (2.01) and in control it was 1.64.

### 4.3.2. Maize (Table: 29) (Plate: 3)

#### 4.3.2.1 Morphological Characters

##### 4.3.2.1.1. Plant height (cm)

Brassinolide foliar application significantly increased the plant height compared to the control. There was an increase in the plant height up to 0.1 ppm BL, after that, in the higher concentration, the plant height was less, but it was higher than the control. The increase in the plant height was noticed in the treatment 0.10 ppm BL (222.9) and it was the least in the control (191.7).

##### 4.3.2.1.2. Leaf Area Index

The leaf area index was calculated at the flowering stage. A significant difference was observed in the leaf area index due to different concentrations of the brassinolide. The highest leaf area index was recorded by the treatment 0.10 ppm BL (5.02) than other treatments and control (4.29). The leaf area index increased concomitantly with increased concentration of BL.

#### 4.3.2.2. Yield parameters

##### 4.3.2.2.1. Cob length (cm)

The cob length has certainly influenced the yield of the crop. The cob length was highest in the 0.1 ppm of BL foliar-sprayed plant than control. The cob length showed significant difference between treatments and controls. The highest Cob length was 14.78 recorded by the treatment 0.10 ppm BL and the least was 14.21 in the control. The Cob length was increased with the increase in the concentration of brassinolide.

##### 4.3.2.2.2. Number of grains per cob

The Number of grains per cob was highly significant with the different treatments. The number grains ranged from 308 to 366. More number of grains was recorded in the treatment 0.10 ppm BL (366) followed by 0.20 ppm BL (343) and in control it was 308. The foliar application of BL significantly increased the number grains and the increase in the best treatment was 18.8 over the control.

#### 4.3.2.2.3. 100 Seed weight (g)

100 Seed weight had significant role to increase the yield. It showed significant difference between the different treatments of brassinolide. 100 seed weight was more in the foliar application of BL at 0.10 ppm (22.50) followed by 0.20 ppm (22.45) and in control (21.86) it was the least.

#### 4.3.2.2.4. Grain yield (q ha$^{-1}$) (Fig. 19)

The grain yield showed a significant difference among the treatments. It ranged from 33.50 to 37.23. The maximum yield was recorded in the treatment 0.10 ppm BL (37.23) which was on par with the treatment 0.20 ppm BL and in the control the grain yield was 33.50. The percent increase of the grain yield in the best treatment was 11 over the control.

#### 4.3.2.2.5. Stover yield (q ha$^{-1}$)

The Stover yield is the biological yield produced by the crop. Application of BL had significant influenced on the production of biological yield. The stover yield was ranged from 82.00 to 84.98. The highest stover yield was recorded by 0.10 ppm BL, which was on par with 0. 20 ppm BL, and the control recorded 82.00 of biological yield. The percent increase of the best treatment over the control was 3.9.

#### 4.3.2.2.6. Harvest index

The application of brassinolide showed significant difference on Harvest index (HI). It was ranged from 0.321 to 0.362. The maximum harvest index obtained in foliar application of 0.10 ppm BL (0.362), which was followed by 0.20 ppm BL, and the control the HI was 0.321.

#### 4.3.2.2.7. Benefit cost ratio

The highest Benefit cost ratio was recorded in the treatment 0.10 ppm BL and it was followed by 0.20 ppm BL (2.27) than control (2.21).

### 4.3.3. Sorghum (Table: 30)

#### 4.3.3.1. Morphological Characters.

#### 4.3.3.1.1. Plant height (cm)

A significant variation was observed in plant height among the treatments of brassinolide. The variation was ranged from 175.5 to 196.7. The highest plant height was recorded by foliar application of 0.10 ppm BL (196.7) and was on par with 0.20 ppm BL (189.0) than the control (176.5). The treatment 0.01 ppm BL (182.2) was on par with 0.05 ppm BL (185.5).

#### 4.3.3.1.2. Leaf Area Index

The leaf area index was ranged from 3.74 to 4.39. The foliar application of brassinolide showed significant difference among the treatments. The highest LAI was recorded by foliar spraying of 0.10 ppm BL (4.39) followed by foliar spraying of 0.20 ppm BL (2.30), which was on par to each other. The lowest LAI was recorded in control (3.74).

#### 4.3.3.2. Yield and Yield parameters

#### 4.3.3.2.1. 1000 grain weight (g)

The foliar application of brassinolide showed non-significant difference for 1000-grain weight. The highest was recorded in the treatment 0.10 ppm (11.5) followed by 0.20 ppm BL (10.9), which were on par to the following treatments, 0.05 ppm BL, 0.01 ppm BL and control.

#### 4.3.3.2.2. Grain yield (q ha$^{-1}$) (Fig. 19)

Significant variation was observed for the grain yield by application of different concentrations of brassinolide. The grain yield ranged from 34.97 to 38.33. The highest grain yield was recorded by 0.01 ppm BL (38.33) followed by 0.20 ppm BL (37.39) than the control (34.97). The percent increased in yield for best treatment was 9.6 over the control.

#### 4.3.3.2.3. Stover yield (q ha$^{-1}$)

Maximum stover yield was recorded by 0.10 ppm BL (88.80) followed by 0.20 ppm BL (87.26). The treatments should significant variation for the stover yield. The stover yield ranged from 84.21 to 87.26.

#### 4.3.3.2.4. Harvest index

Significant difference was noticed for HI by foliar application of BL. The maximum was recorded by 0.10 ppm BL (0.302), which was on par with 0.20 ppm BL, 0.05 ppm BL and 0.01 ppm BL The control (0.293) recorded the lowest HI.

#### 4.3.3.2.5. Benefit Cost ratio

The benefit cost ratio was high for the treatment 0.10 ppm BL (1.77) followed by 0.20 ppm BL (1.72) than the control (1.54).

### 4.3.4. Ragi (Table: 31) (Plate: 4)

### 4.3.5. Morphological Characters

#### 4.3.4.1.1. Plant Height (cm)

Foliar application of brassinolide showed significant difference between the treatments. The Plant height ranged from 107.0 to 122.8. The ragi plant that was sprayed with 0.10 ppm BL (122.8) recorded the highest plant height, which was on par with 0.20 ppm (122.6) where as control recorded the lowest plant height (107.0).

#### 4.3.4.1.2. Leaf Area Index

The values for leaf area index (LAI) by foliar spraying of brassinolide were ranged from 1.84 to 2.59. The LAI was significant for different treatments. Among the different concentrations tried, the foliar application of 0.10 ppm BL (2.59) recorded the highest LAI, which was on par with 0.20 ppm BL (2.43) than the control (1.84).

### 4.3.4.2. Yield and Yield parameters

#### 4.3.4.2.1. Number of tillers plant

The number of tillers produced was significant for foliar application of brassinolide. The number of tillers per plant was ranged form 3.2 to 4.3. The Maximum tillers was produced in foliar application 0.10 ppm BL (4.3) which was on par with 0.20 ppm (4.1) and 0.05 ppm BL (3.9) than the control (3.2).

#### 4.3.4.2.2. Number of Panicle par plant

Number of panicle per plant was highest in the treatment 0.10 ppm (6.7) followed by 0.20 ppm (5.9) than the control (4.5). The number of panicle was highly significant for the different treatments. The number of panicle ranged from 4.5 to 6.7.

### 4.3.4.2.3. Seed weight (g panicle$^{-1}$)

Variation was noticed in seed weight per panicle for different treatments. The seed weight ranged from 11.44 to 17.33. The highest seed weight was recorded in the foliar application of 0.10 ppm BL (17.33) followed by 0.20 ppm BL (15.90) than the control (11.44).

### 4.3.4.2.4. Yield (q ha$^{-1}$) (Fig. 19)

Foliar spraying of brassinolide showed variation on yield for different treatment. The yield was ranged from 18.62 to 21.67. The highest yield was recorded by foliar application of 0.10 ppm BL (21.66) followed by 0.20 ppm BL (20.67). The percent increase in the best treatment was 10.9 over the control.

### 4.3.4.2.5. Total dry matter production (g plant$^{-1}$)

Foliar spraying of brassinolide increases the accumulation of dry matter. It ranged from 92.96 to 109.68. The highest TDMP was produced by the foliar application of 0.10 ppm BL (109.94) that was on par 0.05 ppm BL (98.70) than the control (92.96).

### 4.3.4.2.6. Harvest index.

The foliar spraying of different concentration showed significant variation for Harvest index. The highest HI was recorded by 0.10 ppm BL (0.359), which was on par with 0.20 ppm BL (0.358) than the control (0.326). The treatment 0.05 ppm BL (0.343) was on par with 0.01 ppm BL (0.337).

#### 4.3.4.2.7. Benefit cost ratio

The highest benefit cost ratio was worked in the treatment 0.10 ppm BL (1.58) followed by 0.20 ppm BL (1.54) and 0.05 ppm BL (1.53) than the control (1.42).

## 4.3.5. Green gram (Table: 32)

### 4.3.5.1. Morphological Characters

#### 4.3.5.1.1. Plant height (cm)

Foliar application of brassinolide showed significant variation in the plant height. The plant height ranged from 45.3 to 52.9. The highest plant height was recorded by foliar application of 0.10 ppm BL (52.9), which was on par with the treatments of 0.20 ppm BL (50.3), 0.05 ppm BL (49.9) than the control (45.3). The treatment 0.20 ppm BL was on par with 0.05 ppm BL and 0.01 ppm BL.

#### 4.3.5.1.2. Leaf Area Index

Significant variation was noticed for the leaf area index (LAI). The LAI was ranged from 2.80 to 3.29. The highest LAI was recorded by the foliar application of 0.10 ppm (3.29) followed by 0.20 ppm BL (3.17) than the control (2.80).

### 4.3.5.2. Yield and yield parameters

#### 4.3.5.2.1. Number of pods per plant

The number of pods per plant was significant for the BL foliar application. The number of pods per plant was ranged 22.3 to 32.0. The more number of pods was recorded in 0.10 ppm BL (32.0) foliar application, which was on par with 0.20 ppm BL than the control (22.3).

#### 4.3.5.2.2. Pod weight (g plant $^{-1}$)

Variation was observed in pod weight among the treatment for foliar spraying of brassinolide. The variation ranged from 8.044 to 11.080. The highest pod weight was noticed in 0.10 ppm (11.080) followed by 0.20 ppm (10.217). The lowest was recorded in control (8.044).

#### 4.3.5.2.3. Yield (q ha$^{-1}$) (Fig. 20)

Significant variation was noticed in the grain yield by application of brassinolide. The grain yield ranged from 11.37 to 13.21. The highest grain yield was recorded by 0.01 ppm BL (13.21), which was on par with 0.20 ppm BL (13.04) than the control (11.37). The percent increase for the grain yield in the best treatment was 16.2 over control.

#### 4.3.5.2.4. Total dry mater production (g plant $^{-1}$)

Maximum dry matter was accumulated by foliar application of 0.10 ppm BL (30.53) followed by 0.20 ppm BL (29.75). The lowest TDMP was recorded in control (23.00). The TDMP was showed significant difference for the treatments.

#### 4.3.5.2.5. Harvest Index

The foliar spraying of different concentrations showed significant variation for harvest index (HI). The highest HI was recorded in 0.10 ppm BL (0.361) followed by 0.05 ppm BL (0.347) and was on par with 0.20 ppm BL (0.343) and 0.01 ppm BL (0.330). The lowest HI was recorded in control (0.330).

#### 4.3.5.2.6. Benefit cost ratio

The highest benefit cost ratio was worked in the treatment 0.10 ppm BL (2.23) followed by 0.20 ppm BL (2.19) than the control (1.97).

## 4.3.6. Blackgram (Table: 33) (Plate:4)

### 4.3.6.1. Morphological Characters.

#### 4.3.6.1.1. Plant height (cm)

Variation was observed in plant height among various treatments by foliar spraying of brassinolide. The variations ranged from 55.5 to 62.8. The highest plant height was recorded by foliar application of 0.10 ppm BL (62.8) followed by 0.20 ppm BL (60.8), which was on par with 0.05 ppm BL (59.1). The lowest plant height was recorded in the control (55.5).

#### 4.3.6.1.2. Leaf area index

The leaf area index (LAI) was ranged from 3.33 to 4.00. The foliar application of brassinolide showed significant difference among the treatments. Among the treatments, the highest LAI was recorded by foliar spraying 0.10 ppm BL (4.00) followed by 0.20 ppm BL (3.84). The treatments 0.05. ppm BL was on par with 0.01 ppm BL.

### 4.3.6.2. Yield and Yield parameter

#### 4.3.6.2.1. Number of pods per plant

Foliar application of brassinolide significantly increased the number of pods per plants. It was ranged from 27.3 to 33.1. More number of pods par plant was recorded by 0.10 ppm BL (33.1) followed by 0.20 ppm BL (31.5), which was on par with 0.05 ppm BL (30.6). The lowest number of pods was recorded in control (27.3).

### 4.3.6.2.2. Total dry matters production (g plant$^{-1}$)

Maximum dry matters were accumulated by control (61.518) followed by 0.01 ppm BL (50.246) followed by 0.01 ppm BL. The treatment 0.05 ppm BL (48.258) was on par with 0.10 ppm BL (47.591) and 0.20 ppm BL (47.984). The TDMP was ranged from 47.561 to 61.518.

### 4.3.6.2.3. Pod weight (g plant$^{-1}$)

Foliar application of brassinolide recorded significant variations among the treatments. The pod weight was ranged from 20.717 to 22.998. The highest pod weight was recorded in 0.10 ppm BL (22.998) followed by 0.20 ppm BL (22.283). The treatment 0.05 ppm BL (21.325) was on par with 0.01 ppm BL for pod weight. The low pod weight was recorded by control (20.717).

### 4.3.5.2.5. Yield (q ha$^{-1}$) (Fig. 20)

A significant variation was noticed for pod yield by foliar application of BL at different concentration. The yield was ranged from 7.20 to 8.46. The highest pod yield was recorded by 0.10 ppm BL (8.46) followed by 0.20 ppm BL (8.24). The lowest was recorded in control (7.20). The percent increased in the yield for best treatment was 17.5 over the control.

### 4.3.6.2.4. Harvest Index

The harvest index showed significant difference among the treatments. Among treatments tried, foliar spraying of 0. 10 ppm BL (0.51) recorded the highest HI followed by 0.20 ppm BL (0.48), which was on par with 0.05 ppm BL (0.46). The harvest index was ranged from 0.38 to 0.51.

### 4.3.6.2.5. Benefit Cost ratio

The BC ratio for foliar application brassinolide showed highest ratio in 0.10 ppm BL (2.36) followed by 0.20 ppm BL (2.32) than the control (1.95).

### 4.3.7. Groundnut (Table: 34) (Plate: 5)

### 4.3.7.1. Morphological Characters

### 4.3.7.1.1. Plant Height (cm)

Significant difference was recorded for different treatments of brassinolide. The plant height was ranged from 36.6 to 40.8. As the concentration increases the plant height also increased. The highest plant height was recorded by 0.20 ppm BL (40.8) followed by 0.10 ppm BL (39.5) and lowest in control (36.6).

### 4.3.7.1.2. Leaf area Index

The leaf area index (LAI) showed significant difference among the treatments. The LAI was ranged from 3.5 to 3.9. The highest LAI was recorded by 0.20 ppm BL (3.9) followed by 0.10 ppm BL (3.7) than the control (3.5). The treatments 0.01 ppm BL and 0.05 ppm BL (3.6) recorded no difference in LAI.

### 4.3.7.2. Yield and Yield parameters

### 4.3.7.2.1. Numbers of pegs per plants

The number of pegs produced per plant was significant among the treatments. The number of pegs per plant ranged from 19.9 to 24.1. More number of pegs was produced by the foliar application of 0.20 ppm BL (24.1) than other treatments and control (19.9).

### 4.3.7.2.2. Number of pods per plants

Application of brassinolide at different concentration recorded significant effect on the number of pods per plant. It was ranged from 16.8 to 21.7. The more pods were recorded in foliar application of 0.20 ppm BL (21.7) followed by 0.10 ppm BL (20.8) compared to control (16.8). The percent increase over the control was 29.1

### 4.3.7.2.3. Pod weight (g plant$^{-1}$)

Pod weight per plant was showed significant difference among the treatments. It was ranged from 12.60 to 14.95. The highest pod weight was recorded in the treatment 0.20 ppm BL (14.95) followed by 0.10 ppm BL (14.40) than the control (12.60).

### 4.3.7.2.4. Kernel weight (g plant$^{-1}$)

The highest kernel weight was recorded by foliar application of 0.20 ppm BL (5.149) followed by 0.10 ppm BL (4.927) and the lowest was recorded by control (4.108). The kernel weight showed significant difference among the treatments.

### 4.3.5.2.6. Pod yield (qha$^{-1}$) (Fig. 20)

The pod yield was significantly influenced by the different concentration of brassinolide. The pod yield recorded was ranged from 22.86 to 25.91. The maximum pod yield was recorded in foliar application of 0.20 ppm BL (25.91) followed by 0.10 ppm BL (24.87) and the lowest pod yield was recorded in control (22.89). The percent increase of pod yield in the best treatment over control was 13.3.

### 4.3.7.2.5. Total dry matter production (g plant$^{-1}$)

The highest dry matter was produced in foliar application of 0.20 ppm (61.46) followed by 0.10 ppm BL (58.44) and the lowest was recorded in control (45.50). The TDMP was ranged from 45.50 to 61.46.

#### 4.3.7.2.6. Harvest index

The harvest index (HI) showed significant difference among the treatments and it ranged from 0.252 to 0.336. The highest harvest index was recorded in foliar application of 0.20 ppm BL (0.336) followed by 0.10 ppm BL (0.312) and the lowest was recorded by control (0.252).

#### 4.3.7.2.7. Benefit Cost ratio

The foliar application of 0.20 ppm BL (2.08) recorded the best benefit cost ratio followed by 0.10 ppm BL (1.95) and the least in control (1.71).

## 4.3.8. Sunflower (Table: 35) (Plate: 6)

### 4.3.8.1. Morphological Characters

#### 4.3.8.1.1. Plant height (cm)

Foliar application of brassinolide showed significant difference between the treatments. The plant height was ranged from 172.1 to 186.6. The plant that was sprayed with 0.10 ppm BL (186.6) recorded the highest plant height was on par with 0.2 ppm BL (180.6) and 0.05 ppm BL, while control recorded the lowest plant height.

#### 4.3.8.1.2. Leaf area Index

The Values for leaf area index (LAI) by foliar spraying of brassinolide was ranged from 1.86 to 2.30. The LAI was highly significant for different treatments. Among the different treatment tried, foliar application of 0.10 ppm BL (2.30) recorded the highest LAI, which was on par with 0.20 ppm BL (2.20) and 0.05 ppm BL (2.15). The least was recorded by control (1.86).

### 4.3.8.2.Yield and Yield parameters

#### 4.3.8.2.1. Capitulam diameter (cm)

Variation in the capitulam diameter was more pronounced among the treatments. The capitulam diameter ranged from 11.5 to 12.6. The highest capitulam diameter was recorded by 0.10 ppm BL (12.6) that was on par with 0.05 ppm BL (12.2) and the lowest was recorded by the control (11.5).

#### 4.3.8.2.2. Number of seeds per capitulam

Increasing the concentration of brassinolide increased the number of seeds per capitulam. The number of seeds ranged from 355.9 to 432.4. The foliar application of 0.10 ppm BL (432.4) recorded more number of seeds per capitulam, which was on par with 0.20 ppm BL (432.8) and 0.05 ppm BL. The less number of seeds per capitulam was recorded by control (355.9). The treatments recorded significant difference for the number of seeds per capitulam.

#### 4.3.8.2.3. Percent grain filling

The percent grain filling showed a significant difference among the treatments. It ranged from 84.8 to 89.1. The plant where 0.10 ppm BL (89.1) was foliar sprayed recorded the maximum percent grain filling and was on par to 0.20 ppm BL than the control (84.8).

#### 4.3.8.2.4. 100 seed weight (g)

The 100 seed weight for brassinolide foliar spraying ranged from 4.06 to 4.24. The highest 100 seed weight was registered in 0.10 ppm BL (4.24) which was on par with 0.20 ppm BL (4.18) and that was followed by other treatments. The lowest seed weight was recorded by control (4.06). The treatments showed significant difference for 100 seed weight.

#### 4.3.8.2.5 Grain yield (kg ha$^{-1}$) (Fig. 20)

The grain yield ranged from 1240 to 1343. The foliar application of 0.10 ppm BL (1343) recorded the maximum grain yield, followed by 0.05 ppm BL (1300). The lowest grain yield was recorded in control (1240). Foliar application of BL showed high significant variations among the treatments and control. The percent increase for the best treatment was 8.3 over the control.

#### 4.3.8.2.6. Total Dry Matter Production (g plant$^{-1}$)

The sunflower plant showed significant difference for the total dry matter production among different concentrations of brassinolide foliar spray. The TDMP was ranged from 181.2 to 225.9. The highest amount of dry matter was produced in foliar spraying 0.10p ppm BL which was followed by 0.20 ppm BL (216.6) and less amount of TDMP was recorded in control (181.2).

#### 4.3.8.2.7. Harvest Index

The value for harvest index was ranged from 0.239 to 0.243. Among the treatments tried, the foliar application of 0.10 ppm BL (0.248) recorded the highest HI that was on par with 0.05 ppm BL (0.246), 0.01 ppm BL (0.243) and 0.20 ppm BL (0.245) and the least was recorded in control (0.239).

#### 4.3.8.2.5. Benefit Cost ratio

The benefit cost ratio was highest in the foliar spraying of 0.10 ppm BL (1.70) and the least in control (1.55).

### 4.3.9. Cotton (Table: 36) (Plate: 5)

#### 4.3.9.1. Morphological Characters

#### 4.3.9.1.1. Plant Height (cm)

The value for plant height by foliar spraying of brassinolide was ranged from 113.4 to 125.6. In cotton, plant height showed variations when the plant received different concentrations of brassinolide. Among the treatments, foliar application of 0.10 ppm BL (125.6) recorded the maximum height and was on par with 0.20 ppm BL (123.1). The lowest plant height was recorded by control (113.4).

#### 4.3.9.1.2. Leaf area Index

Foliar application of brassinolide showed significant difference between the treatments for leaf area index (LAI). The LAI was ranged from 2.51 to 2.90. Foliar application of 0.10 ppm BL (2.90) recorded the highest LAI and was on par with 0.20 ppm BL (2.81) and the lowest was recorded in control (2.51).

### 4.3.9.2. Yield and Yield parameters

#### 4.3.9.2.1. Number of sympodia per plant

The sympodia is the branches which bears the fruiting bodies, so increase in number of sympodia will have definite increase in the yield. The foliar spraying of brassinolide showed variation in number of sympodia per plant among the treatments. The number of sympodia was more in the treatment 0.10 ppm BL (19.4) and was on par with 0.20 ppm BL (18.8) and in the control (16.3) it recorded the lowest.

#### 4.3.9.2.2. Number of bolls per pant

Increasing the concentration of BL increased the number of bolls per plant. The foliar application of brassinolide had significant influence on the number of bolls. The treatment 0.10 ppm BL (19.4) recorded the maximum bolls per plant followed by 0.20 ppm BL (18.3). The number of bolls per plant was very low in control (17.2) compared to other treatments.

#### 4.3.9.2.3. Percent boll set.

The percent boll set showed significant variations among the treatments. It ranged from 33.2 to 39.4. The plant where 0.10 ppm BL (41.4) was foliar sprayed, recorded the maximum boll set followed by 0.20 ppm BL (39.4). The less number bolls set was observed in control (33.2).

#### 4.3.9.2.4. Boll weight (g)

The boll weight was ranged from 3.53 to 3.73 by foliar spraying of brassinolide. The treatments showed significant difference to boll weight. Among the treatments tried, the foliar application of 0.10 ppm BL (3.73) recorded the highest, which was on par with 0.20 ppm BL (3.68). The treatments 0.01 ppm BL (3.57) and 0.05 ppm BL (3.59) were on par for boll weight. The lowest boll weight was recorded in control (3.53).

#### 4.3.9.2.5. Kapas yield (ha$^{-1}$) (Fig. 21)

Foliar application of brassinolide showed significant difference among the treatments for kapas yield. The kapas yield ranged from 17.05 to 18.61. The foliar application of 0.10 ppm BL (18.61) recorded the highest kapas yield followed by 0.20 ppm BL (17.76) and the control recorded the lowest kapas yield. The percent increase of kapas yield for the best treatment was 9.1% over the control.

#### 4.3.9.2.6. Total dry mater production (g plant $^{-1}$)

Significant variation was showed by the foliar application of brassinolide for total dry mater production (TDMP). The TDMP was ranged from 222 to 253 and the highest was recorded by foliar application of 0.10 ppm BL (253) followed by 0.20 ppm BL (247). The lowest was recorded in the control (222).

#### 4.3.9.2.7. Harvest Index

The harvest index (HI) was ranged from 0.255 to 0.289. The best treatment to obtain the highest treatments was 0.10 ppm BL (0.289), which was on par with 0.20 ppm BL (0.279). The treatment 0.01 ppm BL (.0269) was on par with 0.05 ppm BL (0.265) foliar spraying. The lowest was recorded in control (0.255).

#### 4.3.9.2.8. Benefit Cost ratio

The benefit cost ratio was high in foliar spraying of 0.10 ppm BL (2.29) followed by 0.20 ppm BL (2.18) than the control (1.86).

## 4.3.10. Banana (Table: 37 ) (Plate: 6)

### 4.3.10.2. Morphological Characters

#### 4.3.10.1.1. Pseudostem height (cm)

The pseudo stem height showed significant difference between the treatments. The height was ranged from 243.6 to 282.0. As the concentration increases the pseduo stem height also increased. The highest stem height was recorded in 0.20 ppm BL (282.0) followed by 0.10 ppm BL (275.6). The lowest was recorded by the control (243.6.).

#### 4.3.10.1.2. Leaf area index

The LAI showed significant difference for the brassinolide foliar application. The LAI was ranged from 3.95 to 5.26. The height LAI was recorded by 0.20 ppm BL (5.26) followed by 0.10 ppm (4.89) than the control (3.95) at shooting stage.

### 4.3.10.2. Yield and yield parameters

#### 4.3.10.2.1. Total dry matter production (kg plant$^{-1}$)

The TDMP was ranged from 5.54 to 6.26. The highest TDMP was recorded in foliar application of 0.20 ppm BL (6.26) followed by 0.10 ppm BL (6.08) than the control (5.54). The TDMP showed significant variations between the treatments.

### 4.3.10.2.2. Bunch weight (g)

Significant variations were recorded for the bunch weight by foliar application of BL. The bunch weight was ranged from 23.14 to 28.29. The highest was recorded by 0.20 ppm BL (28.29) followed by 0.10 ppm (26.92), which was on par with 0.05 ppm BL than the control (23.14).

### 4.3.10.2.3. Number of finger per bunch

Significant difference was noticed by foliar spraying of BL for number of fingers per bunch. It was ranged from 146.8 to 169.8. Maximum number was observed in 0.20 ppm BL (169.8) followed by 0.10 ppm BL. The less number of fingers was recorded in the control (146.8).

### 4.3.10.2.4. Finger weight (g)

The finger weight was ranged from 165.3 to 184.5 for various treatments. It was high in 0.20 ppm BL followed by 0.10 ppm (178.5), which was on par with 0.05 ppm BL (170.9). The lowest finger weight was recorded by control (165.3).

### 4.3.10.2.5. Yield (q ha$^{-1}$) (Fig.21)

Difference observed for various treatments in yield was highly significant. The yield was ranged from 73.43 to 89.11. The maximum yield was recorded by 0.20 ppm BL (98.11) followed by 0.10 ppm BL (86.55) compared to control (73.43). The percent increase for the best treatment was 21.4 over the control.

### 4.3.10.2.6. Benefit cost ratio

The BC ratio was highest in the treatment 0.20 ppm BL (2.82) than the other treatments and control (2.09).

### 4.3.11. Grapes (Table: 38) (Plate: 7)

### 4.3.11.1. Yield and Yield Parameters.

### 4.3.11.1.1. Number of bunches per vein

Significant difference was noticed among the treatments for number of bunches per vein. The bunch weight ranged from 112.3 to 15.2. More number of bunches was recorded in 0.10 ppm BL (15.2) followed by 0.20 ppm (13.7) compared to control (12.3).

### 4.3.11.1.2. Bunch weight (kg vein$^{-1}$)

Variation was recorded for the bunch weight in foliar application of BL. The bunch weight ranged from 4.43 to 5.05. The maximum bunch weight was recorded by 0.10 ppm BL (5.15) followed by 0.20 ppm BL (5.05) when compared to control (4.43).

### 4.3.11.1.3. 100 Berry weight (g)

The highest 100-berry weight was recorded in foliar application of 0.10 ppm BL (269.0) followed by 0.20 ppm BL (258.5) than other treatments and control (199.0). The 100-berry weight was highly significant for various treatments.

### 4.3.11.1.4. Yield (q ha$^{-1}$) (Fig.21)

Foliar application of brassinolide showed significant difference for yield. The yield was ranged from 18.53 to 22.40. The maximum yield was recorded by 0.10 ppm BL (22.40) followed by 0.20 ppm BL (21.07). The lowest yield was recorded in control (18.53). The percent increase over control for the best treatment was 20.9.

### 4.3.11.2. Quality Parameters

### 4.3.11.2.1. Total soluble solids (˚Brix)

The total soluble solids (TSS) were highest in foliar application of 0.1 ppm BL (15.7) followed by 0.20 ppm BL (14.8), which was on par with 0.05 ppm BL (14.6). The lowest TSS was recorded in control (13.5).

#### 4.3.11.2.2. Reducing sugars (mg $g^{-1}$)

The reducing sugars were ranged from 13.0 to 14.8. The highest was recorded in the 0.10 ppm BL (14.8) foliar spraying followed by 0.20 ppm BL (14.1), which was on par with 0.05 ppm BL (13.9) when compared to control (13.0).

#### 4.3.11.2.3. Titrable acidity (%)

The titrable acidity was highly significant for treatments. The maximum titrable acidity was recorded in control (1.35) followed by 0.01 ppm (1.24). The lowest was recorded in 0.10 ppm BL (0.97), which was on par to 0.20 ppm (0.98) foliar application.

#### 4.3.11.2.4. Benefit cost ratio

Foliar application of 0.10 ppm (3.27) recorded the maximum BC ratio followed by 0.20 ppm (3.15) than the control (2.56).

### 4.3.12. Tomato (Table: 39) (Plate: 8)

#### 4.3.12.1.Morphological Characters

#### 4.3.12.1.1. Plant height (cm)

Significant difference was obtained for plant height by foliar application of brassinolide. The plant height ranged from 85.8 to 92.8. The highest plant height was recorded by 0.05 ppm BL (92.8) followed by 0.10 ppm BL (89.5), which was on par with 0.20 ppm BL (89.3) compared to control (85.8).

#### 4.3.12.1.2. Leaf Area Index

The LAI was ranged from 1.59 to 2.01. The maximum LAI noticed in 0.05 ppm BL (2.01) followed by 0.10 ppm BL (1.95) than the control (1.59). The LAI was significant to the treatments.

### 4.3.12.2. Yield and Yield Parameters

#### 4.3.12.2.1. Percent fruit set

Significant variations were noticed for percent fruit set by foliar application of brassinolide. The maximum fruit set was observed in 0.05 ppm BL (39.4) followed by 0.01 ppm BL (38.1) when compared to control (33.1). The percent fruit set was ranged from 33.1 to 39.4.

#### 4.3.12.2.2. Fruit weight (g)

By foliar application of brassinolide significant difference was observed among the treatments for fruit weight. The fruit weight was ranged from 28.9 to 41.2. Among the treatments tried 0.05 ppm BL (41.2) recorded the maximum followed by 0.01 ppm BL (36.5). The lowest fruit weight was recorded by control (28.9).

#### 4.3.12.2.3. Yield (q ha$^{-1}$) (Fig. 21)

Significant difference was noticed for in yield by the foliar spraying of different concentrations of brassinolide. The yield was ranged from 24.8 to 26.5. The maximum yield was recorded in 0.05 ppm BL (26.5) followed by 0.10 ppm BL (25.5), which was comparable with 0.20 ppm BL (25.4). The lowest yield was recorded in control (24.8). The percent increase for the best treatments was 6.9 over the control.

#### 4.3.12.2.4. Total dry matter production (g plant$^{-1}$)

The maximum dry matter was accumulated by the foliar application of 0.05 ppm BL (86.45), which was on par with 0.10 ppm BL (85.12) when compared to control (60.21). The TDMP was ranged from 60.21 to 86.45. They showed significant difference the treatments.

### 4.3.12.2.5. Harvest index

Significant variation was noticed for HI among the treatments. The highest was recorded by 0.05 ppm BL (0.49) followed by 0.01 ppm BL (0.45), which was on par with 0.10 ppm BL (0.43). The treatment 0.10 ppm was on par with 0.20 ppm BL (0.42). The lowest HI was recorded in control (0.41).

### 4.3.12.2.6. Benefit cost ratio

The highest BC ratio was worked in foliar application of 0.05 ppm BL (2.85) followed by 0.10 ppm BL (2.73) and the minimum BC ratio was observed in control (2.25).

## CHAPTER V

# DISCUSSION

Plant growth is a complex phenomenon, yet it is a well organized and coordinated process. Plant growth regulators bring coordination among different parts of the plant for better growth. Brassinosteroids as a sixth group of plant hormones are having significant growth promoting activity at low concentration. Their occurrence has been reported in more than 58 plant species. The effects of BRs are pleiotropic influencing in many developmental processes like growth, germination, rhizogenesis, flowering and senescence. Apart from this, they also confer resistance to abiotic stresses. They had ability to induce cell elongation, swelling and stimulating growth in young vegetative tissues as reported in many plants and enhanced root biomass. The present investigation had been under taken to elucidate the effect of BL on the physiology, biochemistry and molecular action and its effects on influencing the yield in selected agricultural and horticultural crops. Those collected data were scrutinized through statistical analysis wherever possible and discussed here under.

### 5.1. Effect of brassinolide on maize seedling

Brassinolide is highly effective in stimulating growth in young vegetative tissues (Sasse, 1991). It promoted elongation of cells in soybean (Yopp *et al*., 1981) wheat, mung bean and maize (Roddick and Ikekawa, 1992). BRs also did increase the root growth in cress seedlings (Yopp *et al*., 1981) and enhanced root biomass in *Pinus radiata* (Sasse, 1994) with root soaked in 24-epi brassinolide.

### 5.1.1. Seedling growth

The promotion of growth by brassinolide is due to both cell division and cell elongation. Elongation, curvature and splitting occurred when 0.01mg of brassinolide was applied, even 0.01µg induced spillting (Maeda, 1965). Wheat seed treated with µm concentrations of 28-

HBL had enhanced fresh and dry weight (Hayat *et al.*, 2001). In another experiment, pretreatment of wheat seeds with 24-EBL at concentrations ranging from 0.04nm to 40µm led to a biphasic promotion of root length (Shakirova *et al.*, 2002). In rice, time of application and length of exposure to brassinolide were important; shoot lengths of resulting seedlings were significantly promoted by first day treatment but not after 3 and 4th day of germination. (Fujii and Saka, 2001). In the present study also there was an increased shoot and root length due to 0.10ppm brassinolide treatment to the seedlings. This increase in shoot and root length had increased the biomass. This finding was conformity with the findings of Vardhini and Rao (1996) in groundnut where dry weight of seedlings was increased by seed treatment of BRs. Amzallag (2001), reported that the roots of *Sorghum bicolar* seedling treated with 0.1 – 10nm 24-EBL showed significant increase in shoot and root fresh weight.

### 5.1.2. Biochemical characters

Chlorophyll pigments play a vital role in plant productivity, as it is the main pigment responsible for photosynthesis. And the chloroplast contains approximately half of the total protein in leaves and about one fourth to one half of their total protein as Rubisco and this enzyme accounts to one eight to one fourth of leaf protein. Increase in soluble protein content had significantly increased the chlorophyll with the treatment of brassinolide at 0.10ppm concentration. The increase in the chlorophyll content might be due to increase in enzyme protein and also increased chlorophyll synthesis and reduced chlorophyll degradation. BRs application had increased the chlorophyll content in wheat (Sairam, 1994), green gram (Bhatia and Kaur, 1997) and maize (Hao *et al.*, 1990).

Brassinolide application did increase the soluble protein content in wheat (Sairam 1994), epibrassinolide in Chinese cabbage (Nakajima *et al.*, 1996), HBL in groundnut (Vardhini and Rao, 1998) and tomato (Mazorra and Nunez, 2000). The increased soluble protein by BR application was associated with enhanced nucleic acid levels. RNA polymerase activity got increased and reduced the activities of RNAase and DNAase (Wu, 1993). Photosynthetic enzyme RuBPcase forms nearly 50% of the soluble protein in many plants (Joseph *et al.*, 1981), thus in the present study increase in the soluble protein content in BL treated plants could be due to enhanced activation of RuBPcase and other enzymes which were responsible for the production of proteins.

Treatment of brassinolide increased the activity of nitrate reductase enzyme. BR enhanced the NRase activity in wheat (Hayat *et al*., 2001), and *Lens culinaris* (Hayat and Ahmed, 2003). Significant increase in DNA, RNA and protein in BR treated bean (Kalinich *et al*., 1985) suggested that BR involved in transcription and replication leading to increase the enzyme activities during tissue growth. Increase in enzyme proteins leads to increase the rate of photosynthesis and nitrate assimilation and increased the activity of NRase (Sairam, 1994).

Brassinolide treatments exhibited higher amount of unoxidesed auxin compared to control. This shows low activity of IAA oxidase and therefore high auxin content in 0.10ppm BR treatment. The BRs act synergistically with auxin in stimulating cell elongation and suggesting that BR effects are mediated through auxin (Takeno and Pharis, 1982) or that BRs enhance tissue sensitivity to auxin (Mandava, 1988). BRs might affect auxin-dependent targeted protein degradation (Leyser 2001). On the other hand, as Ullah *et al*., (2003) proposed, BRs might modulate auxin action upstream of its transcriptional control by coupling via AGB1 in a heterotrimeric G-protein complex.

### 5.2. Influence of Brassinolide on abiotic stress tolerance in maize seedlings

Brassinosteroids treatment can ameliorate various biotic and abiotic stresses in plants as discussed by Khripach *et al*. (1999 and 2000) and Krishna (2003). They and others emphasized the importance of the induction of antioxidant enzymes to protect cells, for example, in temperature stress (Mazorra *et al*., 2002). Brassinolide treatment enhanced early seedling growth, grain ripening, and lamina inclination in chilling stress in rice (Fujii and Saka 2001), and Yu *et al*., (2002) found that pretreatment with 24- epibrassinolide or abscisic acid increased tolerance and photosystem II efficiency in cucumber. In salt stress, pretreatment of rice seeds with 24- epi- or 28-homobrassinolide promoted germination in the presence of sodium chloride. Lengths, fresh and dry weights, and DNA, RNA, and soluble protein contents of the resulting seedlings were also enhanced (Anuradha and Rao, 2001).

### 5.2.1. Drought

Exposure of sugar beet plants to drought stress led to reduction in taproot mass in proportion to stress severity. Treatment with BR fully compensated for the reduction in biomass as caused by mild drought stress. The increase in root growth in BR-treated plants versus untreated plants was seen only under water stress condition. Increase in biomass was correlated with increases in acid invertase activity in young leaves, which likely provided more assimilates to the plant due to their larger sizes (Schilling *et al*., 1991). Applied either as seed treatment or foliar spray to drought-tolerant and drought susceptible wheat varieties, BR had a stimulatory growth effect under stress conditions. Increased water uptake and membrane stability and higher carbon dioxide and nitrogen assimilation rates in BR-treated plants under stress were correlated with BR-induced drought tolerance (Sairam, 1994).

#### 5.2.1.1. Seedling growth

Brassinosteroids increased the resistance of plants against various abiotic stresses. Exposure of maize seedling to drought stress led to reduction on the seedling growth. Treatment with BL fully compensated for the reduction in shoot, root growth and dry weight as caused by drought stress. Increase in seedling growth in BL treated seedlings versus untreated was highly significant. The increased dry weight might be correlated with increase in shoot and root growth and acid invertase activity in young leaves, which likely provided more assimilates to the plant only under water stress condition (Schilling *et al.*, 1991). The seed treatment or foliar spray to wheat varieties, BR had a stimulatory growth effect under stress condition (Sairam, 1994). The plants treated with EBL at concentrations of $10^{-11}$ to $10^{-6}$ M improved the resistance and accelerated the plant growth under deficiency (Pustovoitova *et al.*, 2001).

5.2.1.2. Biochemical characters

The loss of chlorophyll under drought stress condition is deleterious to plant productivity. In wheat, under moisture-stress condition maximum chlorophyll content was induced by 0.05ppm application. The stability of chlorophyll content for drought stress could be regarded as index for tolerance, which might sustain high photosynthetic efficiency and eventually showed higher yield (Sriram, 1994). In the current experiment the maximum chlorophyll 'a', 'b' and total chlorophyll content was recovered in 0.10ppm BL seedling treatment under drought stress condition.

The soluble protein content was estimated in order to find out the photosynthetic capacity of the plant under drought stress. The highest soluble protein was recovered by the seedling treatment with 0.10ppm BL under drought stress. The HBL application as seed treatment in wheat resulted in increased soluble protein under induced moisture-stress (Sairam *et al.*, 1996). Treatment of BR analogues at six days with 0.01ppm BB6 and 0.05ppm of MH5 significantly increased the soluble protein content. This finding suggested that they involved in molecular process leading to increase in protein content (Mazorra and Nunez, 2000).

Proline is the free amino acids that accumulated during stress condition and act as osmoticum. Treating the plants with EBL at $10^{-11}$ to $10^{-6}$ M improved the resistance by accumulation of free amino acids in particular, which evidently contributed to plant osmoregulation (Pustovoitova *et al.*, 2001). In the present study, the less amount of proline was accumulated in pre-treated seedlings with 0.10ppm BL than in control. This showed that brassinolide had the ability to withstand the drought stress increase in the content of free amino acids.

In the present study, treating the maize seedling with BL increased the NRase activity and the increase activity of NRase was due to application brassinolide before imposing the drought. This finding was similar to the findings of Sairam *et al.* (1994) in wheat. Increased

NRase activity could be due to the improvement in leaf water balance as indicated by increased RWC under stress and specific enzyme activity. Foliar spraying of BR at 0.1ppm and 1.0ppm increased the NRase activity in wheat under water stress condition (Sairam, 1994).

Under drought stress condition, there was an increased activity of IAA oxidase resulting in decreased seedling height, but in the present study, seedlings treated with BL showed less activity of IAA oxidase resulting in increased growth of seedlings This result was in accordance with Pustovoitova *et al.* (2001) in cucumber. Under water deficiency, EBL treatment increased the content of tryptophan by 1.7 fold in leaves, which could be related to the activation of IAA synthesis. The tryptophan is the precursor for the synthesis of auxin.

### 5.2.1.3. Free radical scavenging enzymes

It is widely accepted that Active Oxygen Species (AOS) were responsible for various stree-induced damages to macromolecules and ultimately at cellular level. In the antioxidative system of plants, SOD can remove $O_2^-$. As SOD may control other activated species ($H_2O_2$ and OH), it is defined as a key antioxidative enzyme in the system. When $O_2^-$ level was elevated under drought, the activity of SOD and catalase and peroxidase enzymes were triggered up and their led to the tolerance as a results of 0.10ppm BL treatment in the present investigation. These results agreed with the reports of Li *et al.* (1998) and Wu Shaohua (2001). They observed increased activity of SOD by BR treatment to the seedlings and antioxidant substance level.

Catalase is mainly involved in the decomposition of hydrogen peroxide and oxidation of hydrogen donors. Drought causes impaired activity of catalase, which leads to enhanced production of reactive hydroxyl radicals, which in turn cause peroxidation of unsaturated lipids. The catalase activity was effectively increased by the treatment of BR or MJ and maintained a higher level during water stress in the resistant maize seedlings (Li *et al.,* 1998). In the present study, BL treatment had increased the activity of catalase and reduced the level of AOS produced due to drought.

Peroxidase is an important enzyme involved in morphogenesis and auxin oxidation. One protective system to drought tolerance involves SOD converting superoxide radicals to hydrogen peroxide, which is further reduced to water by peroxidase activity. Under stress, BR and Kinetin influenced the activities of POD at initial stage of water stress and the activity of POD was higher in the leaves treated with BR in straw berry (Wu Shaohua 2001). In the present study, there was an increased activity of peroxidase in BL treated seedlings subjected to drought stress. This result was accordance with the findings of Mazorra and Nunez (2000) in tomato.

### 5.2.1.4. Leaf protein profile

The leaf protein profile separated through SDS-PAGE Showed clear distinct bands isolated due to the effect of brassinolide on drought implication. There was a clear distinct five bands with the Rm value of 0.51, 0.68, 0.71, 0.83 and 0.95. Among these the treatment 0.10ppm brassinolide concentration had clearly brought out a significant effect on protein, which could be visualized with an Rm value of 0.83 that could have been synthesized due to BL on drought. The possibility that these stress proteins may act as transcription enhancing factors for the synthesis of specific stress related enzymes. Hence presence of this protein can be taken as an index of tolerance of the particular crop (Hall, 2002).

### 5.2.2. Low temperature

The temperature changes are likely to occur more rapidly than other stress-causing factors in nature. Maize seedlings are highly sensitive to chilling stress at germination and also at the early stages of growth. Treatment with BR promoted growth recovery of maize seedlings following chilling treatment (exposure to 0–3˚C for increasing number of days). BR also promoted greening of etiolated maize leaves, especially at lower temperature in light (He *et al*., 1991). Similarly, cucumber seedlings germinated from seeds soaked in BR solution had greater growth as compared to controls (without BR treatment) under cold condition (5˚C for 3 days) (Katsumi, 1991). The growth-promoting effects of BR in rice also were obvious under low temperature condition but not under optimal growing conditions (Kamuro and Takatsuto, 1991).

### 5.2.2.1. Seedling growth

The dry weight got increased as growth progressed with increase in the root length, shoot length and leaf area. Under low temperature stress, there was reduction in the dry weight noticed, but brassinolide (0.10ppm) treated maize seedling were subjected to chilling stress (4˚C) showed significant improvement over the control. In rice, treating the seedling with BR under low temperature condition enhanced the growth (Wang and Zang, 1993). The maize seedlings treated with BR promoted growth and recovered following chilling treatment (He *et al.,* 1991). Fujii and Saka (2001) reported that BR promoted early shoot and root growth and higher dry weight in rice as a result of increased cell expansion at low temperature. Katsumi (1991) and Asao *et al.* (2002) reported increased plant growth in cucumber seedlings treated with BR solution as compared to control under cold condition.

### 5.2.2.2. Biochemical characters

Usually reduction in chlorophyll would be noticed under low temperature stress. In this study, the chlorophyll ‘a’, ‘b’ and total were improved by BL 0.10ppm treatment. The pre-treatment with 24-EBL or abscisic acid had increased the tolerance and photosynthetic efficiency in cucumber (Yu *et al.*, 2002). Katsumi (1991) reported that chlorophyll content was maintained in BR treated cucumber seedlings during cold treatment.

Pre-treatment of maize seedling with 0.10ppm BL increased the soluble protein content under low temperature stress condition. This result is in conformity with the findings of Wang and Zang (1993) who reported in rice that 24-EBL had increased the resistance against chilling stress and this tolerance was associated with the increased soluble protein. A concentration of 0.10ppm BL pre-treatment to maize seedling reduced the effect of chilling as a result of decreased proline accumulation. In this experiment, reduced accumulation of free amino acid resulting on the increased production of protein and enzymes.

The nitrate reductase activity was improved by pretreatment of BL than the other treatment. In the untreated seedling it recorded the lowest and it was improved by pre treatment with BL. The increased activity was a result of nitrogen assimilation and helps in increased protein synthesis and enzymes, which are responsible for reduction of damages as caused by low temperature stress. The finding was on line with findings of Sairam *et al.* (1994) in wheat.

Significant difference was obtained with pre-treatment of seeding with BL for IAA oxidase. The lesser activity was noticed in 0.10ppm BL through more amount of unoxidised auxin content. Fujii and Saka (2001) reported that, when rice seedling was treated with brassinolide, it promoted the cell elongation along with IAA, resulting in enhanced growth of the seedling at early stage.

### 5.2.2.3. Free radical scavenging enzyme

The SOD, CAT and POD activities were enhanced by BL treatment under the low temperature stress. The increasing activity of SOD will lead to increase in the scavenging ability of the free radicals that were produced during low temperature stress and converting them to water. In moth bean Upadhyaya *et al.* (1991) reported that there was slight increase in SOD activity at 22˚C but at high temperature, the activity decreased. Treatment with homobrassinolide increased the scavenging ability of active oxygen species, in rice seedling and improved the cold resistance to some extent (Chen-Shan Na *et al.* 1997).

### 5.2.2.4. Leaf protein profile

The protein profile isolated from the leaf with pretreatment of BL and subjected to low temperature showed a clear three protein bonds with an Rm value of 0.67, 0.80 and 0.93. This protein band could have been synthesized due to BL treatment, which has caused an additional residue during low temperature stress. The effects of EBR were first examined on a bromegrass cell suspension culture known to develop cold and thermotolerance in response to ABA. EBR increased the freezing tolerance of bromegrass cells by only 3– 5˚C, but markedly enhanced cell viability following exposure to high-temperature stress (Wilen *et al*., 1995).

### 5.2.3. High temperature stress

The effect of high-temperature stress in BL treated and untreated wheat leaves were examined at the level of total protein synthesis and leaf cell ultra structure. Protein synthesis was maintained in BR-treated leaves at 43˚C at levels similar to those at 23˚C, whereas in untreated leaves the total protein decreased 2.5 fold at 43˚ (Kulaeva *et al*., 1991). Application of brassinosteroids had increased tolerance to high temperature in brome grass (Wilen *et al*., 1995).

### 5.2.3.1. Seedling growth

The maize seedling subjected to high temperature showed decreased seedling growth. Where as pre-treated seedling when subjected to induction and lethal stress, the recovery rate in shoot and root length and also dry weight was more than the other treatment. When the seedling was given induction temperature then treated with BL and given lethal stress, the recovery of shoot length was corporately lesser than when it was given as pre-treatment. Kulaeve *et al.* (1991) reported that BR increased the tolerance to high temperature in wheat and there by it reduced the ill effect as caused on root length. The tolerance in plants to high temperature was due to application of BRs and was associated with induction of heat shock protein synthesis. . Krishna *et al.* (1997) reported that EBL enhanced the surviving of swede seedling and increased the dry weight when treated with high temperature. EBL treated tomato seedling showed moderate growth than untreated seedling at 45˚C and recovery in a growth chamber (Sangeeta Dhaubhade *et al.* 1999). Sam *et al.* (2001) reported that high temperature stress in tomato seedling showed the same result.

### 5.2.3.2. Biochemical characters

Pre-treatment of BL at different concentration improved the chlorophyll ‘a’, ‘b’ and total subject to lethal stress. Khripach *et al.* (2000) reported the chlorophyll content was maintained as that of control, when the tomato seedlings were subjected to high temperature.

The soluble protein content was well recovered in the pre-treated seedling with 0.10ppm BL than with the other treatments. Kulaeve *et al.* (1991) reported that protein synthesis was maintained in BR-treated leaves at 43˚C at levels similar to those at 23˚C. Sangeeta Dhaubheade *et al.* (1999) reported soluble protein content was slightly higher in EBL treated seedlings, particularly during the recovery period. Brassinolide pre-treated maize seedling significantly reduced the accumulation of proline and there by increased the protein synthesis. This finding was conformity with the findings of Pustovoitova *et al.* (2001). They reported that in cucumber plant treatment with EBL at concentration from $10^{-11}$ to $10^{-4}$ M improved resistance to desiccation and over heating by the slight accumulation of osmotically active compounds particularly free amino acids than control.

The NRase activity was improved by pretreatment of BL than the other treatment. In the untreated seedling it recorded the lowest activity and it was improved by treatment with BL either as pretreatment or after induction of temperature. This finding was supported by Kulaeva *et al.* (1991)in improving the NRase activity.

IAA oxidase activity was increased in temperature stressed plant. The BL treated seedling showed recovery in the accumulation of IAA resulting in lower level of IAA oxidase activity. Pustovoitova *et al.* (2001) reported that under water deficiency, EBL treatment increased the tryptophan content in leaves, which could be related to the activation of IAA synthesis.

### 5.2.3.3. Free radical scavenging enzyme

In this study, the maximum activity of SOD, CAT and POD was recorded in the pre-treatment of BL at both concentration subjected to lethal stress and recovery, but there was no similar effect in the activity when the BL was treated after induction stress. That shows that induction temperature is not required for the treatment of BL. Mazorra and Nunez (2000) reported that EBL had no effect on SOD activity at 22˚C but increased the activity at 0.01ppm and 0.05ppm. Mazorra *et al.* (2002) reported that EBR promoted activation of free radical scavenging enzymes to decrease the possible toxic concentration of $O_2^-$ radicals and the EBR enhanced activity at 40˚C and this would be for removing any excess $O_2^-$ generated. The catalase enzyme might be thermolabile, a significant reduction in the activity at 40˚C was expected, but presence of EBR increased the activity and these increased activity might be important in eliminating $H_2O_2$ excess.

### 5.2.3.3.Leaf protein profile

The induction response of brassinolide to high temperature stress revealed that a protein profile band, which could be visualized as four with the Rm value 0.54, 0.71, 0.84 and 0.95. The higher concentration of the protein band could have been induced due to BL and induction temperature, synthesizing few additional amino acid residues which formed a lower density of protein resolve at an Rm value of 0.54. This lower density protein could have protected the seedlings from high temperature stress similarly; the breassinosteroids treatment after the induction temperature could not produced any significant effect in synthesizing additional protein with lower density (Dhaubhadel et al., 1999). A link between hsps and thermotolerance is well established (Parsell and Lindquist 1993). Thus, the higher accumulation of hsps in EBR-treated seedlings contributes, at least in part, to enhanced thermotolerance in these seedlings. The results suggest that EBR treatment limits the loss of some of the components of the translation apparatus during a prolonged heat stress and increases the level of expression of some of the components of the translational machinery during recovery, which correlates with higher hsp synthesis during heat stress, a more rapid resumption of cellular protein synthesis following heat stress, and a higher survival rate (Krishna, 2003).

### 5.2.4. Salinity stress

BR enhanced germination of *Eucalyptus camaldulensis* seeds in the presence of 150 mM salt, but when seedlings were grown hydroponically in salt, uptake of BR through roots caused more damage (Sasse 1999). In another study, rice seeds soaked in water or 150 mM NaCl in the presence or absence of BR were tested for germination and seedlings growth. When the salt solution was supplemented with BR, the inhibitory effect of salt on germination was reduced considerably. The promotion of growth by BR under salt stress conditions was associated with enhanced level of nucleic acids and soluble proteins (Anuradha and Rao, 2001).

#### 5.2.4.1. Seedling growth

Brassinolide pre-treated seedling showed maximum recovery of shoot and root length and dry weight under salinity stress, which was nearly equal to the absolute control seedlings.  There was also increase in the plant height when BL was treated after induction of stress but it was not that amount of pre-treated seedlings.  This was in conformity with earlier finding of Vardhini and Rao (1997) where in BR reversed the growth inhibitory effects of salinity stress in groundnut.  The BL at 3μM concentration exhibited more alleviating influence on salinity stress.  The promotion of growth by BL under salt stress condition was associated with enhanced level of nucleic acid and soluble protein (Anuradha and Rao, 2002). Filiz Ozdemir *et al.* (2004) also confirmed this type of result with 24-EBL that improved rice seedling growth under saline stress.

### 5.2.4.1. Biochemical characters

Pre sowing treatment of brassinosteroids to seeds considerably restored the pigment level in plants grown in saline medium. A decrease in chlorophyll level due to salt stress had been reported in several plants, such as tomato (Sinel'nikova *et al*., 1998), rice (Pandey and Saxena, 1987), and wheat (Salma *et al*., 1994). Growth promotion in bean by brassinolide (Krizek and Mandava, 1983) and in triticale by epibrassinolide (Kalituho *et al*., 1996) was associated with higher levels of chlorophyll. Brassinolide pre-treatment considerably restored the pigment level in plant grown in saline medium and the values were only slightly lower than those of plants grown in non-saline condition. In the present study, brassinosteroids removed the inhibitory effect of salt stress on pigment levels and this could be one of the reasons for growth stimulation by brassinosteroids under saline condition. This was in agreement with the results obtained by Anuradha and Rao (2003); rice seed application of BR restored the chlorophyll 'a', 'b' and total.

In the present study, brassinolide treatment imparted tolerance to salinity stress in the BL pretreated seedling than the untreated seedlings. In rice, protein and nucleic acid synthesis was suppressed by NaCl treatment and it not only restored with the addition of BR, but also further activated as reflected in the higher soluble protein content (Anuradha and Rao, 2001). The maize seedlings that were pre-treated with BL reduced the formation of proline content, under induced salinity stress. Jain *et al.* (2004) reported that proline had been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger. Free proline content was increased remarkably in rice seedlings with salinity but decreased with 24EBL + NaCl treatment. It seems possible that 24EBL showed a protective role for rice seedling to prevent them from being severally affected by salinity stress (Filiz Ozdemir *et al*., 2004).

Brassinosteroids improved the nitrate reductase activity in rice plants as compared to the untreated plants, when grown in saline condition. Although the nitrate reductase enzyme itself represented a very small proportion of leaf protein (Calza *et al*., 1987), the activity of the enzyme played a pivotal role on the supply of nitrogen and the growth and productivity of plants, especially in cereals (Srivastava, 1995). The activity of nitrate reductase, a key enzyme in nitrogen assimilation, was a measure of the habitat-dependent nitrate utilization of a plant (Larcher, 1995). It is a well-established fact that high salt concentration inhibited nitrate reductase activity. The reduced nitrate reductase activity in the leaves of salt-stressed plants is attributed to salinity inhibited nitrate transport to the shoot, which in turn is due to interference with nitrate uptake and xylem loading (Cramer et al., 1995). In the present investigation, the NRase activity was increased with the pre-treatment of BL to maize seedling than the untreated and also after induction of salinity treatment. Anuradha and Rao (2003) reported that seed application of BRs improved the NRase activity rice plants grown in saline medium. It was well-established fact that high salt concentration inhibited NRase activity, but seed treatment of BRs improved the activity.

The BL pre-treatment reversed the effect of IAA oxidase under saline condition. The activity was reduced in the BL pre-treated than untreated seedlings grown under saline environment. This was conformity with the findings of Nakajima *et al.* (1996) in Chinese cabbage; EBL reduced the activity of IAA oxidase.

### 5.2.4.3. Free radical scavenging enzyme

Salinity stress exerted oxidative stress due to the production of variety of AOS such as superoxide anion, hydrogen peroxide and hydroxyl radicals, which caused oxidative damage to plants (McCord, 2000). To scavenge these toxic species, plants developed antioxidant enzymes, such as SOD, POD and CAT. Since their activities and transcripts were altered when plants were subjected to stress, changes in the levels of antioxidant enzymes had been used to assess the effect of different stresses including salinity (Hasegawa *et al*., 2000).

In the present study, BL pre-treatment enhanced the activity of SOD, CAT and POD under saline condition than the untreated seedlings. The increased activity would enhance the scavenging of free radical formed during the salinity stress. This finding was in accordance with the finding of Singh and Choudhuri (1990). They reported that BR treatment to *vigna* and rice seedlings increased the scavenging of free radical by the SOD than the untreated seedling under induced salinity. This was in accordance with the findings of Jain *et al.* (2001), who reported that groundnut seedlings in 24EBL + NaCl group might be scavenging free radical more effectively than the seedlings treated with NaCl alone by means of 24-EBL applications.

### 5.2.4.4. Leaf protein profile

The induction response of brassinolide to salinity stress revealed that a protein band which was visualized as four with the Rm value of 6.2, 0.74, 0.81 and 0.88. Among the treatment tried, the pretreatment of BL to the maize seedling then subjected to induction response produce a significant thicker band. At the higher concentration of the treatment, the protein band could been induced due to BL and induction of salinity synthesized few addition amino acid residues which formed the low density of protein resolved at an Rm value of 0.62. Similarly, the BL treatment after the induction of salinity could not produced any significant effect in synthesizing additional protein with lower density. BRs treatment under salinity stress condition decreases the basal activity of P5CS1 gene which response for the accumulation of free aminoacid (Abraham, et al., 2003).

## 5.3. Greenhouse experiment

### 5.3.1. Plant Height

The plant height showed a linear trend of increase during growth stages of crop. In the present study it was revealed that, brassinolide increased the plant height individually and also in combination with the other regulatory. Noteable result was obtained at higher concentration of BL, BA and NAA treatment than the other treatments and control. Amzallag (2001) observed similar increase in the shoot length and shoot fresh weight with the application of 1.0 – 10nm 24-EBL. Meudt (1983) reported that the effect of BR and IAA on growth and acid secretion of Azuki bean epicotyls were clearly additive even at optimal concentration of the compounds. Katzumi (1985) reported that the interaction of HBL with IAA and GA3 in cucumber seedlings showed that HBL acted as synergistically with IAA and not with GA3 and the effect was only an additive effect. And it is concluded that the increase in plant height by BRs might be attributed to its powerful synergistic interaction with available endogenous auxin and it could be observed in terms of increased cell wall plasticity and cell elongation.

### 5.3.2. Root length

The root growth tended to increase over the crop growth period. In the combined effect of BL, BA and NAA, the higher concentration produced the maximum root length. The rooting ability of rice seedlings was significantly increased by foliar application of BRs (Wang and Cheng, 1992). Vardhini and Rao (1998) confirmed the above finding that the foliar application of 28-HBL, significantly increased the root length in green gram.

### 5.2.3. Leaf Area

Area of photosynthetically active leaves is a major determinant of the rate of photosynthesis by the plant. In the present study the leaf area was increased up to 60DAS and it slowly declined till harvest. The maximum leaf area was attained with foliar spraying of higher concentration of BL, BA and NAA and the increased leaf area was 16.8 percent over the control. Iwashori *et al.* (1990) observed that BR was effective than IAA in preventing leaf abscission. Diz *et al.* (1995) reported that in tobacco foliar application of synthetic BR, DAA-6 increased the leaf length and width. In baby corn Nagasubramaniam (2003) reported BR showed positive effect in improving number of leaves per plants and also on leaf area index.

### 5.3.4. Specific leaf weight and Specific leaf area

Specific leaf weight was highly correlated with the stress tolerance and development of flowers and yield (Arnon, 1975). Krizek and Mandava (1983) recorded significant increase in SLW in bean plant. Higher SLW indicated increased leaf thickness and high density of chlorophyll per unit leaf area and hence had a greater photosynthesis capacity (Crayural *et al.*, 1999). Results obtained from the present experiment showed increased chlorophyll content and biomass for combined foliar spraying of BL, BA and NAA and the results further revealed that the auxin had synergistic effect on brassinolide. The above finding was in agreement with the findings of Lini (2002) in cotton.

The minimum SLA was noticed at the higher concentration and the less SLW was recorded in combined application of BL, BA and NAA indicating that foliar application of BR showed positive effect for higher photosynthesis. This finding was conformity with the finding of Sujatha (2001) in green gram where in foliar application of 0.1ppm BR decreased the SLA.

### 5.3.5. Net assimilation rate

Net assimilation is the amount of photosynthetic assimilated by the plant and mainly depends upon the chlorophyll and leaf area. In the present experiment, the NAR was increased at the initial stages and after 30DAS it slowly declined as assimilates were transferred to the reproductive and storage structures. Foliar spraying of BL, BA and NAA increased the NAA at higher concentration than other treatments. This finding was in line with the findings of Nagasubramaniam (2003) in baby corn.

### 5.3.6. Crop Growth Rate

Crop growth rate is a linear function of intercepted irradiance and maintaining higher LAI and had positive effect for higher dry matter production through increased CGR and resulted in higher yield. Brassinolide along with BA and NAA at higher concentration increased the CGR in the present investigation. The increased CGR due to BL application might be the result of increased leaf area, as CGR had positive association with LAI. This finding was in agreement with the findings of Rajasekaren and Blake (1998) who observed that HBL application increased the growth rate by 19 percent in jack pine seedlings.

### 5.3.7. Relative Growth Rate

Relative Growth rate is an index of the amount of growing material per unit dry weight of the plant. In the present study RGR showed a gradual increase from 30 to 60 DAS and there after it steadily declined. When the growth regulators were combined at their highest concentrations, there was an enhancement of RGR than other treatments and control. Pandy *et al.* (1981) observed a positive relationship between RGR and biomass production in cowpea. Bindu (2000) reported that foliar application of BR in groundnut had higher relative growth rate than the control.

### 5.3.8. Total Dry Matter Production.

Dry matter production is an important criterion as it determines the source sink relationship. The first pre requisite for increased yield is an increased total dry matter per unit area. Among the treatments the highest TDMP was produced by the higher concentration of BL, BA and NAA. That showed that brassinolide has synergistic effect with auxin. The above report was agreement with the findings of Pipattanawong *et al.* (1996). Hayat *et al.* (2000) reported that spraying of 28-HBL increased the total dry matter in mustard plant. Similar result was obtained by Lini (2001) and Nagasubramainam (2003) in cotton and baby corn respectively.

### 5.3.9. Chlorophyll

Chlorophyll plays an important role in photosynthesis and it increased the plant productivity. Foliar application of brassinolide and other hormones significantly increased the chlorophyll content at 30 and 60 DAS and after that there was a decrease in chlorophyll content. The maximum chlorophyll content was attained with foliar spraying of higher concentration of BL, BA and NAA. The increase in chlorophyll content might be due to increase in enzyme protein by the brassinolide foliar application. The above finding was in accordance with Kulaeva *et al.* (1991). There was increase in chlorophyll ‘a’, ‘b’ and total in the mung bean with HBL foliar application at three different stages of growth. Hayat *et al.* (2000) observed 0.5ppm BR foliar spraying had increased the chlorophyll content in rice. Similar result was obtained by Senthil *et al.* (2003) in soybean using BR.

### 5.3.10. Chlorophyll fluorescence

Plant physiologist characterized chlorophyll fluorescence as stethoscope, because they are extremely useful in the diagnosis of photosynthesis activity of plants under normal and stressed conditions (Krause and Weis, 1991). In the present study, the maximum total and variable fluorescence were increased with the foliar application of plant growth regulators. The chlorophyll fluorescence ratio increased up to 60DAS only. The ratio of variable and maximum fluorescence indirectly proportional to the quantum yield of photochemistry and showed high degree of correlation to quantum yield on net photosynthesis of intact leaves. The treatment of BL, BA and NAA at higher concentration showed increased chlorophyll fluorescence ratio. This might be due to the excitation of energy captured in PS II and was high in foliar sprayed plant. This was in accordance with the findings of Nagasubramaniam (2003) in baby corn.

### 5.3.11. Leaf soluble protein

Soluble protein content being a measure of RuBPcarboxylase activity is considered as an index for photosynthetic efficiency. RuBPcase contribute for 50% of total soluble protein in the leaf extract. Significant variation in soluble protein content was noticed in all the growth stages of maize. The increased soluble content was notice with the foliar spraying of higher concentration of BL with BA and NAA. BL had significant synergistic effect with NAA than BL. The soluble protein content was increased by foliar spraying of EBL in groundnut (Vardhini and Rao, 1998) and this increase in soluble protein was due to higher DNA and RNA contents; enhanced the activity of RNA polymerase; reduced the activity of RNAase and DNAase. Thangaraj *et al.* (1998) reported similar findings in rice. Prakash *et al.* (2003) reported that in groundnut BR application had increased the soluble protein content. This increased soluble protein content in treated plants could be due to enhanced activation of RuBPcase.

### 5.3.12. Nitrate reductase activity

NRase plays a key role on the regulation of assimilatory nitrate reduction. The present study revealed that BL with BA and NAA increased the NRase activity irrespective of their concentrations. BL in combination with NAA showed maximum activity than when it combined with BA. This indicated that BA had no interaction effect and NAA had synergistic effect. BR induced increase in NRase activity could be due to improvement in leaf water balance and or specific enzyme activity (Kulaeva *et al.*, 1991). Hayat *et al.* (2001) reported that seed treatment of wheat with BL did enhance the NRase activity. Soaking *Lens culinaris* seeds with HBL improved the NRase activity (Hayat and Ahmed, 2003).

### 5.3.13. IAA oxidase activity

IAA oxidase activity determines the auxin level in the plant for the growth. When BL was combined with NAA, the activity was very less when compared to BL with BA. And the lowest activity was observed in higher concentration of BL, BA with NAA. From the above, it could be seen that brassinolide had negative effect on IAA oxidase activity and it was still lowered when NAA was combined. Katsumi (1985) reported that BR acted synergistically with auxin in stimulating cell elongation. Umadevi (1998) observed lowest IAA oxidase activity in sesame with the application of BR. Karnachuk *et al.* (2002) observed similar effect in *A. thaliana* seedling with EBL. In EBL treated seedlings there was an increase in the content of free IAA by several times.

### 5.3.14. Yield and Yield components

Cob length had significant role in deciding the source size. The highest Cob length was achieved in higher concentration of BL with BA and NAA. The NAA showed synergistic effect with BL and increased the Cob length than BL with BA. Since BR enhanced cell division and cell elongation in meristematic tissue, it was possible that BR application improved the pod growth (Ramraj *et al.*, 1997). Nagasubramaniam (2003) reported same trend in baby corn with foliar spraying of BR and they had increased the cob length.

In the present study the maximum number of grains was recorded under combined foliar spray at higher concentration of BL, BA and NAA. When BL was combined with NAA, more number of grains was recorded as a result of synergistic effect. Ravichandran and Pathmanbahan (2000) reported increased number of grains in pearl millet by BR application. Maibangsa *et al.* (2000) reported increased number of grains per panicle in rice.

The grain yield was highly significant for all treatments. The grain yield was increased by foliar application of BL, BA and NAA in combination than the other treatments and control. The increase in grain yield might be due to increased cell elongation and resulted in increased chlorophyll, soluble protein, NRase activity, cob length and increased grains number. The BL with NAA showed synergistic effect and increased the yield over BL with BA. The above finding was supported by Sivakumar *et al.* (2002) in pearl millet where in BR increased the number of grains, 1000grain weight and yield. Seed treatment of wheat with EBL increased the crop yield (Nilovskaya *et al.*, 2001).

Harvest index indicates the proportion of photosynthetic partition to the economic part. The yield can be improved by increasing the proportion of photosynthates allocated to the grains. The results of the present study revealed that BL in combination with BA and NAA increase the HI in maize. The highest HI was noticed due to increase grain weight and heavier translocation in BL treated Plants. The above finding was in line with the finding of Fuji *et al.* (1991). Sairam (1994) reported that BR treatment caused higher grain weight and HI in wheat suggesting more translocation to reproductive sink.

## 5.4. Effect of brassinolide on the productivity of crops

Application of brassinolide increased the growth, enhanced maturation and increased the crop yield of lettuce, pepper and beans (Meudt et al., 1983 and 1984). Brassinosteroids increased the growth and yield of potato, wheat (Brun and wild, 1984) and mustard (Hayat *et al.*, 2000). Brassinosteroids sprayed in corn plant increased the yield to 18-33 per cent. Foliar application of brassinosteroids increased the yield of groundnut crop (Vardhini and Rao, 1998). The yield of the fruit crops and vegetable crops such as tomato, cucumber and egg plants treated with BR at flowering stage gave higher yield (Ikekawa and Zhao, 1991).

### 5.4.1. Rice

Foliar application of BL 0.2ppm increased the yield. The increase in yield was 12.4 per cent and this was due to increase in number of productive tillers and grains. The increased tillers number and grains number as reported in the present study might be due to synergistic action of BR with indigenous auxin in cell elongation and cell proliferation of meristematic tissues (Sairam, 1994). Thangaraj *et al.* (1998) found similar results in *Thaladi* rice; 0.1ppm BR as foliar spray at panicle initiation and flowering increased the yield. Foliar application of BR at tillering and ear emergence, had increased rice yields significantly (Krishnan *et al.*, 1999). Thirthalingappa *et al.* (1999) reported that 0.1ppm HBR + 60ppm GA increased the number of tillers, productive tillers, number of spiklets, seed set per cent and 100 seeds weight in rice. Maibangsa *et al.* (2000) also had the same findings in CO 45 rice variety with 0.5ppm BR.

### 5.4.2 Maize

Foliar application of 0.10ppm BL increased the plant height, LAI, Cob length, number of grains, 100 seed weight, grain yield and Harvest index. BR increased the length of the cob by promoting the elongation of cell. This might be the reason for obtaining longest ear in treated plants (Heping Cob and Shankum Chen, 1995). BL enhanced water and nutrient uptake, which in turn increased the partitioning per centage and translocation of photosynthates and increased the cob weight. This was in accordance with the finding of Bhatia and Kaur

(1997) and Thirthalingappa *et al.* (1999). Increase in cob yield due to foliar spray of BR was also attributed to increase cob length; cob diameter and cob weight in baby corn as reported by Nagasubramaniam (2003).

### 5.4.3. Sorghum

Application of brassinolide significantly increased the yield and yield parameters in sorghum. Foliar application of 0.10ppm BL had increased the plant height, LAI, 1000grain weight, grain yield, stover yield and harvest index. The increase in yield was 9.6 per cent and this might be due to increase in LAI and 1000grain weight. The translocation of assimilate from source to sink was enhanced by the application of brassinolide and this improved yield (Sivakumar *et al.* 2002) in pearl millet. Under rainfed condition, pre-sowing seed hardening with brassinolide significantly increased ear number, ear length and 1000grain weight as reported by Ravichandran and Pathmanabhan (2000).

### 5.4.4. Ragi

In the present study brassinolide at 0.1ppm foliar spray increased the yield and yield parameters. The increase in yield was 10.96 percent over control mainly by increase in number of tillers, number of panicle and seed weight. This showed that brassinolide had significant influence in translocation of photosynthate from the sink to the source. The above findings was on line with the finding of Sairam (1994) who reported that HBL applied either as seed treatment or foliar spray in wheat, increased the number of ears per plant, number of grains per ear, 1000grain weight, yield and HI.

### 5.4.5. Green gram

In the present study, foliar application of 0.10ppm increased the yield attributing character by which the yield was increased by16.2 percent. BRs were involved in the process of cell enlargement through their effects on gene expression and enzyme activity. Thus increase in the source sink size resulted in high yield (Mussig and Altmann, 1999). Bhatia and Kaur (1997) and Sujatha (2001) in mungbean, reported that application of HBL increased the number or pods, number of seeds, 100 seed weight and seed yield. Findings from this study were in agreement with those of Nakaseko and Yoshida (1989) in soybean and *vigna ungularis*.

### 5.4.6. Black gram

Foliar application of brassinolide at 0.1ppm significantly increased the yield and yield attributing characters like number of pods, pod weight, yield and Harvest index. The increase in yield was 17.5 percent over the control. The increased yield might be due to increase in translocation of photosynthate by BL treatment. Significant increase in DNA, RNA and protein in BR treated mung bean and beans resulted in transcription and replication leading to increase in enzyme activities during tissue growth (Kalinich *et al.*, 1985). Kamal *et al.* (1995) reported that BR application increased the seed and pod numbers in soybean.

### 5.4.7. Groundnut

Foliar spraying of 0.20ppm increased the LAI, number of pegs, number of pods, pods weight, kernel weight, TDMP and pod yield. The increase in yield was 13.3 percent over control. Due to LAI increase the photosynthate accumulation would be higher and brassinolide had the capacity to translocate this to the pods and kernels. The above findings was earlier reported by Li *et al.* (1993) who observed in groundnut that, treatment of BR may enhance transport of assimilates to the developing pod and resulted in higher yield. BR application improved the efficiencies of peg and pod growth in groundnut due to enhanced cell division and cell elongation in meristematic tissue (Ramraj *et al.*, 1997). Bindu (2000) reported that foliar application of BR had increase the number of pegs and pods per plant and also increased the yield by 28.4% over unsprayed control.

### 5.4.8. Sunflower

Maximum LAI, number of seeds, per cent grain filling 100seed weight, TDMP and grain yield were obtained by foliar spraying of 0.10ppm BL. The increase in yield was 8.3 percent by foliar application of BL. The increased yield may be due to increase in sink size (mainly number of seeds) and per cent grain filling by brassinolide application and translocation of the photosynthate. There fore the availability of the photosynthates in larger quantities during the reproductive phase significantly favored the pod bearing capacity of this plant. In mustard, higher photosynthetic capability generated in the HBR treated plants was further reflected in their better vegetative growth and increased TDMP (Hayat *et al.*, 2000).

### 5.4.9. Cotton

Foliar spraying of 0.10ppm BL increased yield by 9.1 per cent and this was due to increase on the number of sympodia, number of bolls, percent boll set and boll weight. BL application enhanced the cell division and helped in have more number of sympodia, bolls and kapas yield. Bhat *et al.* (1982) reported that abscission of reproductive organ might be one of the possible reasons for the lowest yield in cotton. The number of boll set or shed was decided by auxin – abscisin interaction. BR application increased the endogenous auxin content and this might have resulted in increased boll set and retention. Ramraj *et al.* (1997) reported that foliar application of 28-HBL increased the seed cotton yield. The results of Lini (2001) in cotton brought information that BR application had increased the seed cotton yield through increased per cent of fruit set.

### 5.4.10. Banana

Foliar application of brassinolide significantly increased the yield. Foliar spray of 0.20ppm BL increased the LAI, bunch weight, finger weight, finger number and yield in the present investigation. The increased yield might be due efficient translocation of photosynthate by brassinolide foliar spraying. Anitha (2003) reported that foliar spray of BR increased the bunch weight and finger weight in banana. It was confirmed by Ganesen and Raman (2004) that spraying of BL at 100ml per acre with developing fingers recorded highest bunch weight in Nendran and Lally poovan varieties.

### 5.4.11. Grapes

In the present study, foliar spraying of brassinolide at 0.10ppm significantly increased the number of bunches, bunch weight, 100 berry weight and yield. The increased yield might be due to the efficient translocation of the photosynthates and increase in the TSS and reducing sugar of the berries. The above finding was in line with the finding of Han *et al.* (1988) who reported that foliar application of BR increased the reducing sugar content in tobacco. Xu *et al.* (1994) reported that EBL increased the fruit yield in grapes. Watanabe *et al.* (1997) reported that brassinolide analogues TS303 promoted fruit set in grapes.

### 5.3.12. Tomato

Exogenous application of brassinolide resulted in promotion of growth and yield of tomato plants. BL at 0.05ppm increased the yield and yield attributing characters. The increase in yield was due to increase in percent fruit set, fruit weight, TDMP and yield. Altman (1998) expressed the view that BRs elicit strong growth responses when applied exogenously to the plants. The growth promotion in tomato plants was influenced by BR treatment and this was associated with enhancement on the yield of the plants. Vardhini and Rao (2001) reported in tomato that among BL, HBL and EBL, BL was most effective in accounting increase in the number of fruits per plant and yield.

## CHAPTER VI

# SUMMARY

The present study was carried out to standardize the protocol for the biological activity of brassinolide and to understand the physiological, biochemical and molecular action of brassinolide on the productivity as well as identify optimum concentration of BL for improving the yield in selected agricultural and horticultural crops. The final outcome of the detailed study is summarized below.

Brassinolide 0.10 ppm in maize seedling has increased the shoot length, root length, dry weight, chlorophyll content, soluble protein, nitrate reductase and IAA oxidase activities.

Brassinolide increased resistance of plants against various abiotic stresses. Exposure of maize seedlings to drought leads to reduction in the growth and enzyme activity. Pre-treatment of maize seedlings with 0.10 ppm BL reduced the impact of drought stress by improving the shoot and root length, chlorophyll, soluble protein content and reduced the formation of proline. In pre-treated seedlings, the enzyme NRase activity was improved resulting in more synthesis of protein by way of nitrogen assimilation. There was an increased level of auxin due to BL pretreatment as shown by reduced activity of IAA oxidase.

The free radical scavenging enzymes, superoxide dismutase, catalase and peroxidase activities were increased in the BL pre treated seedlings when they were subjected to drought stress. The increased activity of the enzymes reduced the free radical content of the seedlings.

When the maize seedlings were exposed to low temperature stress, it has resulted the reduction of the growth and enzymatic activity. But the pre-treatment of 0.10 ppm brassinolide ameliorated the low temperature stress and it recovered the shoot and root growth, soluble protein but reduced proline content. It also encouraged the activity of NRase, and free radical scavenging enzymes like catalase, peroxidase and superoxide dismutase.

The pretreatment of brassinolide at 0.10 ppm concentration improved the activity of enzymes like NRase, catalase, peroxidase and SOD under high temperature stress also, thus resulting in the formation of soluble protein and reduction in the free amino acid accumulation mainly proline. Due to higher activity of the enzymes and synthesis of protein, the growth of shoot and root was not affected by high temperature stress. The pretreatment with BL was superior having significant effect than when the seedling was treated with BL after induction temperature.

The pretreatment of maize seedling with brassinolide at 0.10 ppm concentration showed maximum recovery of growth, enzyme activity and synthesis of protein, when subjected to salinity stress and was nearly equal to the absolute control.

Foliar application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA increased the plant height. But when BL was combined with BA, the plant height was comparatively less than BL that with NAA. This showed that brassinolide had synergistic effect with auxin rather than cytokinin. Foliar application of BL, BA and NAA in combined form at higher concentrations had increased the root length than when they were applied individually.

Foliar application of brassinolide along with BA and NAA increased the leaf area and SLW but it decreased the SLA. The NAR, CGR, RGR and TDMP were increased in the combined application of 0.10 ppm BL + 20 ppm BA + 10 ppm NAA. The increase in the growth parameters significantly increased the yield of the maize.

Foliar application of BL, BA and NAA increased the chlorophyll content and chlorophyll fluorescence. Increased soluble protein content was noticed in the BL, BA and NAA sprayed maize plants indicating enhanced photosynthetic efficiency owing to higher RuBPcase activity.

NRase activity was increased with combined foliar spry (BL, BA and NAA) at higher concentration. The increased activity was recorded on 60 DAS. The activity of IAA oxidase was decreased resulting in higher auxin content in treated plants.

Yield and yield related attributes in BL, BA and NAA foliar sprayed plants were significantly higher compared to other treatments. This combined treatment produced more number of grains per cob and increased cob length. The yield per plant was found to be the highest in the same treatment resulting in higher harvest index.

Foliar application of 0.20 ppm brassinolide in rice significantly increased the plant height, LAI, number of tillers, productive tillers, numbers of grains and 1000 grain weight. As a result of the increase in yield attributes, brassinolide increased the yield and harvest index. The benefit cost ratio for BL was 2.02 and that of control (1.64).

Foliar application 0.10 ppm brassinolide increased the plant height, LAI, cob length, number of grains per cob, 100 seed weight, grain yield and HI in maize. By foliar spraying of BL, had a higher BC ratio was (2.33) than control (2.12).

In ragi, foliar spraying of 0.10 ppm brassinolide increased the yield and yield attributing characters. The increase in the yield, by 0.10 ppm BL foliar application was 10.96 per cent over the control and the benefit cost ratio was 1.58 and 1.42 respectively.

Foliar spraying of 0.10 ppm BL increased the yield and yield attributing characters of sorghum. The percent increase in the yield was 9.6 over the control and BC ratio was 1.77.

In green gram, foliar spraying of 0.10 ppm BL increased the pod numbers, pod weight and yield. The percent increase in the brassinolide treatment was 16.2 over the control with BC ratio of 2.23.

Foliar application of 0.10 ppm BL in black gram having a BC ratio of 2.36 increased the pod yield by 17.5 percent over the control.

Foliar spaying of 0.20 ppm BL treatment increased the groundnut yield and the percent increase was 13.3 over the control with a cost benefit ratio of 2.08.

The capitulam diameter, number of seeds, percent grain filling and 100 seed weight of sunflower were increased by the foliar spraying of 0.10 ppm BL resulting in 8.3 percent increase in the yield over control.

Foliar application of brassinolide at 0.10 ppm increased the number of sympodia, number of bolls, percent boll set and boll weight in cotton, resulting in the increased yield of kapas by 9.1 percent over the control with BC ratio 2.29.

The brassinolide at 0.20 ppm increased the bunch weight, number of fingers, finger weight and yield in banana. The percent increase in yield was 21.4 over control with BC ratio of 2.82.

In grapes foliar spraying of 0.10 ppm BL increased the bunch weight, 100 berry weight and yield by 20.9 percent over control. Foliar spraying also increased the quality of the berry.

In vegetable crops, brassinolide at 0.05 ppm increased the plant height, LAI, percent fruit set, fruit weight TDMP and HI in tomato. As a result of the improved the yield attributes, the fruit yield was increased by 6.9 percent over the control with a BC ratio was 2.85.

The brassinolide, an unique plant growth regulator at concentrations of 0.10 and 0.20 ppm. It had significantly ameliorated the abiotic stress effects (drought, low and high temperature, salinity) and prevented damage to specific enzymes and biochemical pathways by synthesizing substantial amounts of scavenging enzymes and that helped in maintenance of growth, dry matter accumulation and ultimately resulted in increased yield. Effect of brassinolide on different crops revealed that in rice, groundnut and banana foliar application of 0.20 ppm BL increased the yield and yield attributing characters. Where as in maize, sorghum, ragi, black gram, green gram, sunflower, cotton and grapes 0.10 ppm BL increased the yield and yield characters. In tomato, foliar spraying of 0.05 ppm BL increased the yield and yield attributing characters.

**Table: 2. Effect of Brassinolide on physiological and biochemical characters of maize seedlings**

| Treatment | Shoot length (cm) | Root length (cm) | Seedling dry weight (g) | Chlorophyll a (mg $g^{-1}$) | Chlorophyll b (mg $g^{-1}$) | Total chlorophyll (mg $g^{-1}$) | Soluble protein (mg $g^{-1}$) | NRase activity (μ mole $NO_2^-$ $g^{-1}$ $hr^{-1}$) | IAA oxidase activity (μg unoxidised auxin $g^{-1}$ $hr^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| **$T_1$** | 13.4 | 12.5 | 0.165 | 0.245 | 0.173 | 0.418 | 4.76 | 3.42 | 54.3 |
| **$T_2$** | 13.6 | 12.1 | 0.186 | 0.257 | 0.182 | 0.437 | 5.50 | 3.48 | 57.2 |
| **$T_3$** | 16.4 | 12.7 | 0.203 | 0.271 | 0.176 | 0.447 | 6.11 | 3.80 | 56.5 |
| **$T_4$** | 18.3 | 13.9 | 0.219 | 0.364 | 0.295 | 0.659 | 6.62 | 4.65 | 58.9 |
| **$T_5$** | 17.4 | 13.5 | 0.209 | 0.347 | 0.276 | 0.623 | 6.40 | 4.34 | 58.3 |
| **$T_6$** | 16.8 | 13.8 | 0.206 | 0.339 | 0.275 | 0.614 | 5.95 | 4.23 | 57.8 |
| **$T_7$** | 16.3 | 13.3 | 0.205 | 0.339 | 0.273 | 0.611 | 6.13 | 4.00 | 56.4 |
| **$T_8$** | 16.2 | 12.9 | 0.200 | 0.347 | 0.236 | 0.583 | 6.15 | 4.19 | 55.4 |
| **S Ed** | **0.3** | **0.3** | **0.005** | **0.004** | **0.004** | **0.006** | **0.22** | **0.11** | **0.3** |
| **CD (P= 0.05)** | **0.7** | **0.6** | **0.009** | **0.009** | **0.008** | **0.012** | **0.46** | **0.22** | **0.6** |

**Table: 3. Influence of brassinolide on physiological and biochemical parameters of maize seedlings under drought stress**

| Treatment | Shoot length (cm) | Root length (cm) | Seedling dry weight (g) | Chlorophyll a (mg $g^{-1}$) | Chlorophyll b (mg $g^{-1}$) | Total chlorophyll (mg $g^{-1}$) | Soluble protein (mg $g^{-1}$) | Proline (µmol $g^{-1}$ $hr^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **$T_1$** | 8.3 | 6.5 | 0.103 | 0.131 | 0.106 | 0.237 | 2.26 | 59.42 |
| **$T_2$** | 10.5 | 9.6 | 0.122 | 0.189 | 0.150 | 0.339 | 2.77 | 40.25 |
| **$T_3$** | 10.8 | 10.3 | 0.130 | 0.206 | 0.155 | 0.361 | 3.34 | 28.43 |
| **$T_4$** | 12.4 | 10.8 | 0.145 | 0.227 | 0.164 | 0.391 | 3.67 | 24.12 |
| **$T_5$** | 11.5 | 10.2 | 0.140 | 0.216 | 0.160 | 0.376 | 3.55 | 26.36 |
| **S Ed** | **0.5** | **0.5** | **0.005** | **0.008** | **0.007** | **0.009** | **0.09** | **2.06** |
| **CD (P= 0.05)** | **1.0** | **0.10** | **0.010** | **0.016** | **0.014** | **0.019** | **0.19** | **4.24** |

**Table: 4. Influence of brassinolide on enzyme activity of maize seedlings under drought stress**

| Treatment | NRase activity (µ mole $NO^-_2$ $g^{-1}$ $hr^{-1}$) | IAA oxidase activity (µg unoxidised auxin $g^{-1}$ $hr^{-1}$) | Catalase activity (enzyme units x$10^4$ $g^{-1}$ $min^{-1}$) | Peroxidase activity (enzyme units $l^{-1}$ $h^{-1}$) | Superoxide Dismutase enzyme units mg (protein$^{-1}$ min$^{-1}$) |
|---|---|---|---|---|---|
| **$T_1$** | 2.04 | 62.5 | 1.52 | 16.2 | 1.37 |
| **$T_2$** | 2.87 | 69.7 | 2.39 | 26.3 | 1.89 |
| **$T_3$** | 3.10 | 73.6 | 2.57 | 34.7 | 2.00 |
| **$T_4$** | 3.28 | 76.0 | 2.69 | 38.3 | 2.12 |
| **$T_5$** | 3.14 | 70.3 | 2.55 | 34.3 | 2.06 |
| **S Ed** | **0.07** | **1.7** | **0.10** | **1.9** | **0.09** |
| **CD (P= 0.05)** | **0.15** | **3.6** | **0.22** | **4.1** | **0.18** |

**Table: 5. Influence of brassinolide on physiological and biochemical parameters of maize seedlings under low temperature stress**

| Treatment | Shoot length (cm) | Root length (cm) | Seedling dry weight (g) | Chlorophyll a (mg $g^{-1}$) | Chlorophyll b (mg $g^{-1}$) | Total chlorophyll (mg $g^{-1}$) | Soluble protein (mg $g^{-1}$) | Proline (µmol $g^{-1}$ $hr^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **$T_1$** | 6.5 | 4.2 | 0.095 | 0.117 | 0.076 | 0.193 | 2.25 | 52.42 |
| **$T_2$** | 8.9 | 7.4 | 0.126 | 0.165 | 0.126 | 0.291 | 2.84 | 39.26 |
| **$T_3$** | 9.3 | 8.2 | 0.134 | 0.195 | 0.132 | 0.327 | 2.96 | 35.41 |
| **$T_4$** | 10.8 | 9.1 | 0.148 | 0.208 | 0.146 | 0.354 | 3.23 | 21.05 |
| **$T_5$** | 10.6 | 8.7 | 0.142 | 0.198 | 0.136 | 0.334 | 3.01 | 24.32 |
| **S Ed** | **0.4** | **0.8** | **0.022** | **0.009** | **0.007** | **0.014** | **0.12** | **2.31** |
| **CD (P= 0.05)** | **0.8** | **1.7** | **0.046** | **0.019** | **0.014** | **0.029** | **0.26** | **4.72** |

**Table: 6. Influence of brassinolide on enzyme activity of maize seedlings under low temperature stress**

| Treatment | NRase activity (µ mole $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$) | **IAA oxidase activity** (µg unoxidised auxin $g^{-1}$ $hr^{-1}$) | Catalase activity (enzyme units x$10^{4}$ $g^{-1}$ $min^{-1}$) | Peroxidase activity (enzyme units $l^{-1}$ $h^{-1}$) | Superoxide Dismutase enzyme units mg (protein$^{-1}$ min$^{-1}$) |
|---|---|---|---|---|---|
| **$T_1$** | 1.82 | 69.5 | 1.25 | 15.4 | 1.26 |
| **$T_2$** | 2.65 | 67.3 | 1.87 | 23.2 | 1.96 |
| **$T_3$** | 2.88 | 67.6 | 2.35 | 30.8 | 2.11 |
| **$T_4$** | 2.97 | 62.6 | 2.42 | 36.5 | 2.27 |
| **$T_5$** | 2.90 | 64.7 | 2.38 | 32.3 | 2.23 |
| **S Ed** | **0.08** | **0.4** | **0.25** | **1.4** | **0.04** |
| **CD (P= 0.05)** | **0.18** | **0.8** | **0.53** | **2.9** | **0.09** |

**Table: 7. Influence of brassinolide on physiological and biochemical parameters of maize seedlings under high temperature Stress**

| Treatment | **Shoot length** (cm) | Root length (cm) | Seedling dry weight ( g) | Chlorophyll ‘a’ (mg $g^{-1}$) | Chlorophyll ‘b’ (mg $g^{-1}$) | Total chlorophyll (mg $g^{-1}$) | Soluble protein (mg $g^{-1}$) | Proline (µmol $g^{-1}$ $hr^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **$T_1$** | 12.9 | 11.3 | 0.155 | 0.234 | 0.175 | 0.409 | 4.89 | 11.24 |
| **$T_2$** | 6.3 | 5.2 | 0.087 | 0.065 | 0.058 | 0.123 | 2.29 | 55.43 |
| **$T_3$** | 4.8 | 3.8 | 0.063 | 0.053 | 0.042 | 0.095 | 2.05 | 57.42 |
| **$T_4$** | 10.1 | 8.7 | 0.136 | 0.195 | 0.149 | 0.344 | 3.82 | 24.34 |
| **$T_5$** | 11.3 | 10.2 | 0.138 | 0.217 | 0.156 | 0.373 | 4.10 | 22.42 |
| **$T_6$** | 8.8 | 7.5 | 0.123 | 0.168 | 0.130 | 0.298 | 3.25 | 45.65 |
| **$T_7$** | 9.5 | 8.1 | 0.134 | 0.182 | 0.136 | 0.318 | 3.36 | 49.32 |
| **S Ed** | **0.7** | **0.4** | **0.012** | **0.003** | **0.010** | **0.011** | **0.18** | **3.06** |
| **CD (P= 0.05)** | **1.5** | **0.8** | **0.03** | **0.006** | **0.022** | **0.024** | **0.39** | **6.17** |

**Table: 8. Influence of brassinolide on enzyme activity of maize seedlings under high temperature stress**

| Treatment | NRase activity ($\mu$ mole $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$) | IAA oxidase activity ($\mu$g unoxidised auxin $g^{-1}$ $hr^{-1}$) | Catalase activity (enzyme units x$10^{4}$ $g^{-1}$ $min^{-1}$) | Peroxidase activity (enzyme units $l^{-1}$ $h^{-1}$) | Superoxide Dismutase enzyme units mg (protein$^{-1}$ $min^{-1}$) |
|---|---|---|---|---|---|
| **$T_1$** | 3.29 | 58.5 | 1.06 | 20.54 | 1.80 |
| **$T_2$** | 1.52 | 24.5 | 1.20 | 25.09 | 1.42 |
| **$T_3$** | 1.38 | 22.2 | 0.98 | 21.30 | 1.03 |
| **$T_4$** | 2.93 | 40.5 | 2.73 | 48.23 | 2.62 |
| **$T_5$** | 3.01 | 42.2 | 2.95 | 52.13 | 2.95 |
| **$T_6$** | 2.42 | 33.5 | 2.09 | 31.66 | 2.25 |
| **$T_7$** | 2.78 | 35.5 | 2.39 | 42.58 | 2.55 |
| **S Ed** | **0.15** | **1.02** | **0.14** | **1.83** | **0.22** |
| **CD (P= 0.05)** | **0.32** | **2.10** | **0.31** | **3.91** | **0.49** |

**Table: 9. Influence of brassinolide on physiological and biochemical parameters of maize seedlings under salinity stress**

| Treatment | *Shoot length* (cm) | **Root length** (cm) | Seedling dry weight (g) | **Chlorophyll a** (mg g$^{-1}$) | **Chlorophyll b** (mg g$^{-1}$) | **Total chlorophyll** (mg g$^{-1}$) | *Soluble protein* (mg g$^{-1}$) | *Proline* (µmol g$^{-1}$ hr$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **T$_1$** | 13.6 | 10.5 | 0.181 | 0.226 | 0.153 | 0.379 | 3.83 | 10.14 |
| **T$_2$** | 7.2 | 4.3 | 0.073 | 0.068 | 0.081 | 0.149 | 2.26 | 41.32 |
| **T$_3$** | 4.3 | 3.1 | 0.061 | 0.057 | 0.060 | 0.117 | 2.03 | 45.45 |
| **T$_4$** | 10.3 | 8.9 | 0.149 | 0.183 | 0.128 | 0.311 | 3.25 | 20.12 |
| **T$_5$** | 11.4 | 9.3 | 0.164 | 0.202 | 0.136 | 0.338 | 3.72 | 16.86 |
| **T$_6$** | 8.1 | 7.1 | 0.109 | 0.142 | 0.116 | 0.225 | 3.35 | 38.46 |
| **T$_7$** | 8.9 | 7.6 | 0.121 | 0.150 | 0.123 | 0.273 | 3.23 | 33.23 |
| **S Ed** | **0.7** | **0.5** | **0.008** | **0.005** | **0.007** | **0.008** | **0.23** | **2.17** |
| **CD (P= 0.05)** | **1.5** | **0.9** | **0.018** | **0.012** | **0.015** | **0.015** | **0.45** | **4.32** |

**Table: 10. Influence of brassinolide on enzyme activity of maize seedlings under salinity stress**

| Treatment | NRase activity (µ mole $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$) | IAA oxidase activity (µg unoxidised auxin $g^{-1}$ $hr^{-1}$) | Catalase activity (enzyme units x$10^{4}$ $g^{-1}$ $min^{-1}$) | Peroxidase activity (enzyme units $l^{-1}$ $h^{-1}$) | Superoxide Dismutase enzyme units mg ($protein^{-1}$ $min^{-1}$) |
|---|---|---|---|---|---|
| **$T_1$** | 4.02 | 57.3 | 1.1 | 25.36 | 1.67 |
| **$T_2$** | 1.75 | 26.3 | 1.3 | 31.42 | 1.44 |
| **$T_3$** | 1.34 | 21.4 | 1.2 | 26.48 | 1.22 |
| **$T_4$** | 3.72 | 43.7 | 2.7 | 39.63 | 2.42 |
| **$T_5$** | 3.90 | 48.2 | 2.8 | 43.15 | 2.73 |
| **$T_6$** | 3.03 | 34.5 | 2.3 | 33.43 | 2.01 |
| **$T_7$** | 3.42 | 37.3 | 2.6 | 38.54 | 2.15 |
| **S Ed** | **0.16** | **0.7** | **0.1** | **0.50** | **0.14** |
| **CD (P= 0.05)** | **0.35** | **1.6** | **0.3** | **1.01** | **0.30** |

Table: 11. Effect of BL, BA and NAA on the plant height (cm) of maize under Pot culture

| **Treatment** | **30DAS** | **60DAS** | **90DAS** |
|---|---|---|---|
| **$T_1$** | 115.2 | 146.0 | 173.7 |
| **$T_2$** | 120.8 | 162.4 | 183.2 |
| **$T_3$** | 121.6 | 163.4 | 186.3 |
| **$T_4$** | 116.2 | 154.4 | 177.7 |
| **$T_5$** | 117.5 | 156.3 | 181.3 |
| **$T_6$** | 119.5 | 161.6 | 182.8 |
| **$T_7$** | 116.2 | 150.2 | 178.5 |
| **$T_8$** | 118.5 | 152.1 | 180.5 |
| **$T_9$** | 121.4 | 158.1 | 185.1 |
| **$T_{10}$** | 122.2 | 159.7 | 186.0 |
| **$T_{11}$** | 123.3 | 165.4 | 191.5 |
| **$T_{12}$** | 126.0 | 166.1 | 190.3 |
| **$T_{13}$** | 126.5 | 168.7 | 193.7 |
| **$T_{14}$** | 129.0 | 175.1 | 194.4 |
| **$T_{15}$** | 128.5 | 171.6 | 193.2 |
| **$T_{16}$** | 132.5 | 186.3 | 199.2 |
| **S Ed** | **0.77** | **1.97** | **1.49** |
| **CD (P= 0.05)** | **1.58** | **4.03** | **3.05** |

**Table: 12. Effect of BL, BA and NAA on the root length (cm) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 30.7 | 40.0 | 44.8 |
| **$T_2$** | 31.5 | 47.0 | 52.0 |
| **$T_3$** | 33.0 | 48.0 | 54.0 |
| **$T_4$** | 30.9 | 47.0 | 48.3 |
| **$T_5$** | 31.5 | 48.1 | 54.0 |
| **$T_6$** | 32.3 | 43.1 | 48.3 |
| **$T_7$** | 33.5 | 49.2 | 49.9 |
| **$T_8$** | 34.3 | 50.2 | 52.1 |
| **$T_9$** | 35.2 | 51.2 | 58.5 |
| **$T_{10}$** | 36.0 | 52.8 | 60.0 |
| **$T_{11}$** | 33.4 | 48.8 | 55.2 |
| **$T_{12}$** | 34.0 | 50.8 | 56.0 |
| **$T_{13}$** | 36.5 | 54.4 | 60.7 |
| **$T_{14}$** | 37.7 | 56.3 | 65.2 |
| **$T_{15}$** | 37.0 | 55.9 | 62.0 |
| **$T_{16}$** | 38.5 | 59.1 | 67.5 |
| **S Ed** | **0.66** | **0.79** | **0.87** |
| **CD (P= 0.05)** | **1.36** | **1.62** | **1.79** |

**Table: 13. Effect of BL, BA and NAA on leaf area ($cm^2$ $plant^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 723 | 2992 | 2787 |
| **$T_2$** | 780 | 3330 | 2832 |
| **$T_3$** | 798 | 3353 | 2904 |
| **$T_4$** | 736 | 3303 | 2836 |
| **$T_5$** | 750 | 3316 | 2868 |
| **$T_6$** | 787 | 3346 | 2889 |
| **$T_7$** | 730 | 3256 | 2820 |
| **$T_8$** | 735 | 3265 | 2852 |
| **$T_9$** | 803 | 3341 | 2906 |
| **$T_{10}$** | 816 | 3366 | 2922 |
| **$T_{11}$** | 832 | 3388 | 2936 |
| **$T_{12}$** | 858 | 3410 | 2952 |
| **$T_{13}$** | 860 | 3406 | 2945 |
| **$T_{14}$** | 905 | 3453 | 2998 |
| **$T_{15}$** | 885 | 3426 | 2967 |
| **$T_{16}$** | 925 | 3495 | 3021 |
| **S Ed** | **7.3** | **19.7** | **27.9** |
| **CD (P= 0.05)** | **15.0** | **40.3** | **57.2** |

## Table: 14. Effect of BL ,BA and NAA on Specific leaf weight (mg cm$^{-2}$) of maize under pot culture

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| $T_1$ | 6.30 | 3.30 | 3.43 |
| $T_2$ | 6.80 | 4.20 | 4.20 |
| $T_3$ | 7.20 | 4.60 | 4.50 |
| $T_4$ | 6.50 | 3.60 | 3.60 |
| $T_5$ | 6.80 | 3.70 | 3.80 |
| $T_6$ | 7.10 | 4.50 | 4.40 |
| $T_7$ | 6.40 | 3.50 | 3.54 |
| $T_8$ | 6.70 | 3.60 | 3.70 |
| $T_9$ | 6.90 | 4.30 | 4.50 |
| $T_{10}$ | 7.10 | 4.50 | 4.90 |
| $T_{11}$ | 7.60 | 4.60 | 5.10 |
| $T_{12}$ | 7.80 | 4.90 | 5.40 |
| $T_{13}$ | 7.40 | 4.70 | 5.40 |
| $T_{14}$ | 8.10 | 5.10 | 5.90 |
| $T_{15}$ | 7.90 | 4.90 | 5.70 |
| $T_{16}$ | 8.80 | 5.50 | 6.10 |
| **S Ed** | **0.2** | **0.2** | **0.3** |
| **CD (P= 0.05)** | **0.4** | **0.4** | **0.6** |

**Table: 15. Effect of BL, BA and NAA on Specific leaf area ($cm^2 g^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| $T_1$ | 308 | 395 | 402 |
| $T_2$ | 274 | 356 | 365 |
| $T_3$ | 271 | 353 | 360 |
| $T_4$ | 298 | 368 | 375 |
| $T_5$ | 294 | 361 | 379 |
| $T_6$ | 285 | 359 | 369 |
| $T_7$ | 295 | 369 | 376 |
| $T_8$ | 293 | 370 | 381 |
| $T_9$ | 283 | 350 | 372 |
| $T_{10}$ | 282 | 345 | 369 |
| $T_{11}$ | 260 | 349 | 354 |
| $T_{12}$ | 255 | 343 | 346 |
| $T_{13}$ | 261 | 339 | 338 |
| $T_{14}$ | 254 | 327 | 320 |
| $T_{15}$ | 260 | 330 | 330 |
| $T_{16}$ | 235 | 302 | 314 |
| **S Ed** | **5.3** | **3.6** | **4.2** |
| **CD (P= 0.05)** | **10.9** | **7.4** | **8.7** |

**Table: 16. Effect of BL, BA and NAA on Net assimilation rate (mg cm$^{-2}$ day$^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 2.01 | 1.20 | 0.72 |
| **$T_2$** | 2.16 | 1.28 | 0.81 |
| **$T_3$** | 2.23 | 1.32 | 0.83 |
| **$T_4$** | 2.01 | 1.20 | 0.68 |
| **$T_5$** | 2.02 | 1.23 | 0.71 |
| **$T_6$** | 2.15 | 1.37 | 0.81 |
| **$T_7$** | 2.0 | 1.20 | 0.73 |
| **$T_8$** | 2.1 | 1.22 | 0.74 |
| **$T_9$** | 2.06 | 1.26 | 0.76 |
| **$T_{10}$** | 2.09 | 1.28 | 0.78 |
| **$T_{11}$** | 2.28 | 1.42 | 0.84 |
| **$T_{12}$** | 2.35 | 1.46 | 0.86 |
| **$T_{13}$** | 2.45 | 1.51 | 0.92 |
| **$T_{14}$** | 2.48 | 1.56 | 0.95 |
| **$T_{15}$** | 2.47 | 1.53 | 0.94 |
| **$T_{16}$** | 2.51 | 1.58 | 0.96 |
| **S Ed** | **0.03** | **0.02** | **0.02** |
| **CD (P= 0.05)** | **0.06** | **0.05** | **0.05** |

**Table: 17. Effect of BL, BA and NAA on Crop growth rate (g $m^{-2}$ $day^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| $T_1$ | 25.02 | 11.02 | 31.08 |
| $T_2$ | 26.53 | 12.22 | 35.6 |
| $T_3$ | 26.88 | 12.42 | 38.57 |
| $T_4$ | 25.12 | 11.15 | 31.87 |
| $T_5$ | 25.22 | 11.31 | 32.02 |
| $T_6$ | 26.69 | 12.32 | 36.12 |
| $T_7$ | 25.59 | 11.57 | 32.15 |
| $T_8$ | 26.02 | 11.83 | 3312 |
| $T_9$ | 26.43 | 11.98 | 34.96 |
| $T_{10}$ | 26.5 | 12.1 | 35.52 |
| $T_{11}$ | 27.06 | 12.53 | 39.87 |
| $T_{12}$ | 27.22 | 12.75 | 41.02 |
| $T_{13}$ | 27.32 | 12.82 | 42.58 |
| $T_{14}$ | 28.82 | 13.06 | 42.92 |
| $T_{15}$ | 27.81 | 12.93 | 42.8 |
| $T_{16}$ | 29.31 | 13.31 | 43.13 |
| **S Ed** | **0.33** | **0.05** | **0.55** |
| **CD (P= 0.05)** | **0.67** | **0.10** | **1.13** |

**Table: 18. Effect of BL, BA and NAA on Relative growth rate (g $g^{-1}day^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 0.133 | 0.070 | 0.052 |
| **$T_2$** | 0.143 | 0.079 | 0.059 |
| **$T_3$** | 0.148 | 0.082 | 0.062 |
| **$T_4$** | 0.137 | 0.073 | 0.053 |
| **$T_5$** | 0.140 | 0.077 | 0.055 |
| **$T_6$** | 0.143 | 0.079 | 0.057 |
| **$T_7$** | 0.134 | 0.072 | 0.052 |
| **$T_8$** | 0.136 | 0.076 | 0.054 |
| **$T_9$** | 0.149 | 0.079 | 0.063 |
| **$T_{10}$** | 0.152 | 0.082 | 0.065 |
| **$T_{11}$** | 0.151 | 0.080 | 0.068 |
| **$T_{12}$** | 0.155 | 0.082 | 0.073 |
| **$T_{13}$** | 0.157 | 0.085 | 0.071 |
| **$T_{14}$** | 0.163 | 0.093 | 0.079 |
| **$T_{15}$** | 0.160 | 0.089 | 0.075 |
| **$T_{16}$** | 0.167 | 0.098 | 0.082 |
| **S Ed** | **0.002** | **0.002** | **0.002** |
| **CD (P= 0.05)** | **0.004** | **0.004** | **0.005** |

**Table: 19. Effect of BL, BA and NAA on Total dry matter production (g plant$^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 13.06 | 79.32 | 140.62 |
| **$T_2$** | 13.52 | 82.52 | 148.35 |
| **$T_3$** | 13.86 | 82.86 | 150.55 |
| **$T_4$** | 13.26 | 80.51 | 143.65 |
| **$T_5$** | 13.46 | 81.67 | 146.55 |
| **$T_6$** | 13.64 | 82.75 | 149.25 |
| **$T_7$** | 13.30 | 82.50 | 147.50 |
| **$T_8$** | 13.52 | 83.60 | 149.90 |
| **$T_9$** | 13.56 | 82.06 | 150.98 |
| **$T_{10}$** | 13.86 | 82.59 | 151.35 |
| **$T_{11}$** | 13.92 | 83.07 | 152.06 |
| **$T_{12}$** | 14.02 | 83.45 | 153.25 |
| **$T_{13}$** | 14.32 | 84.55 | 154.06 |
| **$T_{14}$** | 15.32 | 87.32 | 156.88 |
| **$T_{15}$** | 14.82 | 85.71 | 159.59 |
| **$T_{16}$** | 16.28 | 91.35 | 165.36 |
| **S Ed** | **0.22** | **1.76** | **4.32** |
| **CD (P= 0.05)** | **0.46** | **3.61** | **8.86** |

### Table: 20. Effect of BL, BA and NAA on chlorophyll ‘a’ (mg $g^{-1}$) of maize under pot culture

| **Treatment** | **30DAS** | **60DAS** | **90DAS** |
|---|---|---|---|
| **$T_1$** | 0.369 | 0.807 | 0.706 |
| **$T_2$** | 0.468 | 0.901 | 0.856 |
| **$T_3$** | 0.505 | 0.923 | 0.885 |
| **$T_4$** | 0.431 | 0.837 | 0.734 |
| **$T_5$** | 0.434 | 0.867 | 0.821 |
| **$T_6$** | 0.468 | 0.911 | 1.032 |
| **$T_7$** | 0.419 | 0.825 | 0.721 |
| **$T_8$** | 0.425 | 0.851 | 0.805 |
| **$T_9$** | 0.519 | 1.015 | 0.915 |
| **$T_{10}$** | 0.520 | 1.057 | 0.935 |
| **$T_{11}$** | 0.531 | 1.075 | 0.940 |
| **$T_{12}$** | 0.540 | 1.123 | 0.962 |
| **$T_{13}$** | 0.550 | 1.221 | 1.055 |
| **$T_{14}$** | 0.567 | 1.365 | 1.106 |
| **$T_{15}$** | 0.561 | 1.324 | 1.150 |
| **$T_{16}$** | 0.595 | 1.385 | 1.203 |
| **S Ed** | **0.018** | **0.036** | **0.024** |
| **CD (P= 0.05)** | **0.038** | **0.074** | **0.049** |

**Table: 21. Effect of BL, BA and NAA on chlorophyll ‘b’ (mg $g^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 0.301 | 0.537 | 0.361 |
| **$T_2$** | 0.354 | 0.702 | 0.596 |
| **$T_3$** | 0.386 | 0.744 | 0.621 |
| **$T_4$** | 0.315 | 0.521 | 0.482 |
| **$T_5$** | 0.325 | 0.653 | 0.532 |
| **$T_6$** | 0.381 | 0.726 | 0.585 |
| **$T_7$** | 0.306 | 0.561 | 0.453 |
| **$T_8$** | 0.312 | 0.621 | 0.503 |
| **$T_9$** | 0.398 | 0.764 | 0.588 |
| **$T_{10}$** | 0.411 | 0.794 | 0.632 |
| **$T_{11}$** | 0.411 | 0.803 | 0.654 |
| **$T_{12}$** | 0.427 | 0.842 | 0.703 |
| **$T_{13}$** | 0.429 | 0.927 | 0.723 |
| **$T_{14}$** | 0.430 | 0.962 | 0.816 |
| **$T_{15}$** | 0.428 | 0.943 | 0.763 |
| **$T_{16}$** | 0.486 | 1.021 | 0.887 |
| **S Ed** | **0.015** | **0.019** | **0.017** |
| **CD (P= 0.05)** | **0.030** | **0.041** | **0.035** |

**Table: 22. Effect of BL. BA and NAA on total chlorophyll (mg g$^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 0.670 | 1.286 | 1.023 |
| **$T_2$** | 0.822 | 1.603 | 1.285 |
| **$T_3$** | 0.891 | 1.667 | 1.354 |
| **$T_4$** | 0.746 | 1.358 | 1.192 |
| **$T_5$** | 0.759 | 1.520 | 1.245 |
| **$T_6$** | 0.849 | 1.637 | 1.415 |
| **$T_7$** | 0.725 | 1.309 | 1.200 |
| **$T_8$** | 0.732 | 1.430 | 1.209 |
| **$T_9$** | 0.917 | 1.779 | 1.550 |
| **$T_{10}$** | 0.931 | 1.851 | 1.717 |
| **$T_{11}$** | 0.942 | 1.878 | 1.654 |
| **$T_{12}$** | 0.967 | 1.965 | 1.705 |
| **$T_{13}$** | 0.979 | 2.148 | 1.830 |
| **$T_{14}$** | 0.997 | 2.327 | 1.922 |
| **$T_{15}$** | 0.989 | 2.267 | 1.847 |
| **$T_{16}$** | 1.081 | 2.406 | 2.011 |
| **S Ed** | **0.021** | **0.046** | **0.033** |
| **CD (P= 0.05)** | **0.044** | **0.093** | **0.068** |

Table: 23. Effect of BL, BA and NAA on the chlorophyll fluorescence of maize under pot culture

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 0.728 | 0.765 | 0.745 |
| **$T_2$** | 0.748 | 0.786 | 0.758 |
| **$T_3$** | 0.752 | 0.795 | 0.766 |
| **$T_4$** | 0.736 | 0.773 | 0.750 |
| **$T_5$** | 0.740 | 0.780 | 0.754 |
| **$T_6$** | 0.750 | 0.787 | 0.760 |
| **$T_7$** | 0.735 | 0.776 | 0.748 |
| **$T_8$** | 0.744 | 0.781 | 0.751 |
| **$T_9$** | 0.750 | 0.800 | 0.768 |
| **$T_{10}$** | 0.750 | 0.793 | 0.778 |
| **$T_{11}$** | 0.757 | 0.807 | 0.780 |
| **$T_{12}$** | 0.762 | 0.815 | 0.789 |
| **$T_{13}$** | 0.769 | 0.818 | 0.792 |
| **$T_{14}$** | 0.782 | 0.837 | 0.808 |
| **$T_{15}$** | 0.777 | 0.825 | 0.799 |
| **$T_{16}$** | 0.787 | 0.846 | 0.817 |
| **S Ed** | **0.006** | **0.004** | **0.005** |
| **CD (P= 0.05)** | **0.011** | **0.008** | **0.010** |

**Table: 24. Effect of BL,BA and NAA on soluble protein (mg $g^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 7.85 | 13.58 | 10.32 |
| **$T_2$** | 8.95 | 14.27 | 12.25 |
| **$T_3$** | 9.15 | 14.59 | 12.58 |
| **$T_4$** | 8.32 | 13.89 | 12.58 |
| **$T_5$** | 8.58 | 14.05 | 12.98 |
| **$T_6$** | 8.87 | 14.32 | 12.12 |
| **$T_7$** | 8.3 | 13.78 | 12.49 |
| **$T_8$** | 8.3 | 13.82 | 12.87 |
| **$T_9$** | 9.05 | 14.53 | 12.56 |
| **$T_{10}$** | 9.30 | 14.87 | 12.72 |
| **$T_{11}$** | 9.32 | 14.98 | 12.69 |
| **$T_{12}$** | 9.58 | 15.12 | 12.80 |
| **$T_{13}$** | 9.71 | 15.32 | 12.93 |
| **$T_{14}$** | 10.03 | 16.01 | 13.24 |
| **$T_{15}$** | 9.85 | 15.75 | 13.01 |
| **$T_{16}$** | 10.35 | 16.25 | 13.56 |
| **S Ed** | **0.14** | **0.14** | **0.14** |
| **CD (P= 0.05)** | **0.28** | **0.29** | **0.28** |

**Table: 25. Effect of BL, BA and NAA on the Nitrate reductase (µmoles $NO^{-}_{2}$ $g^{-1}$ $hr^{-1}$) of maize under pot culture**

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| **$T_1$** | 4.90 | 6.45 | 3.54 |
| **$T_2$** | 5.04 | 7.14 | 4.04 |
| **$T_3$** | 5.07 | 7.20 | 4.10 |
| **$T_4$** | 4.98 | 7.00 | 3.70 |
| **$T_5$** | 5.04 | 7.05 | 3.89 |
| **$T_6$** | 5.07 | 7.18 | 4.07 |
| **$T_7$** | 4.92 | 6.86 | 3.65 |
| **$T_8$** | 4.95 | 6.97 | 3.80 |
| **$T_9$** | 5.01 | 7.09 | 4.40 |
| **$T_{10}$** | 5.09 | 7.13 | 4.47 |
| **$T_{11}$** | 5.10 | 7.28 | 4.18 |
| **$T_{12}$** | 5.16 | 7.40 | 4.53 |
| **$T_{13}$** | 5.19 | 7.43 | 4.58 |
| **$T_{14}$** | 5.28 | 7.68 | 4.68 |
| **$T_{15}$** | 5.20 | 7.52 | 4.63 |
| **$T_{16}$** | 5.35 | 7.80 | 4.83 |
| **S Ed** | **0.32** | **0.43** | **0.41** |
| **CD (P= 0.05)** | **6.5** | **8.6** | **8.4** |

## Table: 26. Effect of BL, BA and NAA on the IAA oxidase

## (µg of unoxidised auxin $g^{-1}$ $hr^{-1}$) of maize under pot culture

| Treatment | 30DAS | 60DAS | 90DAS |
|---|---|---|---|
| $T_1$ | 160.3 | 120.0 | 80.3 |
| $T_2$ | 170.7 | 139.4 | 98.3 |
| $T_3$ | 173.4 | 141.6 | 100.6 |
| $T_4$ | 161.3 | 130.5 | 87.9 |
| $T_5$ | 163.0 | 132.6 | 90.4 |
| $T_6$ | 172.0 | 143.4 | 104.6 |
| $T_7$ | 152.3 | 125.3 | 85.0 |
| $T_8$ | 160.2 | 126.3 | 88.5 |
| $T_9$ | 170.2 | 136.1 | 93.6 |
| $T_{10}$ | 162.1 | 140.6 | 95.6 |
| $T_{11}$ | 176.5 | 143.1 | 101.2 |
| $T_{12}$ | 178.6 | 145.2 | 103.2 |
| $T_{13}$ | 179.5 | 146.6 | 105.4 |
| $T_{14}$ | 184.2 | 148.5 | 107.6 |
| $T_{15}$ | 180.2 | 147.1 | 106.5 |
| $T_{16}$ | 185.5 | 151.6 | 108.5 |
| **S Ed** | **0.9** | **2.1** | **1.9** |
| **CD (P= 0.05)** | **1.8** | **4.3** | **3.8** |

**Table: 27. Effect of BL, BA and NAA on the Yield and yield parameters of maize under pot culture**

| **Treatment** | **Cob length cm** | **Number of grains cob$^{-1}$** | **Yield g plant$^{-1}$** | **Harvest Index** |
|---|---|---|---|---|
| **$T_1$** | 10.4 | 262 | 61.8 | 0.421 |
| **$T_2$** | 11.7 | 278 | 64.4 | 0.425 |
| **$T_3$** | 11.9 | 282 | 65.3 | 0.436 |
| **$T_4$** | 10.6 | 267 | 62.3 | 0.434 |
| **$T_5$** | 10.7 | 265 | 63.1 | 0.431 |
| **$T_6$** | 11.8 | 279 | 64.8 | 0.434 |
| **$T_7$** | 10.4 | 261 | 61.9 | 0.420 |
| **$T_8$** | 10.5 | 264 | 62.9 | 0.420 |
| **$T_9$** | 11.8 | 278 | 63.6 | 0.421 |
| **$T_{10}$** | 11.9 | 284 | 64.3 | 0.425 |
| **$T_{11}$** | 12.0 | 287 | 66.5 | 0.437 |
| **$T_{12}$** | 12.0 | 293 | 68.4 | 0.446 |
| **$T_{13}$** | 12.3 | 291 | 67.6 | 0.439 |
| **$T_{14}$** | 13.5 | 306 | 73.3 | 0.467 |
| **$T_{15}$** | 13.3 | 295 | 69.3 | 0.419 |
| **$T_{16}$** | 14.4 | 315 | 79.5 | 0.498 |
| **S Ed** | **0.3** | **3.8** | **3.7** | **0.006** |
| **CD (P= 0.05)** | **0.6** | **7.7** | **7.5** | **0.012** |

**Table: 28. Effect of brassinolide on the yield and yield parameters of rice**

| Treatment | Plant height (cm) | Leaf area index | Number of tillers plant$^{-1}$ | Number of productive tillers plant$^{-1}$ | 1000 grain weight (g) | Grains panicle$^{-1}$ | Grain yield (q ha$^{-1}$) | Straw yield (q ha$^{-1}$) | Harvest index | BC ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 66.5 | 4.34 | 12.6 | 8.3 | 14.2 | 184 | 35.60 | 33.06 | 0.479 | 1.64 |
| **$T_2$ 0.01ppm BL** | 67.4 | 4.52 | 13.1 | 8.9 | 14.4 | 189 | 36.00 | 32.61 | 0.489 | 1.72 |
| **$T_3$ 0.05ppm BL** | 69.6 | 4.74 | 14.1 | 8.7 | 14.5 | 206 | 38.74 | 31.27 | 0.516 | 1.83 |
| **$T_4$ 0.10ppm BL** | 71.3 | 4.75 | 14.6 | 9.2 | 14.6 | 209 | 39.30 | 31.45 | 0.521 | 1.91 |
| **$T_5$ 0.20ppm BL** | 77.4 | 4.95 | 14.9 | 9.4 | 14.8 | 221 | 40.11 | 30.08 | 0.528 | 2.02 |
| **S Ed** | **2.1** | **0.04** | **0.2** | **0.3** | **0.2** | **35** | **0.86** | **0.84** | **0.019** | **-** |
| **CD at (P=0.05)** | **4.4** | **0.09** | **0.5** | **0.6** | **0.4** | **75** | **1.82** | **1.58** | **0.039** | **-** |

**Table: 29. Effect of brassinolide on the yield and yield parameters of maize**

| Treatment | Plant height (cm) | Leaf area index | Cob length (cm) | Number of grains per cob | 100 seed Weight (g) | Grain yield (q ha$^{-1}$) | Stover yield (q ha$^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|---|---|
| **T$_1$** Control | 191.7 | 4.29 | 14.21 | 308 | 21.86 | 33.50 | 82.00 | 0.321 | 2.12 |
| **T$_2$** 0.01ppm BL | 208.2 | 4.51 | 14.33 | 318 | 22.18 | 35.25 | 82.41 | 0.329 | 2.13 |
| **T$_3$** 0.05ppm BL | 214.9 | 4.81 | 14.52 | 324 | 22.30 | 36.60 | 84.59 | 0.343 | 2.25 |
| **T$_4$** 0.10ppm BL | 222.9 | 5.02 | 14.78 | 366 | 22.50 | 37.23 | 85.23 | 0.362 | 2.33 |
| **T$_5$** 0.20ppm BL | 218.9 | 4.91 | 14.59 | 343 | 22.45 | 37.04 | 84.98 | 0.353 | 2.27 |
| **S Ed** | **2.1** | **0.05** | **0.02** | **3.1** | **0.02** | **0.25** | **0.31** | **0.003** | - |
| **CD (P = 0.05)** | **4.4** | **0.11** | **0.05** | **6.7** | **0.03** | **0.53** | **0.67** | **0.006** | - |

**Table: 30. Effect of brassinolide on the yield and yield parameters of sorghum**

| Treatment | Plant height (cm) | Leaf area index | 1000 grain weight (g) | Grain yield (q $ha^{-1}$) | Stover yield (q $ha^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 176.5 | 3.74 | 10.7 | 34.97 | 84.21 | 0.293 | 1.54 |
| **$T_2$ 0.01ppm BL** | 182.2 | 3.95 | 10.8 | 36.08 | 85.60 | 0.297 | 1.65 |
| **$T_3$ 0.05ppm BL** | 185.5 | 4.11 | 10.9 | 36.64 | 86.05 | 0.299 | 1.69 |
| **$T_4$ 0.10ppm BL** | 196.7 | 4.39 | 11.5 | 38.33 | 88.80 | 0.302 | 1.77 |
| **$T_5$ 0.20ppm BL** | 189.0 | 4.30 | 10.9 | 37.39 | 87.26 | 0.300 | 1.72 |
| **S Ed** | **3.6** | **0.13** | **0.4** | **0.27** | **0.60** | **0.003** | **-** |
| **CD at (P=0.05)** | **7.7** | **0.29** | **0.8** | **0.58** | **1.28** | **0.006** | **-** |

**Table: 31. Effect of brassinolide on the yield and yield parameters of ragi**

| Treatment | Plant height (cm) | Leaf area index | Number of tillers | Number of panicle plant$^{-1}$ | Seed weight (g Panicle$^{-1}$) | Yield (q ha$^{-1}$) | TDMP (g plant$^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 107.0 | 1.84 | 3.2 | 4.5 | 11.44 | 18.62 | 92.96 | 0.326 | 1.42 |
| **$T_2$ 0.01ppm BL** | 115.4 | 2.16 | 3.6 | 5.0 | 13.22 | 19.98 | 96.38 | 0.337 | 1.48 |
| **$T_3$ 0.05ppm BL** | 108.8 | 2.26 | 3.9 | 5.3 | 14.20 | 20.14 | 98.70 | 0.343 | 1.53 |
| **$T_4$ 0.10ppm BL** | 122.8 | 2.59 | 4.3 | 6.7 | 17.33 | 21.66 | 109.68 | 0.359 | 1.58 |
| **$T_5$ 0.20ppm BL** | 120.6 | 2.43 | 4.1 | 5.9 | 15.90 | 20.67 | 101.94 | 0.358 | 1.54 |
| **S Ed** | **4.1** | **0.09** | **0.2** | **0.3** | **0.42** | **0.31** | **3.54** | **0.007** | **-** |
| **CD at (P=0.05)** | **8.7** | **0.21** | **0.5** | **0.6** | **0.90** | **0.63** | **7.56** | **0.015** | **-** |

**Table: 32. Effect of brassinolide on the yield and yield parameters of green gram**

| Treatment | Plant height (cm) | Leaf area index | Number of pods plant$^{-1}$ | Pod weight (g plant$^{-1}$) | Yield (q ha$^{-1}$) | TDMP (g plant$^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 45.3 | 2.80 | 22.3 | 8.044 | 11.37 | 23.00 | 0.330 | 1.97 |
| **$T_2$ 0.01ppm BL** | 47.6 | 2.92 | 26.5 | 8.532 | 12.15 | 25.36 | 0.340 | 2.06 |
| **$T_3$ 0.05ppm BL** | 49.9 | 3.07 | 29.1 | 9.375 | 12.50 | 26.67 | 0.347 | 2.12 |
| **$T_4$ 0.10ppm BL** | 52.9 | 3.29 | 32.0 | 11.080 | 13.21 | 30.53 | 0.361 | 2.23 |
| **$T_5$ 0.20ppm BL** | 50.3 | 3.17 | 31.2 | 10.217 | 13.04 | 29.75 | 0.343 | 2.19 |
| **S Ed** | **1.9** | **0.08** | **2.4** | **0.109** | **0.21** | **0.41** | **0.008** | **-** |
| **CD at (P=0.05)** | **4.2** | **0.16** | **5.2** | **0.233** | **0.46** | **0.87** | **0.018** | **-** |

**Table: 33. Effect of brassinolide on the yield and yield parameters of black gram**

| Treatment | Plant height (cm) | Leaf area index | Number of pods plant$^{-1}$ | TDMP (g) | Pod weight (g plant$^{-1}$) | Yield (q ha$^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 55.5 | 3.33 | 27.3 | 61.518 | 20.717 | 7.20 | 0.38 | 1.95 |
| **$T_2$ 0.01ppm BL** | 57.5 | 3.60 | 29.1 | 50.246 | 21.325 | 7.55 | 0.43 | 2.01 |
| **$T_3$ 0.05ppm BL** | 59.1 | 3.84 | 30.6 | 48.258 | 21.566 | 7.89 | 0.46 | 2.21 |
| **$T_4$ 0.10ppm BL** | 62.8 | 4.00 | 33.1 | 47.561 | 22.998 | 8.46 | 0.51 | 2.36 |
| **$T_5$ 0.20ppm BL** | 60.8 | 3.96 | 31.5 | 47.984 | 22.383 | 8.24 | 0.48 | 2.32 |
| **S Ed** | **1.9** | **0.28** | **0.95** | **1.023** | **0.222** | **0.22** | **0.02** | **-** |
| **CD at (P=0.05)** | **4.2** | **0.59** | **1.75** | **2.461** | **0.174** | **0.46** | **0.05** | **-** |

**Table: 34. Effect of brassinolide on the yield and yield parameters of groundnut**

| Treatment | Plant height (cm) | Leaf area index | Number of pegs plant$^{-1}$ | Number of pods plant$^{-1}$ | Pod weight (g plant$^{-1}$) | Kernel weight (g plant$^{-1}$) | Pod yield (q ha$^{-1}$) | TDMP (g plant$^{-1}$) | Harvest index | BC ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| $\mathbf{T_1}$ Control | 36.6 | 3.5 | 19.9 | 16.8 | 12.60 | 8.108 | 22.86 | 45.50 | 0.252 | 1.71 |
| $\mathbf{T_2}$ 0.01ppm BL | 37.9 | 3.6 | 21.1 | 18.4 | 13.40 | 8.407 | 24.00 | 51.04 | 0.264 | 1.75 |
| $\mathbf{T_3}$ 0.05ppm BL | 38.7 | 3.6 | 21.9 | 19.8 | 13.65 | 8.614 | 24.20 | 54.44 | 0.280 | 1.82 |
| $\mathbf{T_4}$ 0.10ppm BL | 39.5 | 3.7 | 22.7 | 20.8 | 14.40 | 8.927 | 24.89 | 58.44 | 0.312 | 1.95 |
| $\mathbf{T_5}$ 0.20ppm BL | 40.8 | 3.9 | 24.1 | 21.7 | 14.95 | 9.149 | 25.91 | 61.46 | 0.336 | 2.08 |
| **S Ed** | **0.39** | **0.02** | **0.41** | **0.39** | **0.31** | **0.004** | **0.62** | **2.96** | **0.008** | **-** |
| **CD at (P=0.05)** | **0.84** | **0.05** | **0.87** | **0.84** | **0.66** | **0.008** | **1.32** | **6.31** | **0.016** | **-** |

**Table: 35. Effect of brassinolide on the yield and yield parameters of sunflower**

| Treatment | Plant height (cm) | Leaf area index | Capitulam diameter cm | Number of seeds per capitulam | 100 seed weight (g) | Per cent grain filling | Grain yield (q ha$^{-1}$) | TDMP (g plant$^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| **$T_1$** Control | 172.1 | 1.86 | 11.5 | 355.9 | 4.06 | 84.8 | 12.40 | 181.2 | 0.239 | 1.55 |
| **$T_2$** 0.01ppm BL | 178.9 | 2.07 | 12.1 | 398.3 | 4.13 | 86.5 | 12.73 | 198.8 | 0.248 | 1.58 |
| **$T_3$** 0.05ppm BL | 180.4 | 2.15 | 12.2 | 416.0 | 4.16 | 87.5 | 13.00 | 211.3 | 0.246 | 1.60 |
| **$T_4$** 0.10ppm BL | 186.6 | 2.30 | 12.6 | 432.4 | 4.24 | 89.1 | 13.43 | 225.9 | 0.243 | 1.70 |
| **$T_5$** 0.20ppm BL | 180.6 | 2.20 | 11.8 | 423.8 | 4.18 | 88.6 | 12.81 | 216.6 | 0.245 | 1.62 |
| **S Ed** | **3.3** | **0.07** | **0.15** | **7.84** | **0.07** | **0.3** | **0.99** | **4.84** | **NS** | - |
| **CD at (P =0.05)** | **7.0** | **0.16** | **0.31** | **16.70** | **0.14** | **0.7** | **2.11** | **10.3** | **-** | - |

**Table: 36. Effect of brassinolide on the yield and yield parameters of cotton**

| Treatment | Plant height (cm) | Leaf area index | Number of sympodia plant$^{-1}$ | Number of bolls plant$^{-1}$ | Boll weight (g) | Per cent boll set | Kapas yield (q ha$^{-1}$) | TDMP (g plant$^{-1}$) | HI | BC ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 113.4 | 2.51 | 16.3 | 17.2 | 3.53 | 33.2 | 17.05 | 222 | 0.255 | 1.86 |
| **$T_2$ 0.01ppm BL** | 113.8 | 2.57 | 17.5 | 17.7 | 3.57 | 36.7 | 17.26 | 215 | 0.269 | 1.92 |
| **$T_3$ 0.05ppm BL** | 117.5 | 2.70 | 17.8 | 18.0 | 3.59 | 37.5 | 17.48 | 239 | 0.265 | 2.04 |
| **$T_4$ 0.10ppm BL** | 125.6 | 2.90 | 19.4 | 19.4 | 3.73 | 41.4 | 18.61 | 258 | 0.289 | 2.29 |
| **$T_5$ 0.20ppm BL** | 123.1 | 2.81 | 18.8 | 18.3 | 3.68 | 39.4 | 17.76 | 247 | 0.279 | 2.18 |
| **S Ed** | **3.5** | **0.04** | **0.33** | **0.23** | **0.03** | **0.4** | **0.16** | **3.5** | **0.005** | **-** |
| **CD at (P=0.05)** | **7.4** | **0.09** | **0.71** | **0.49** | **0.07** | **0.8** | **0.35** | **7.2** | **0.010** | **-** |

**Table: 37. Effect of brassinolide on the yield and yield parameters of Banana**

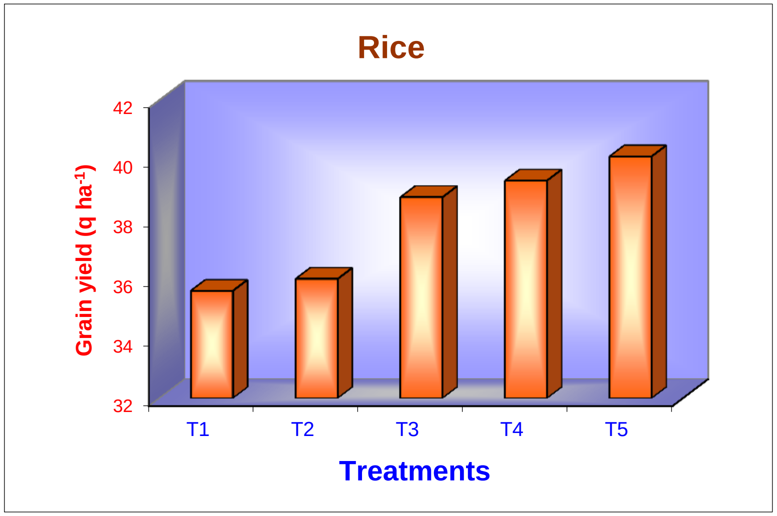
| Treatment | Pseduostem height (cm) | Leaf area index | TDMP (kg plant$^{-1}$) | Number of finger bunch$^{-1}$ | Finger weight (g) | Bunch weight (kg) | Yield (q ha$^{-1}$) | BC ratio |
|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 243.6 | 3.95 | 5.54 | 146.8 | 165.3 | 23.14 | 73.43 | 2.09 |
| **$T_2$ 0.01ppm BL** | 256.4 | 4.22 | 5.69 | 155.4 | 170.9 | 24.66 | 78.11 | 2.17 |
| **$T_3$ 0.05ppm BL** | 261.7 | 4.66 | 5.93 | 160.5 | 177.6 | 26.53 | 82.61 | 2.34 |
| **$T_4$ 0.10ppm BL** | 275.6 | 4.89 | 6.08 | 165.2 | 178.5 | 26.92 | 86.55 | 2.60 |
| **$T_5$ 0.20ppm BL** | 282.0 | 5.26 | 6.26 | 169.8 | 184.5 | 28.29 | 89.11 | 2.82 |
| **S Ed** | **5.46** | **0.10** | **0.13** | **3.13** | **6.58** | **1.20** | **0.96** | **-** |
| **CD at (P=0.05)** | **11.63** | **0.22** | **0.29** | **6.69** | **14.02** | **2.56** | **2.05** | **-** |

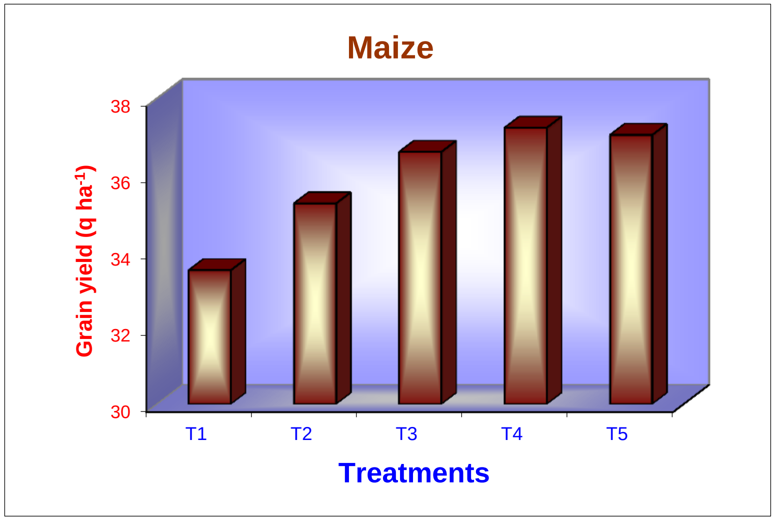
**Table: 38. Effect of brassinolide on the yield and yield parameters of grapes**

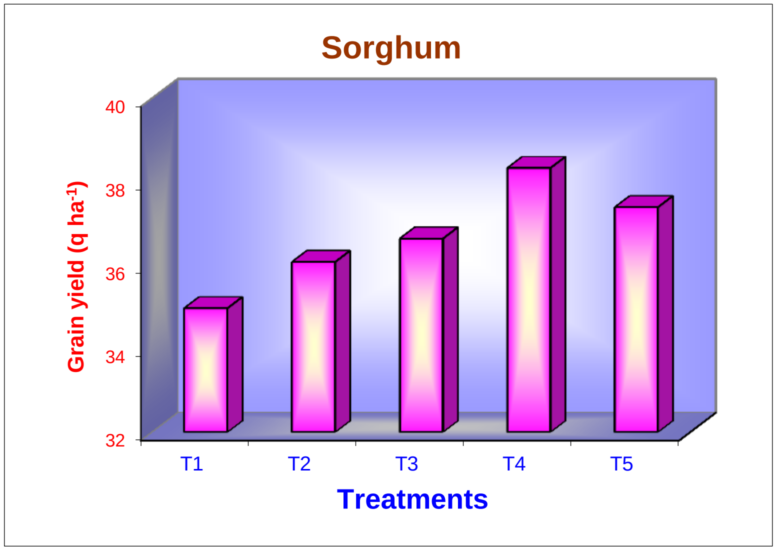
| Treatment | Number of bunch vein$^{-1}$ | Bunch weight (kg vein$^{-1}$) | 100 beery weight (g) | Yield (q ha$^{-1}$) | Titrable acidity (%) | TSS (ºBrix) | Reducing Sugars (mg g$^{-1}$) | BC ratio |
|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 12.3 | 4.43 | 199.0 | 18.53 | 1.35 | 13.5 | 13.0 | 2.56 |
| **$T_2$ 0.01ppm BL** | 12.6 | 4.59 | 216.8 | 19.33 | 1.24 | 14.3 | 13.5 | 2.94 |
| **$T_3$ 0.05ppm BL** | 13.5 | 4.93 | 236.3 | 20.37 | 1.17 | 14.6 | 13.9 | 3.10 |
| **$T_4$ 0.10ppm BL** | 15.2 | 5.15 | 269.0 | 22.40 | 0.97 | 15.7 | 14.8 | 3.27 |
| **$T_5$ 0.20ppm BL** | 13.7 | 5.05 | 258.5 | 21.07 | 0.98 | 14.8 | 14.1 | 3.15 |
| **S Ed** | **0.7** | **0.15** | **0.2** | **0.3** | **0.05** | **2.5** | **0.21** | - |
| **CD at (P=0.05)** | **1.4** | **0.32** | **0.4** | **0.6** | **0.12** | **5.0** | **0.51** | - |

**Table: 39. Effect of brassinolide on the yield and yield parameters of tomato**

| Treatment | Plant height (cm) | Leaf area index | Pre cent fruit set | Fruit weight (g) | Yield (q $ha^{-1}$) | TDMP (g $plant^{-1}$) | Harvest Index | BC ratio |
|---|---|---|---|---|---|---|---|---|
| **$T_1$ Control** | 85.8 | 1.59 | 33.1 | 28.9 | 24.8 | 60.21 | 0.41 | 2.25 |
| **$T_2$ 0.01ppm BL** | 89.3 | 1.74 | 38.1 | 36.5 | 25.1 | 75.35 | 0.45 | 2.64 |
| **$T_3$ 0.05ppm BL** | 92.8 | 2.01 | 39.4 | 41.2 | 26.5 | 86.45 | 0.49 | 2.85 |
| **$T_4$ 0.10ppm BL** | 89.5 | 1.95 | 36.7 | 35.2 | 25.5 | 85.12 | 0.43 | 2.73 |
| **$T_5$ 0.20ppm BL** | 85.7 | 1.87 | 35.3 | 33.7 | 25.4 | 80.26 | 0.42 | 2.62 |
| **S Ed** | **1.8** | **0.04** | **0.4** | **0.5** | **0.1** | **3.21** | **0.02** | **-** |
| **CD at (P=0.05)** | **3.86** | **0.09** | **1.0** | **1.1** | **0.3** | **7.02** | **0.06** | **-** |


Rice
Grain yield (q ha-1)
42
40
38
36
34
32
T1
T2
T3
T4
T5
Treatments


Maize
Grain yield (q ha-1)
38
36
34
32
30
T1
T2
T3
T4
T5
Treatments


Sorghum
Grain yield (q ha-1)
40
38
36
34
32
T1
T2
T3
T4
T5
Treatments

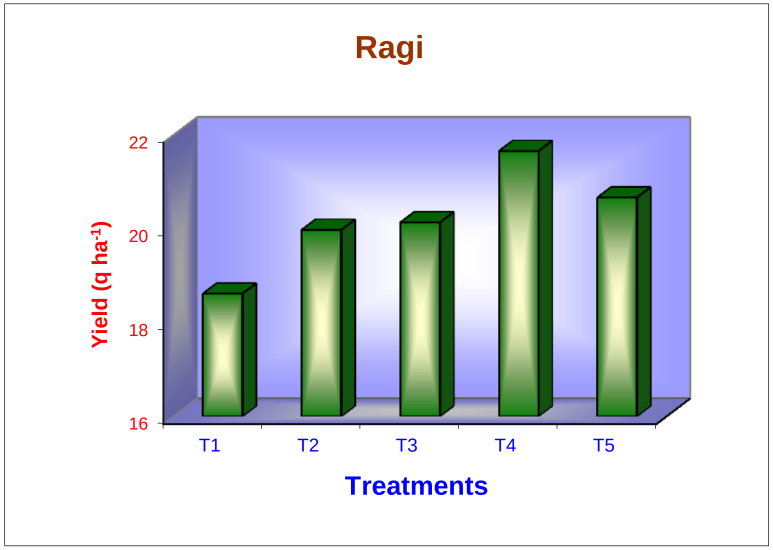

Ragi
Yield (q ha-1)
22
20
18
16
T1
T2
T3
T4
T5
Treatments

Fig. 20. Effect of brassinolide on the productivity of pulses and oil seeds

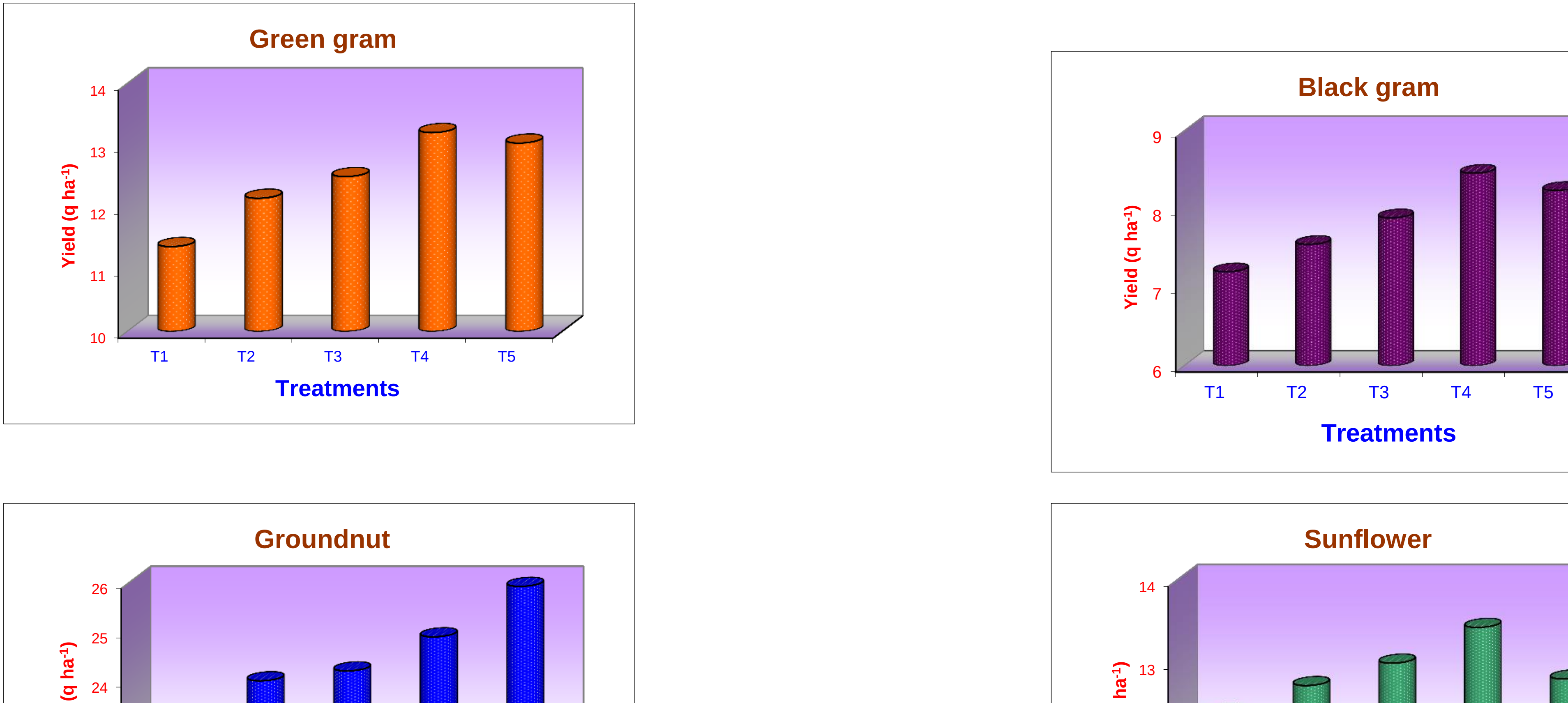


Fig. 21. Effect of brassinolide on the productivity of cotton, banana, grapes and tomato

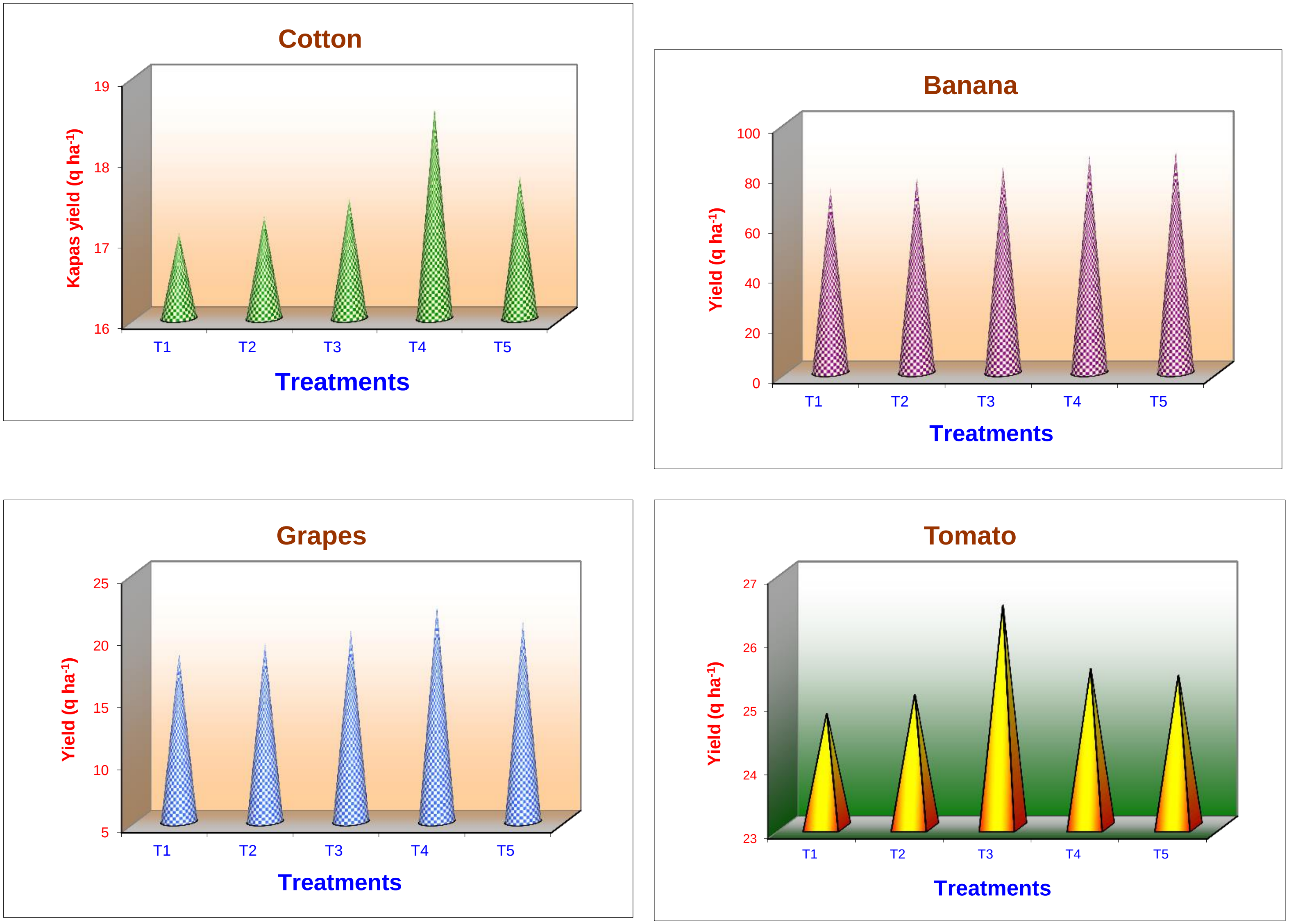
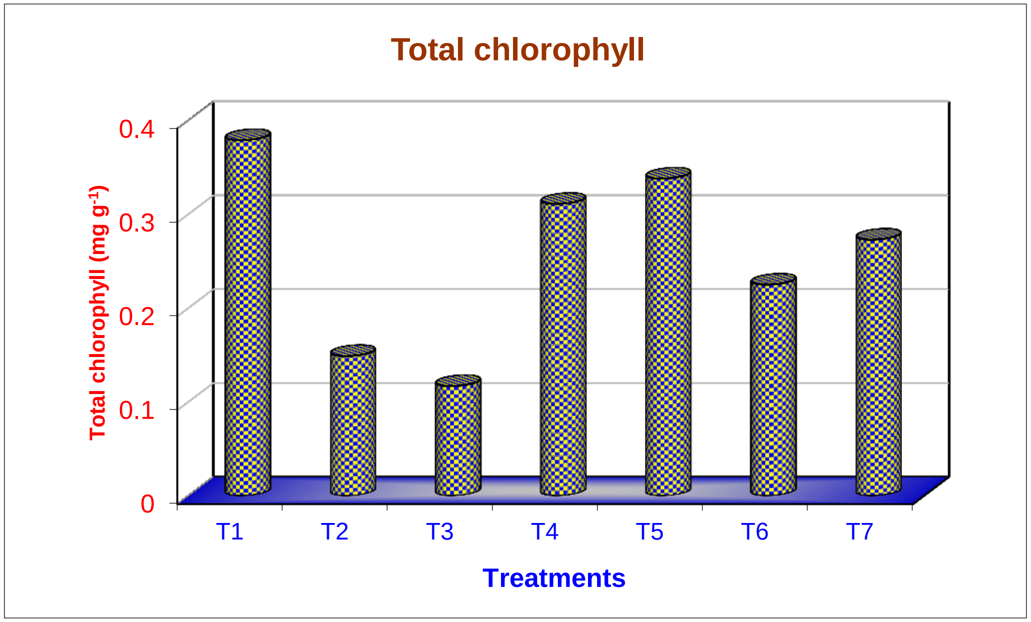


**Fig.8.** Influence of brassinolide on chlorophyll and soluble protein in maize under salinity



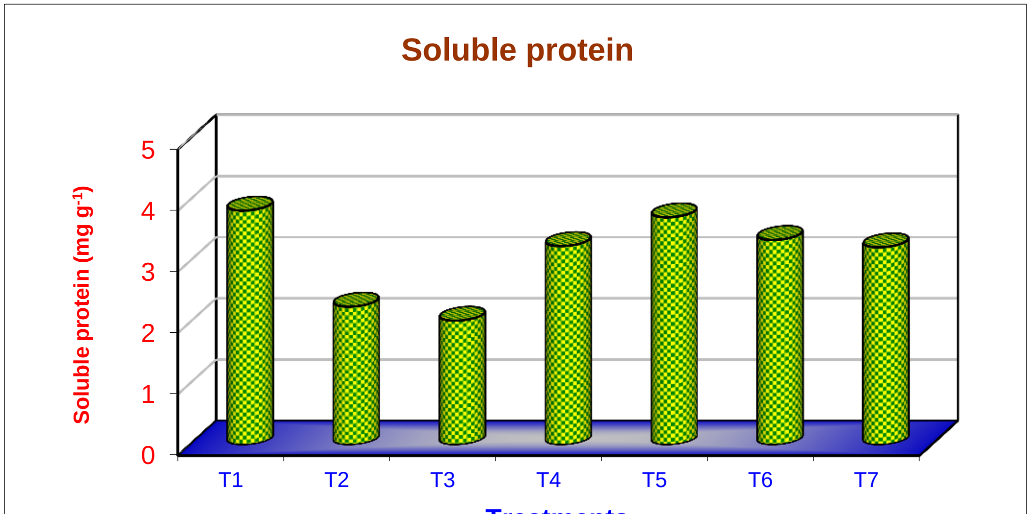
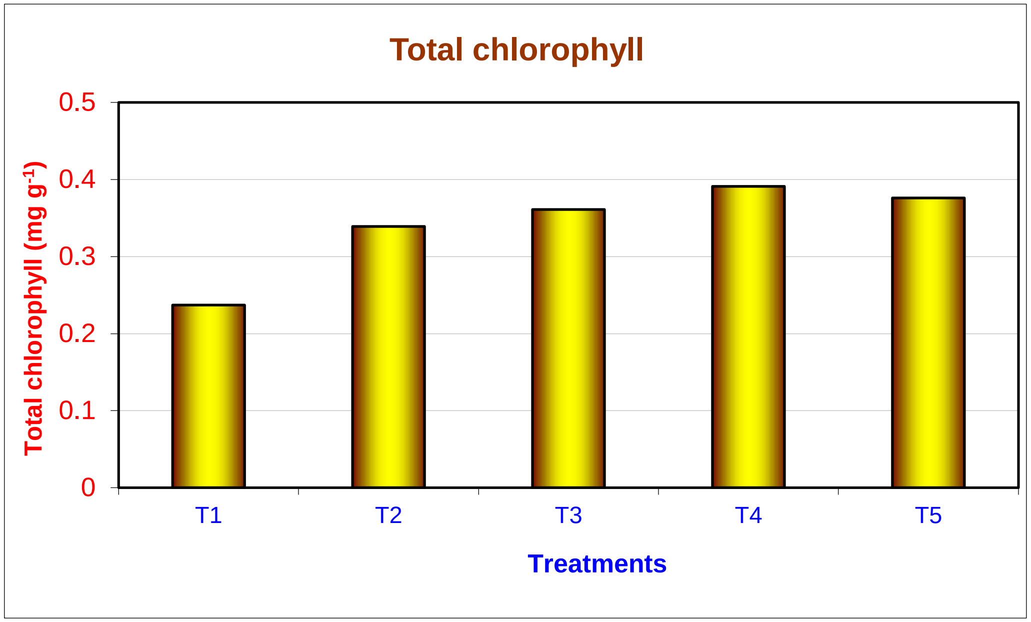


Fig.2. Influence of brassinolide on chlorophyll and soluble protein in maize under drought condition



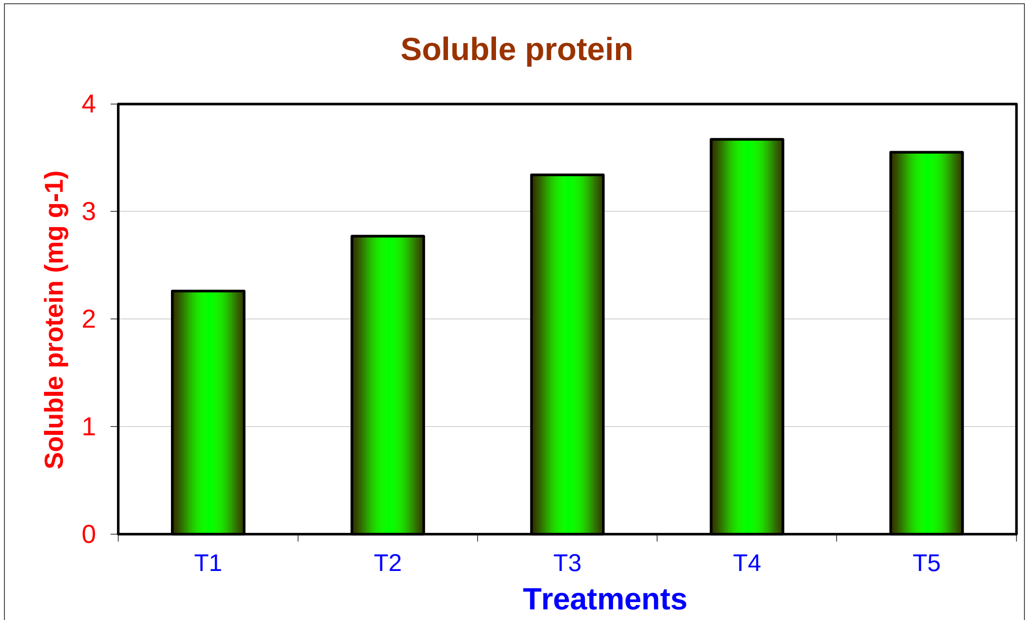


Fig.6. Influence of brassinolide on chlorophyll and soluble protein in maize under high temperature stress

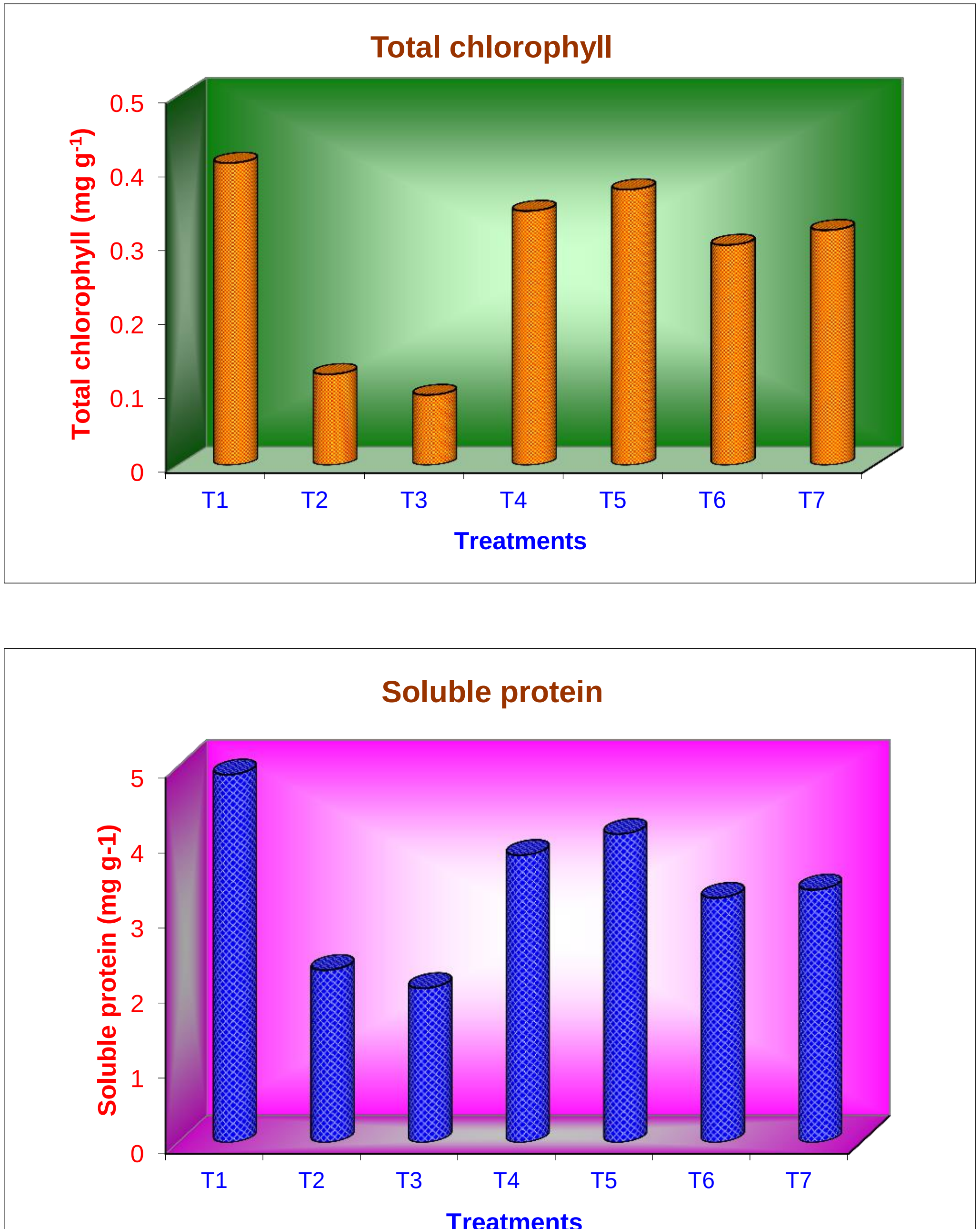
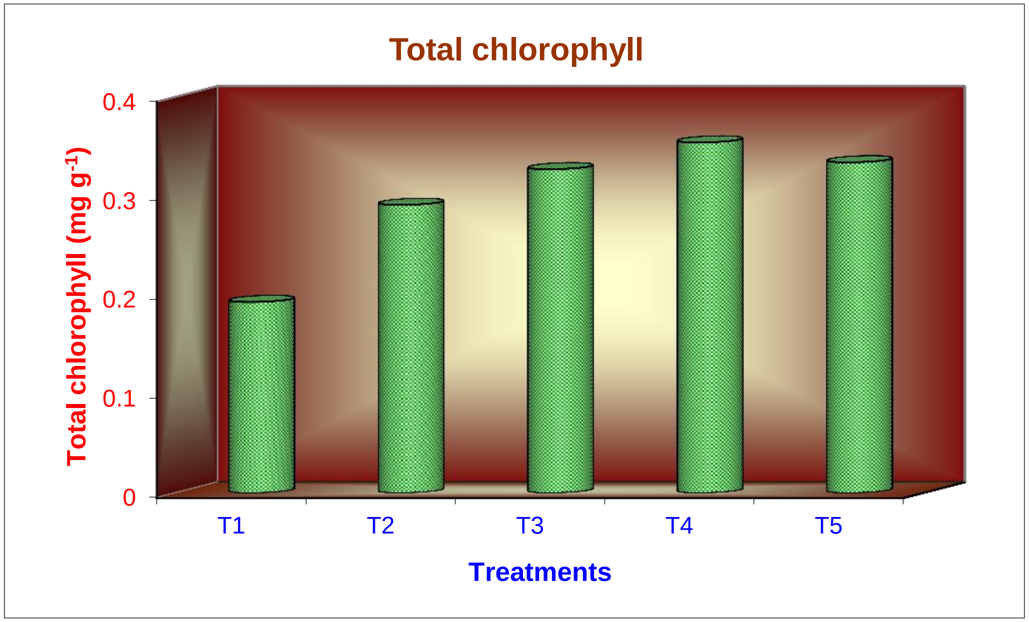


**Fig.4.** Influence of brassinolide on chlorophyll and soluble protein in maize under low temperature stress



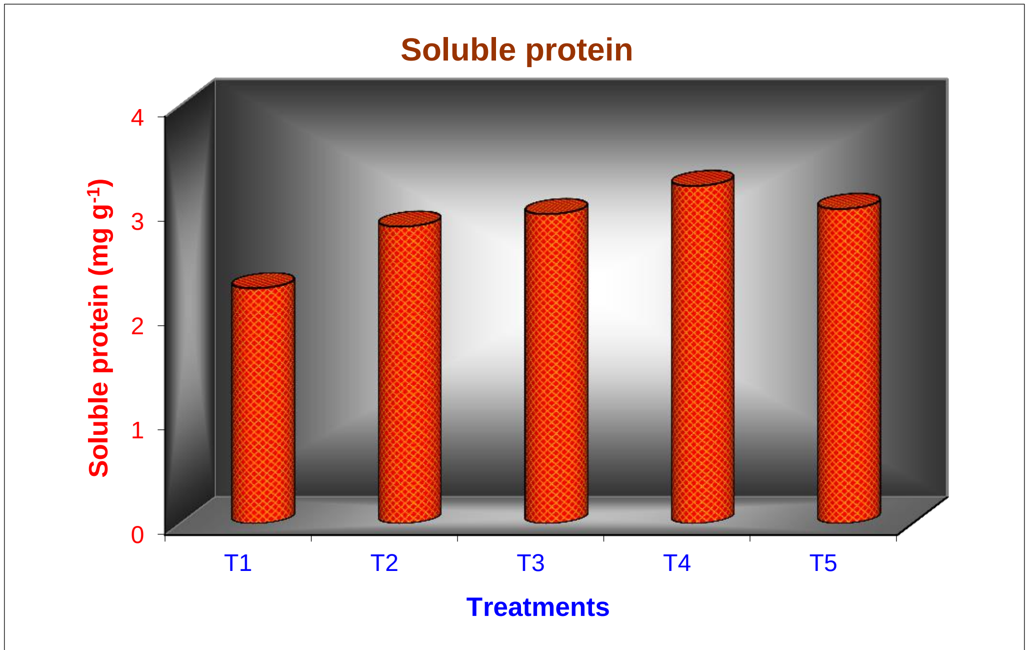


Fig.1. Effect of Brassinolide on chlorophyll, soluble protein, nitrate reductase acitvity and IAA oxidase acitvity in maize

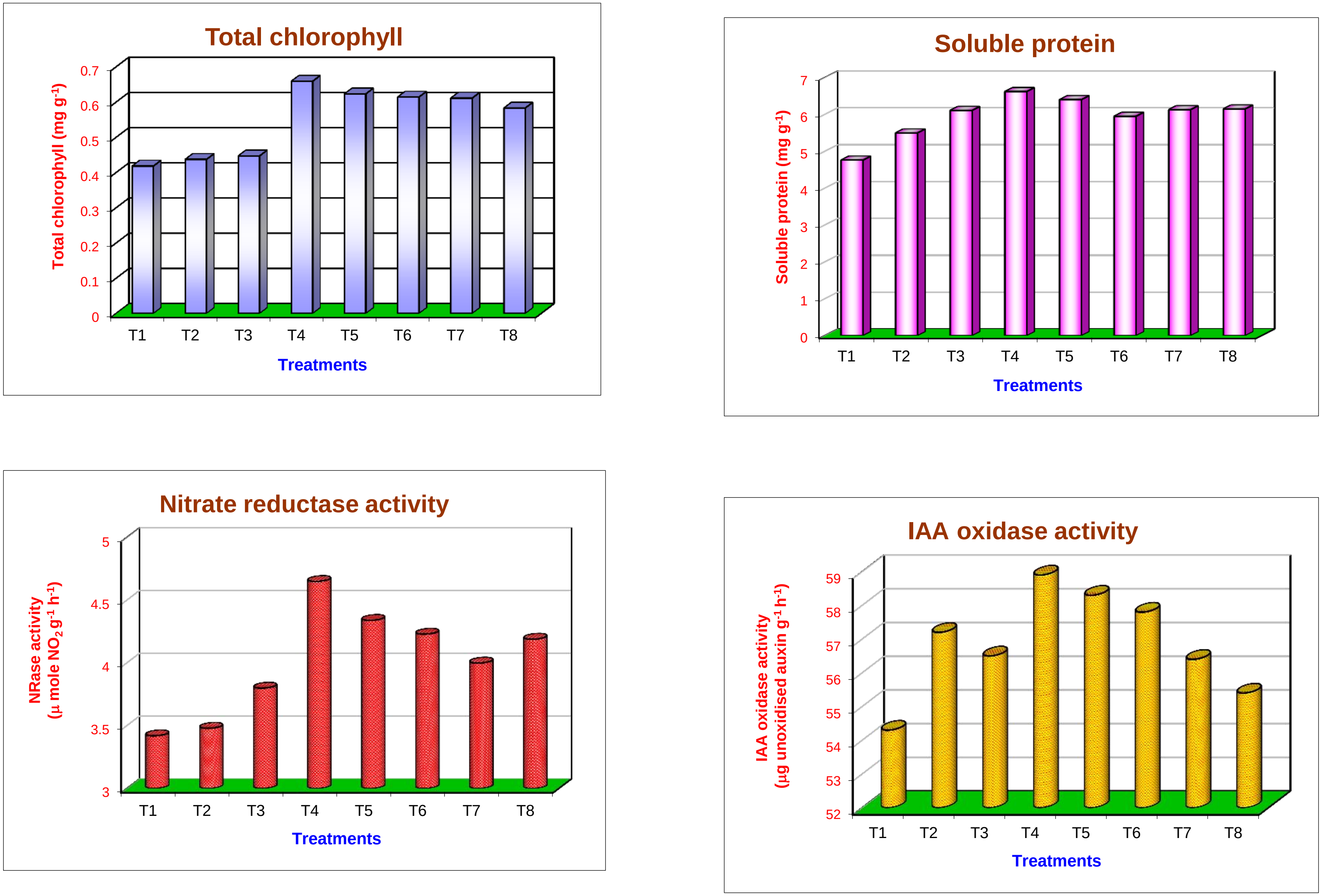


Fig.9. Effect of Brassinolide on enzyme activity in overcoming salinity stress in maize

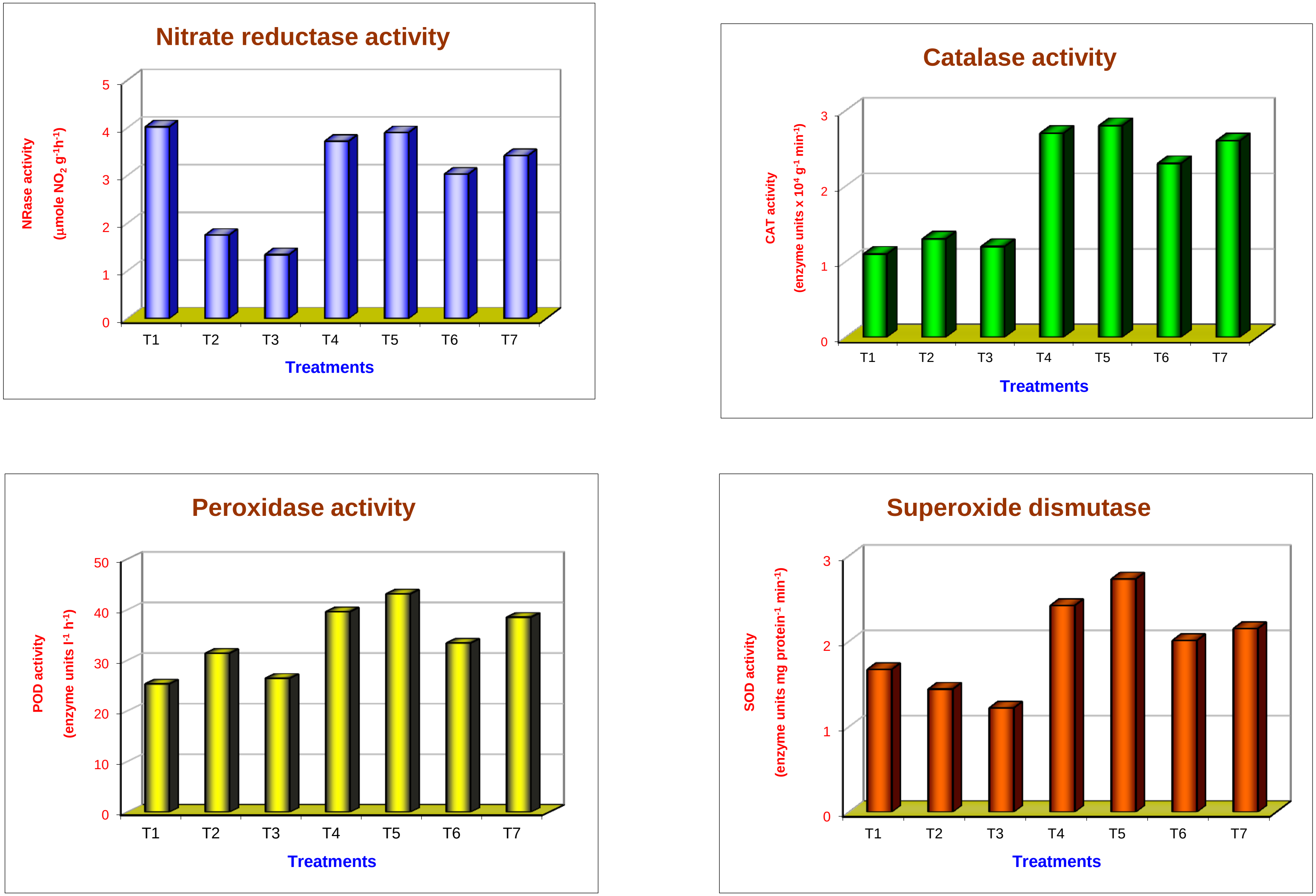


**Fig.5.** Effect of Brassinolide on enzyme activity in overcoming low temperature stress in maize

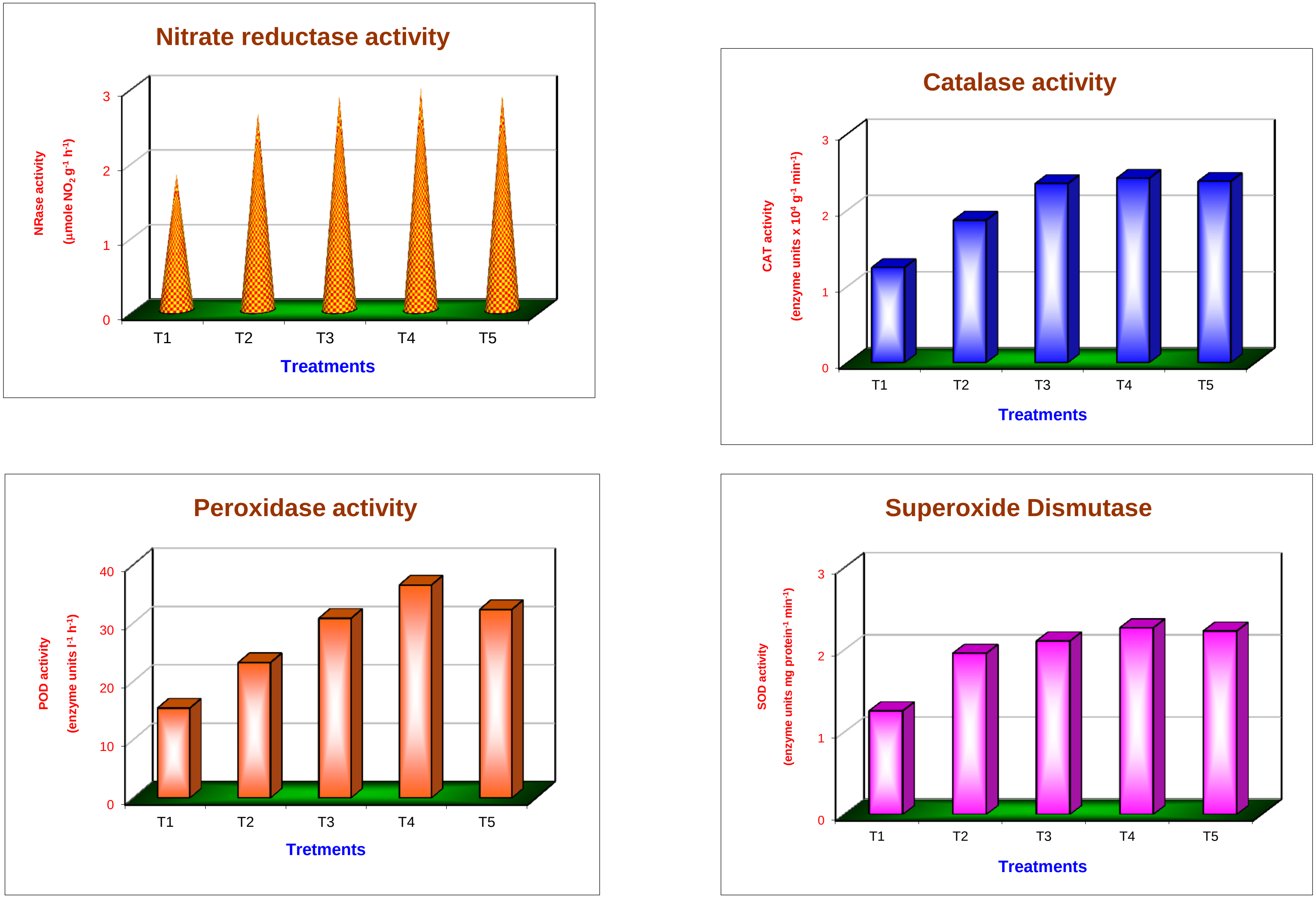


**Fig.7. Effect of Brassinolide on enzyme activity in overcoming high temperature stress in maize**

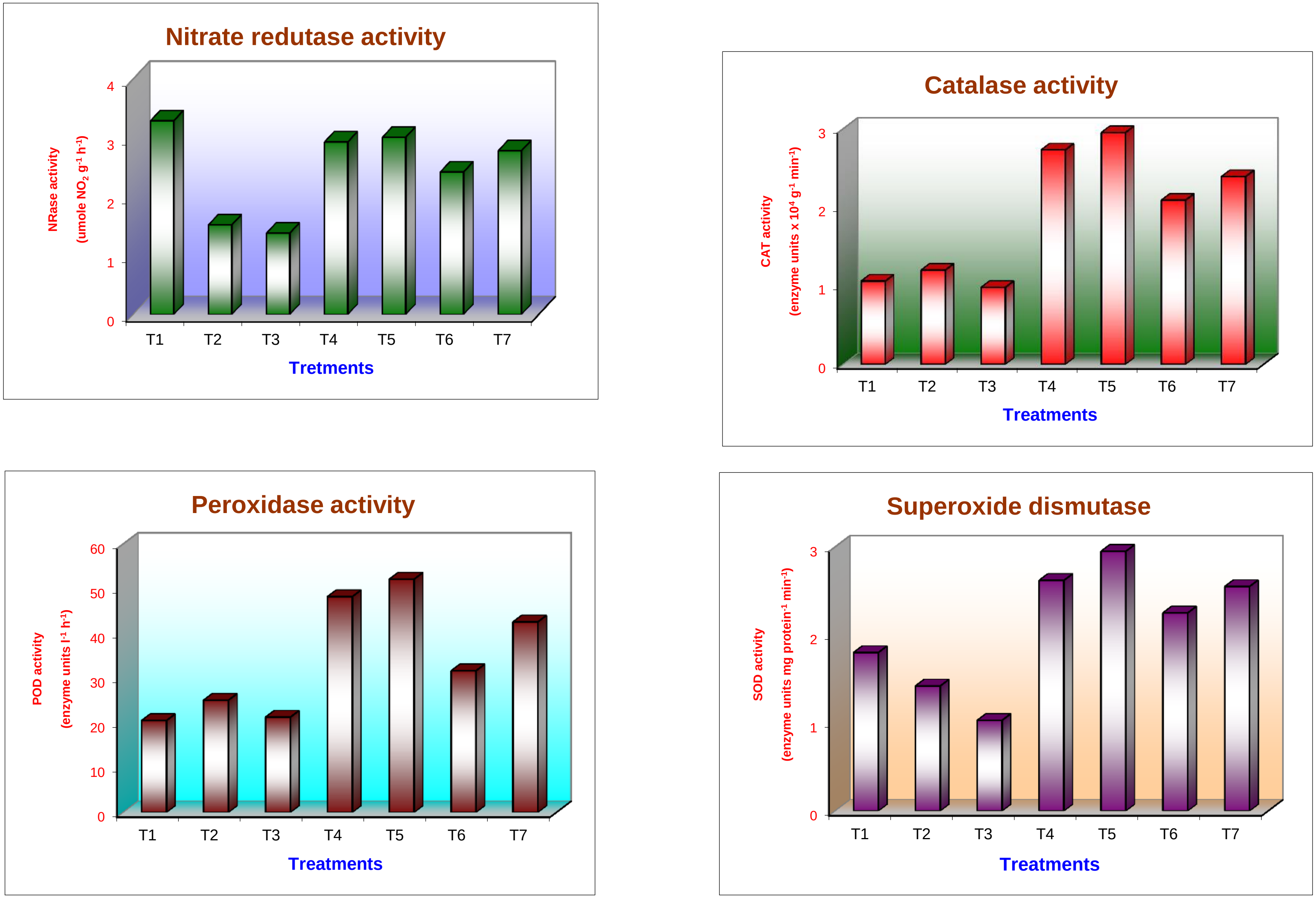


**Fig.3. Effect of brassinolide on enzyme activity in overcoming drought in maize**

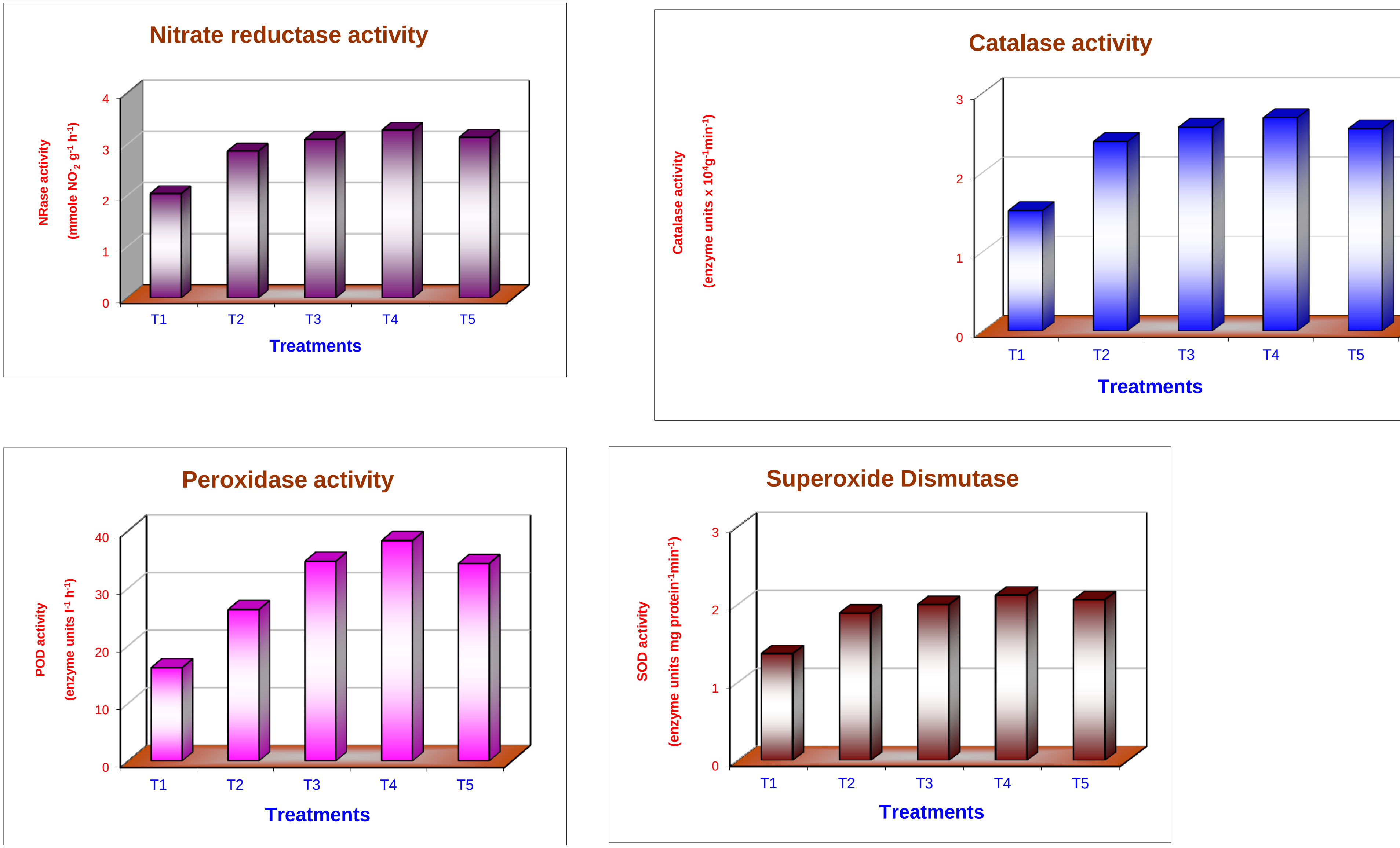


**Fig.10. Interaction effect of BL with BA and NAA on plant height in maize**

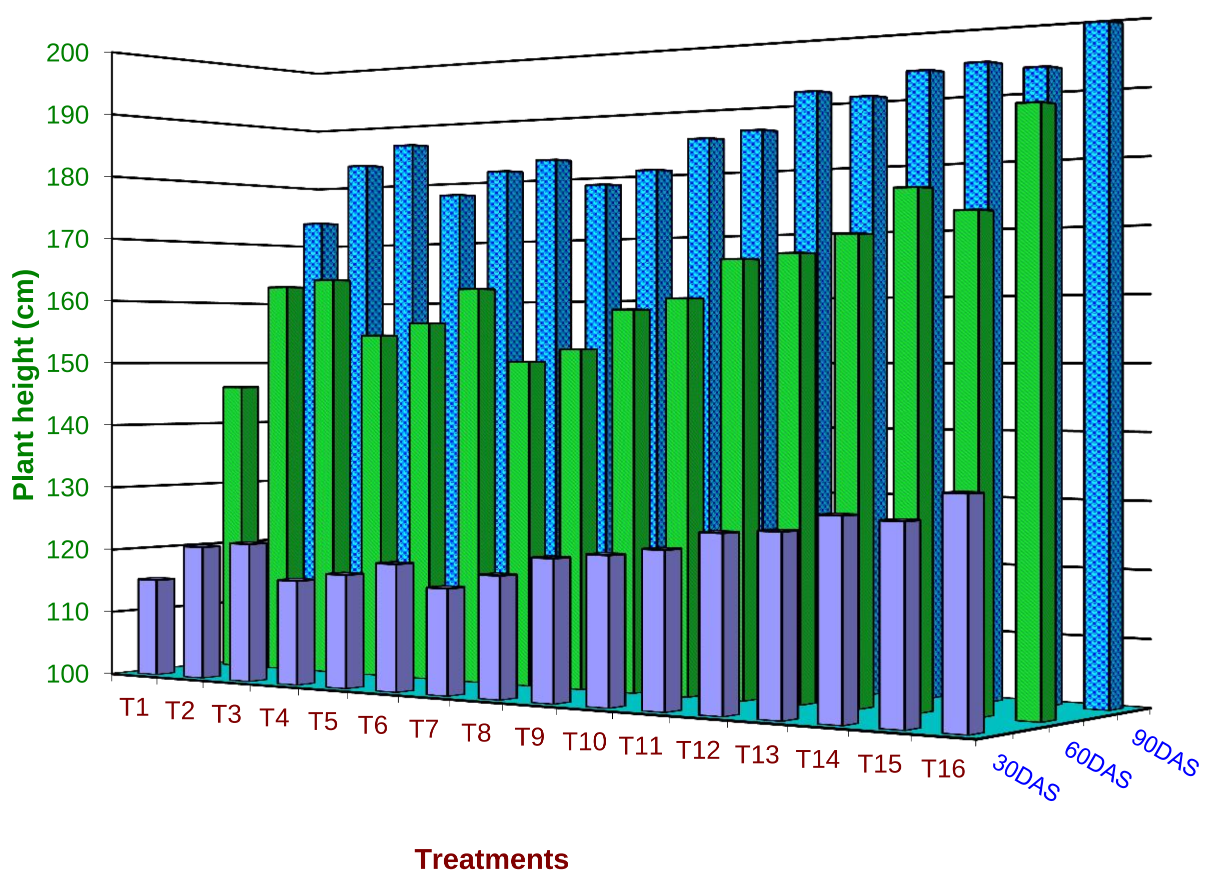


Fig. 11. Effect of BL,BA and NAA on Specific Leaf Weight of maize

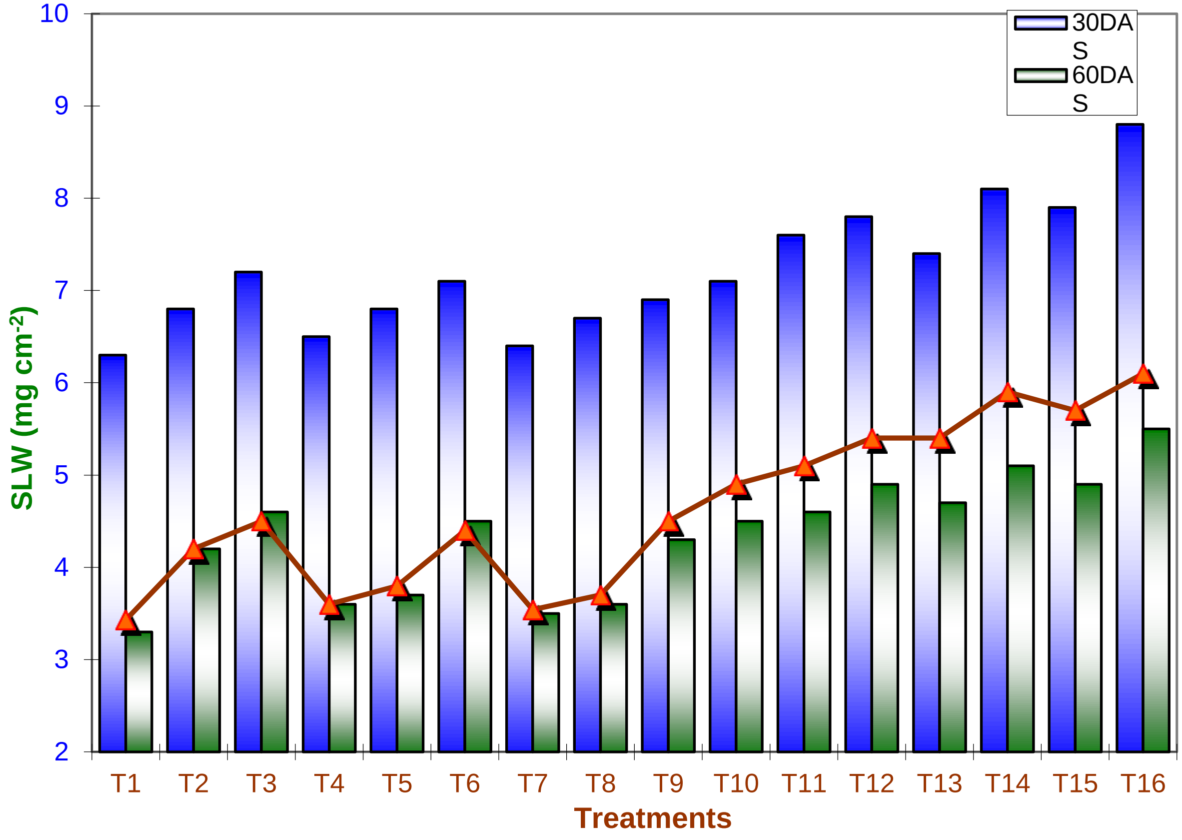


Fig.12. Interation effect of BL with BA and NAA on Specific Leaf Area of maize

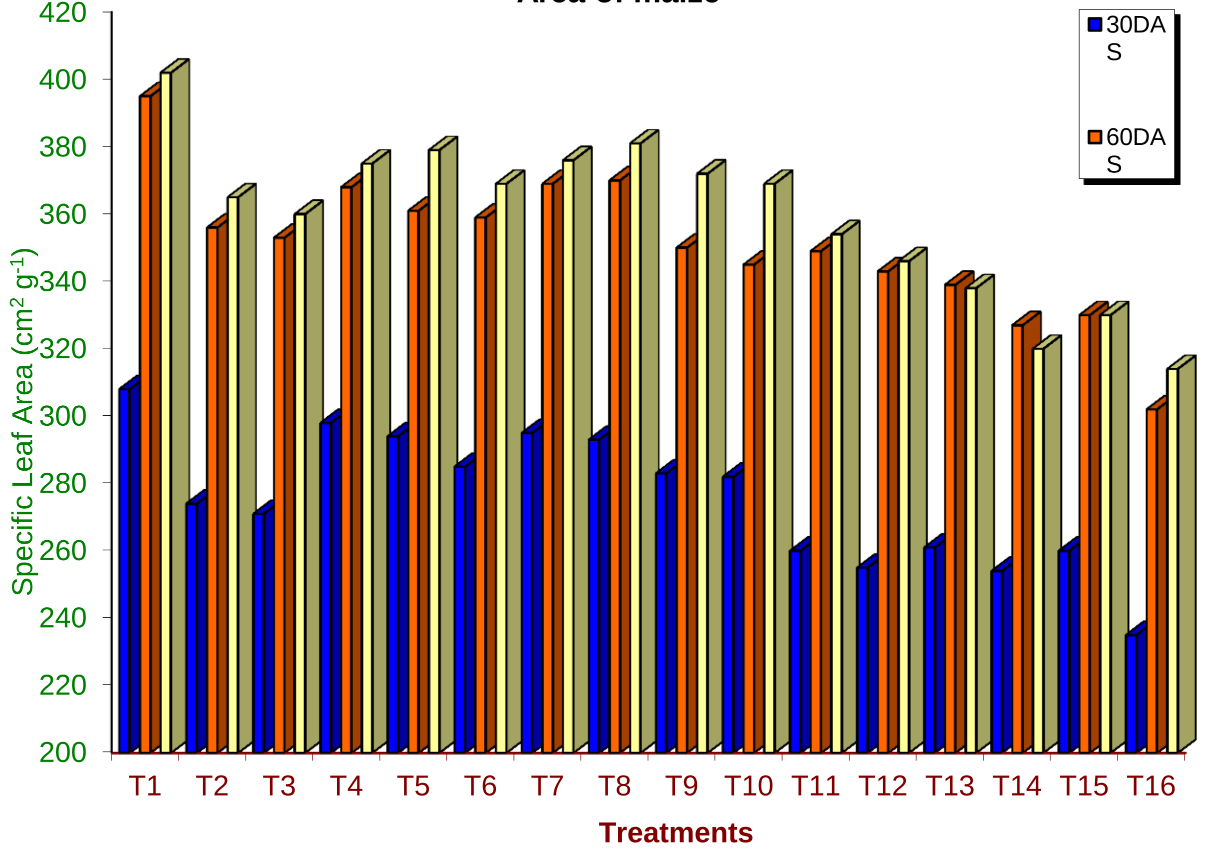
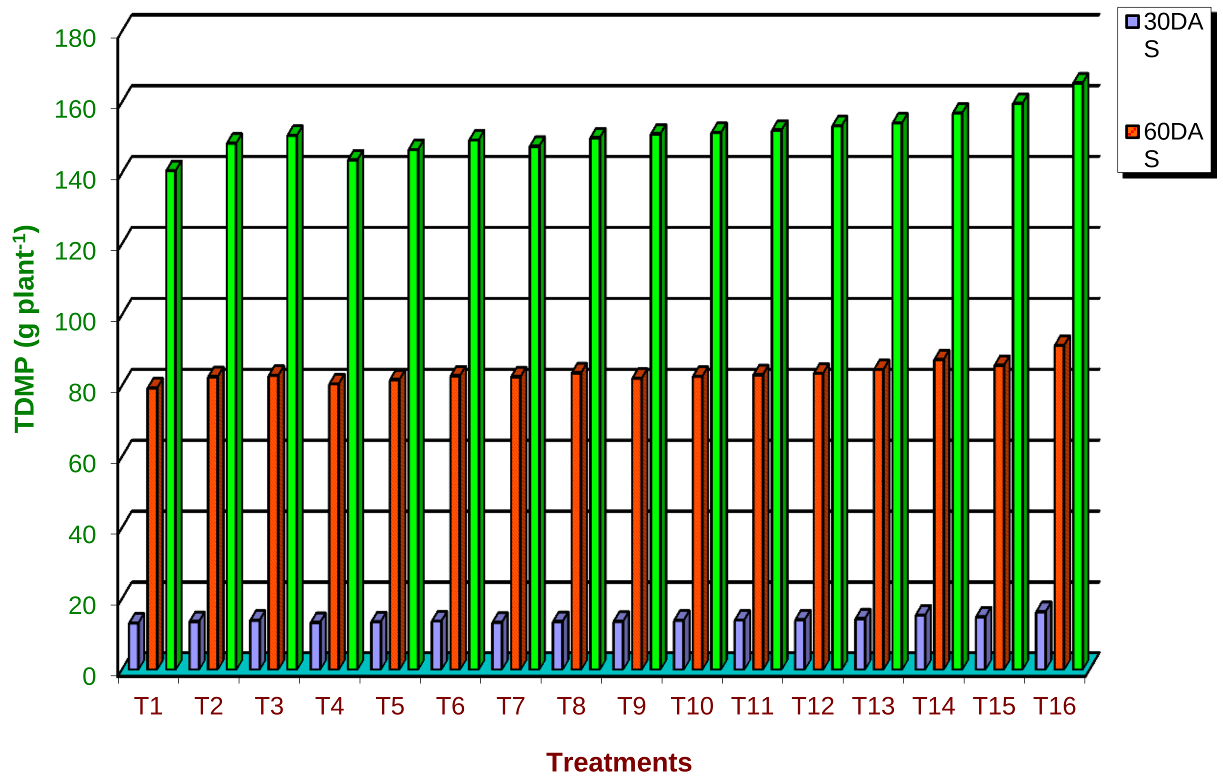


Fig.13. Interaction effect of BL with BA and NAA on Total Dry Matter Production of maize



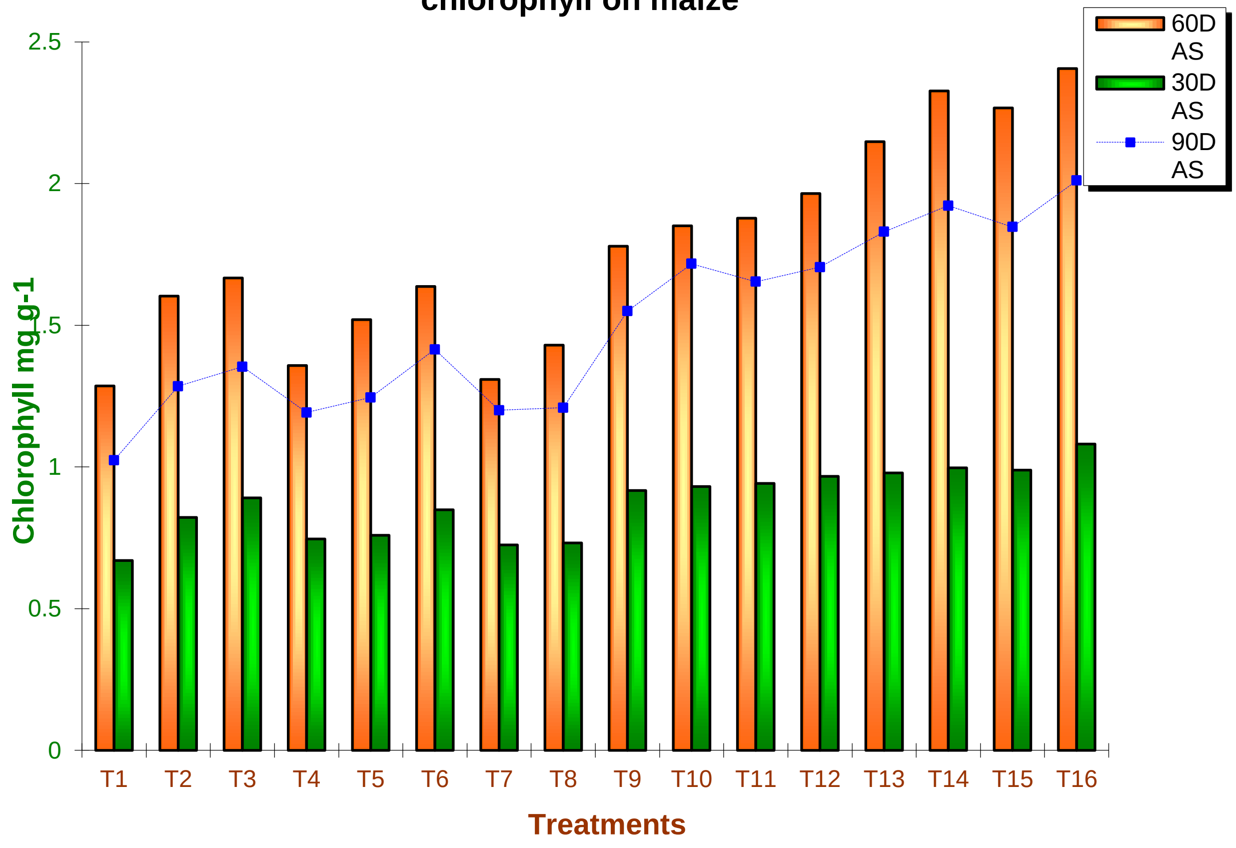


Fig.14. Interaction effect of BL with BA and NAA on total chlorophyll on maize

Fig.15. Interaction effect of BLwith BA and NAA on soluble protein in maize

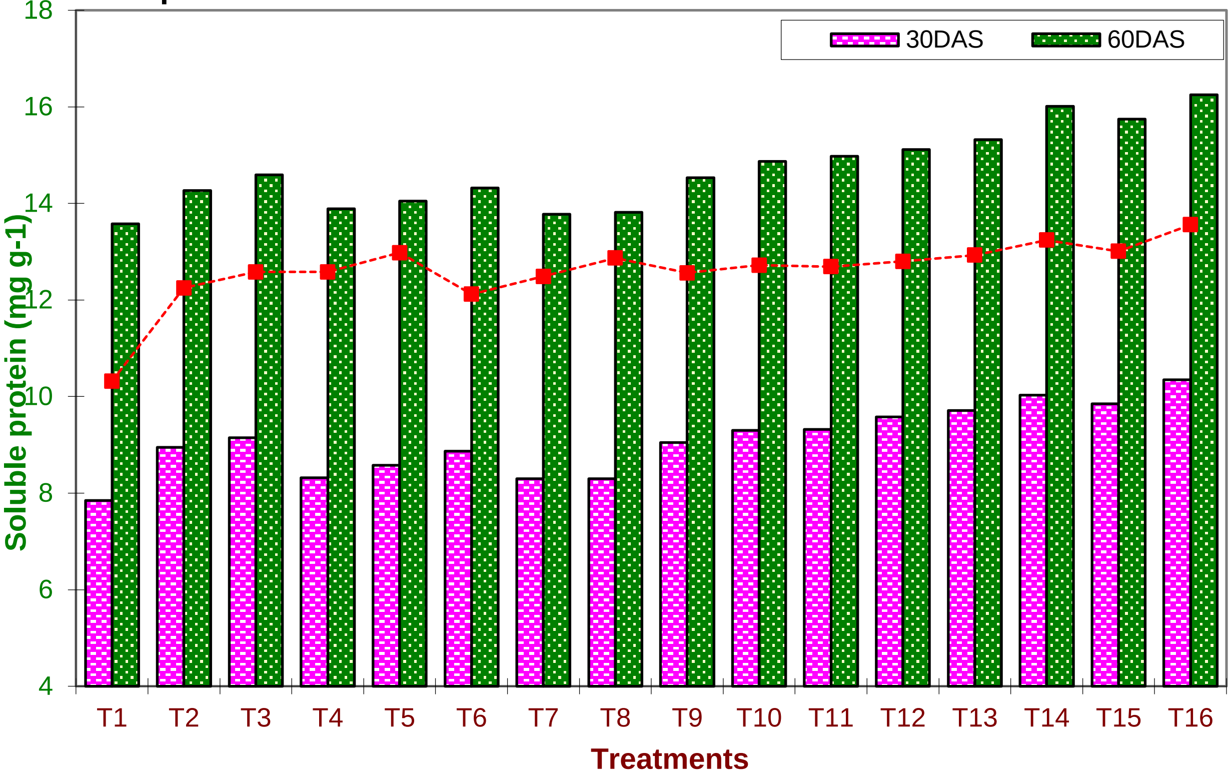
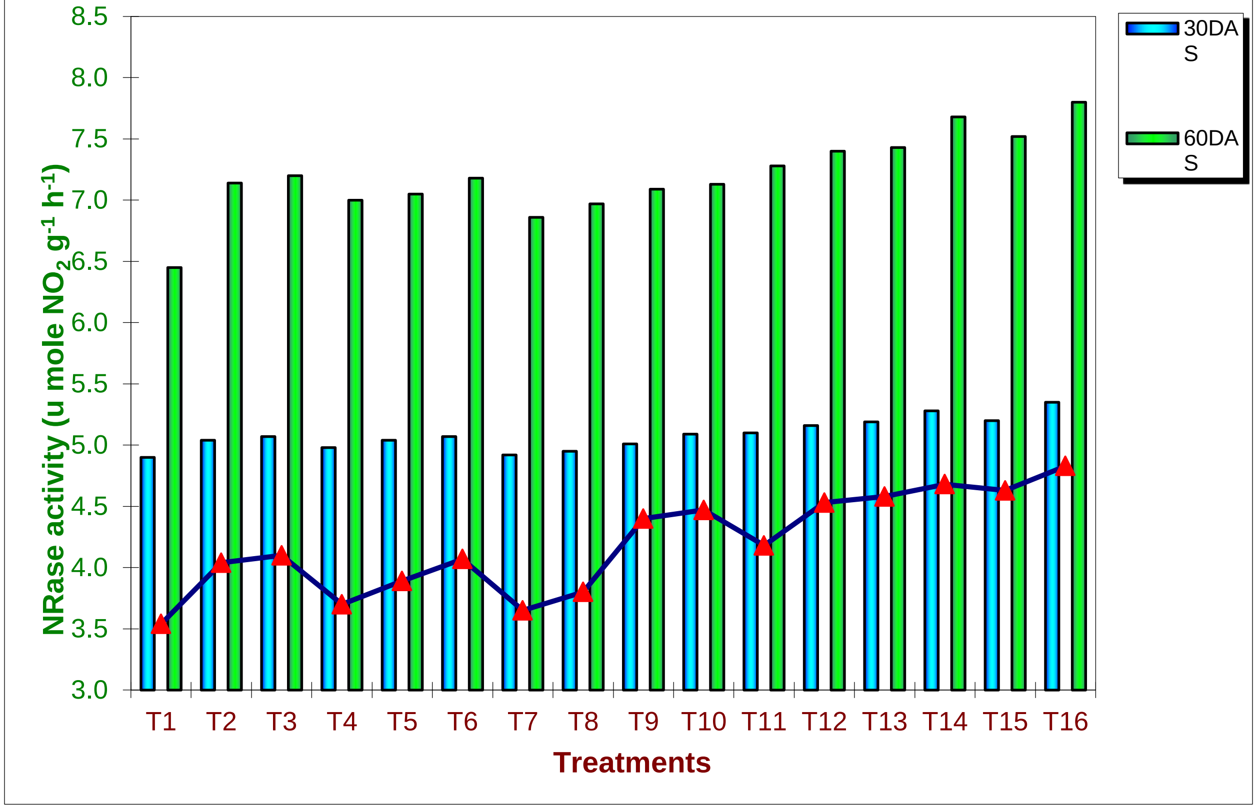


Fig.16. Interation effect of BL with BA and NAA on Nitrate Reductase activity of maize



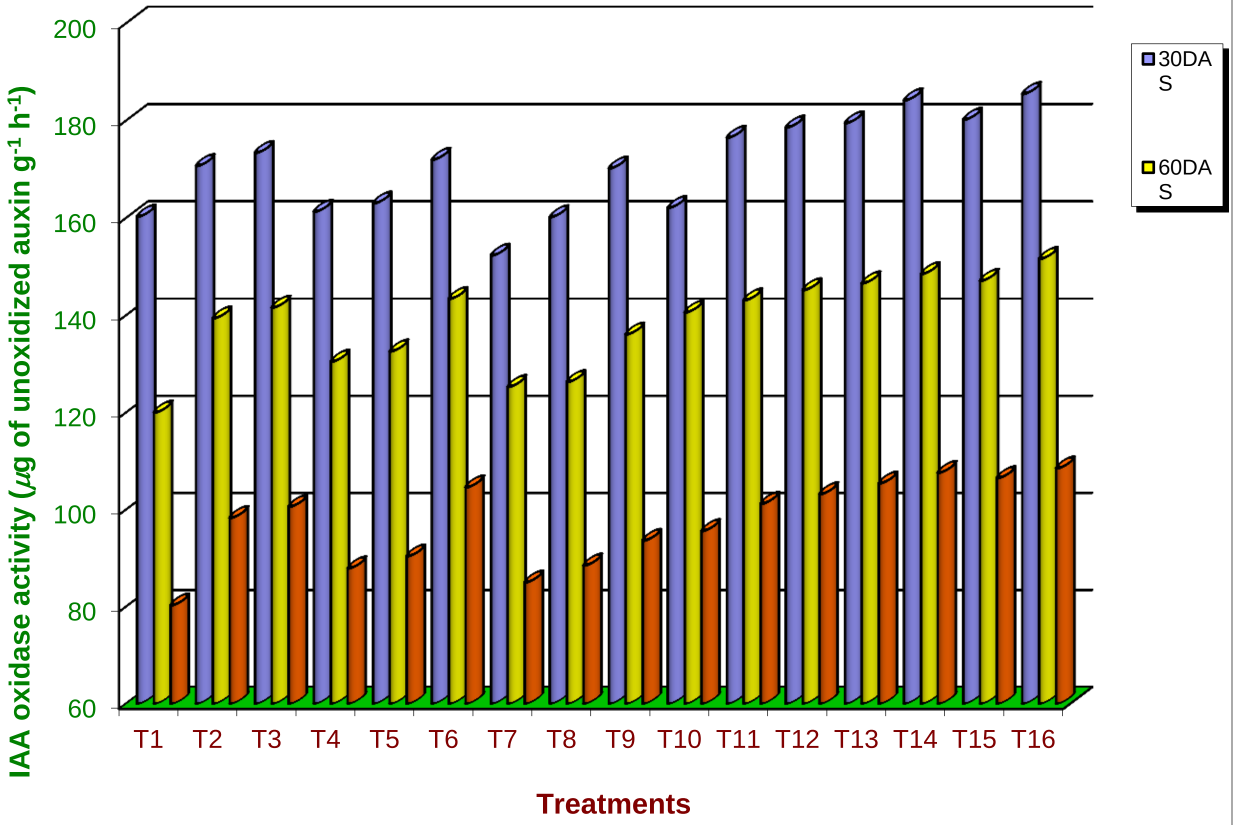
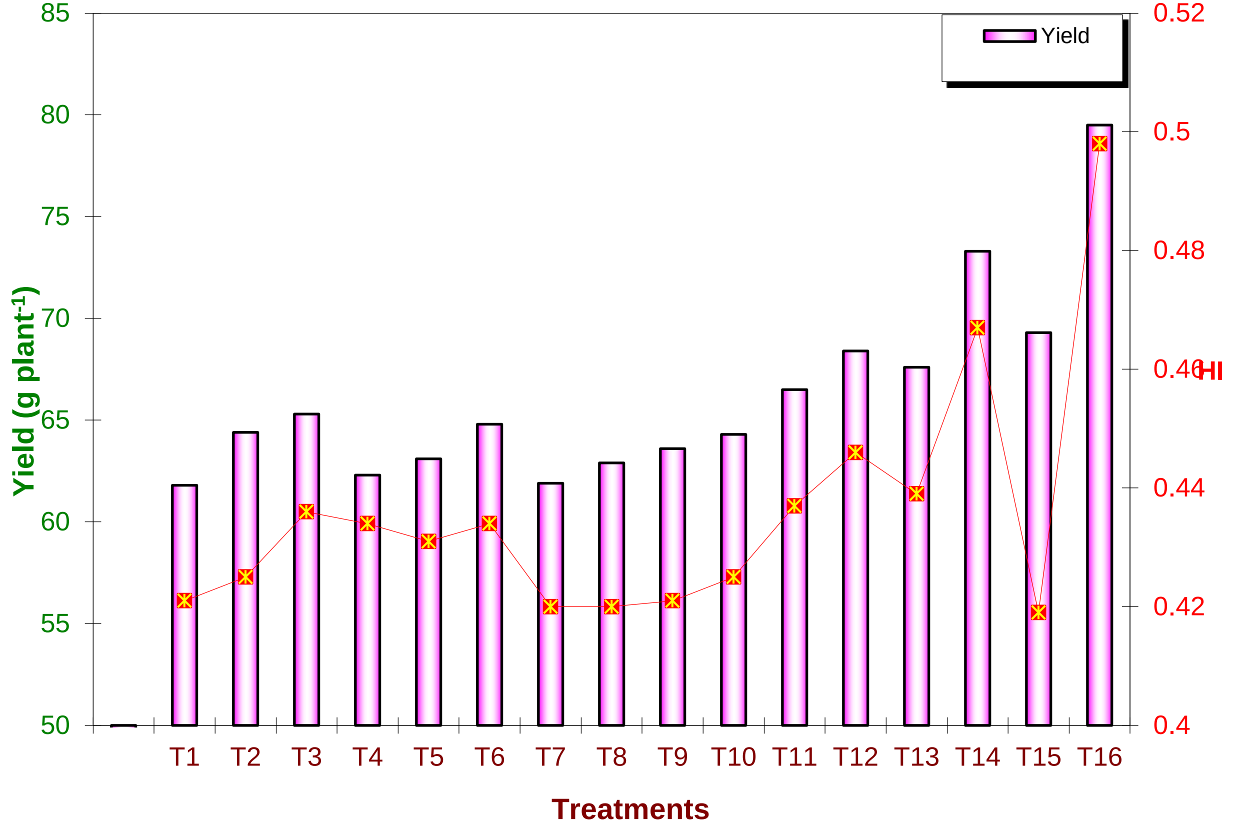
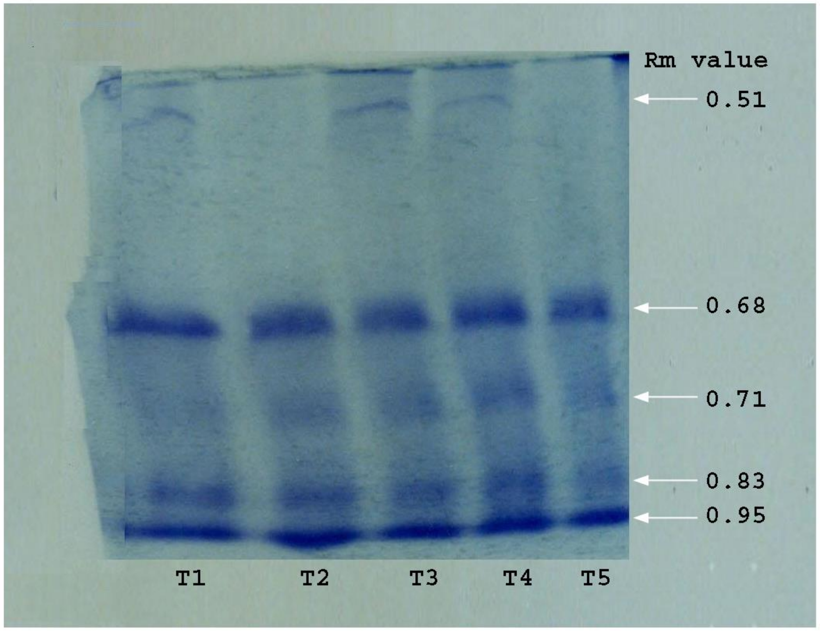


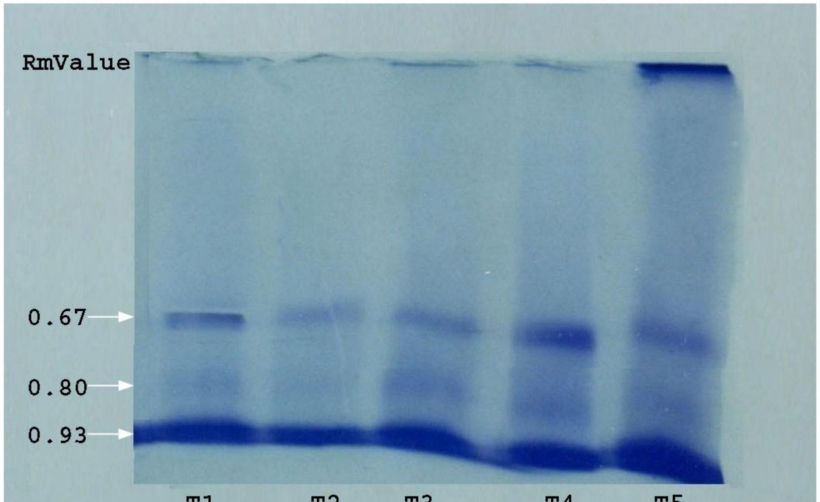
Fig.17. Interaction effect of BL with BA and NAA on IAA oxidase activity in maize

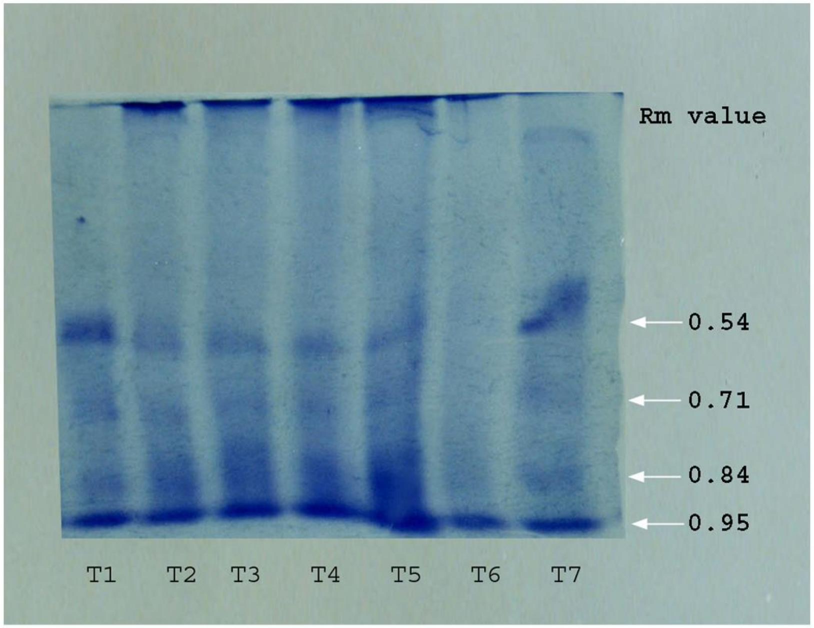
Fig.18. Interaction effect of BL with BA and NAA on Yield and Harvest Index in maize



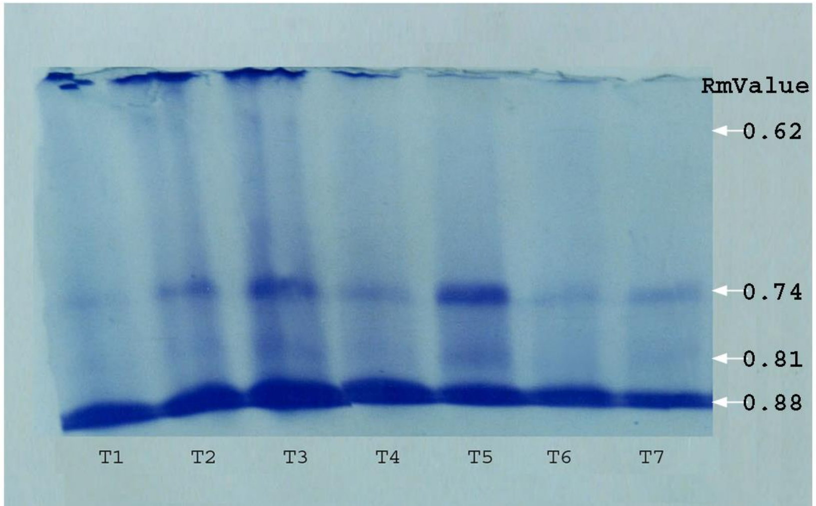


Drought stress

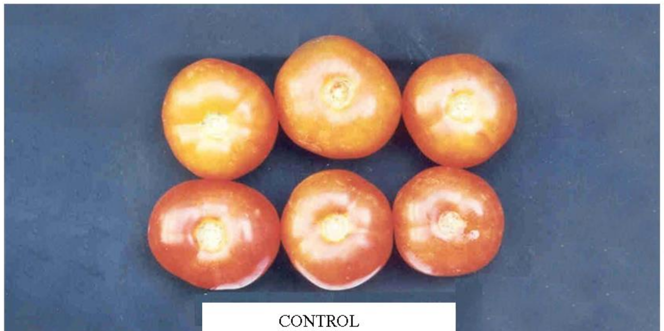




High temperature stress



Field view of the experimental plot



Control

BL sprayed

Control

BL sprayed

Control

Control

BL sprayed

BL sprayed

**Plate 3. Influence of brassinolide on yield of rice and maize (Field view)**

Control

Control

BL sprayed

BL sprayed

**Plate 4. Influence of brassinolide on yield of ragi and blackgram (Field view)**

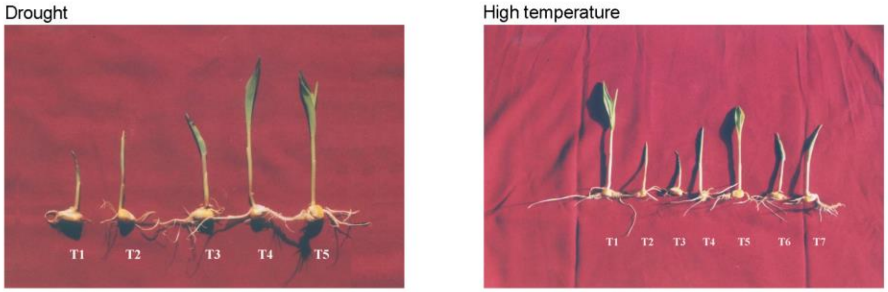

Control

Control


BL sprayed

BL sprayed

**Plate 5. Influence of brassinolide on yield of cotton and groundnut (Field view)**


Drought

High temperature


Low temperature

Salinity

**Plate 1. Influence of brassinolide on maize seedling under stress conditions**

Field view of experimental plot