CHAPTER- II

REVIEW OF LITERATURE

Indian farming is the largest or the second largest in the world in the terms of population dependency. There is no development model available for such a farming system. We have to shape our own model. As, Indian farming is undergoing a rapid change, crop productivity of agricultural and industrial plants is influenced by the interaction of their inherent eco-physiological traits, with physical, chemical, and biological environments under which they grow. Our horticulture, which includes fruit production, floriculture and vegetable production are making a tremendous heading. The developments taking place due to five-year plans and technologies in agriculture. Traditional farming is changing into modern farming, which leads to the rapid depleting of energy resources and world-wide increase in environmental pollution. With increasing population, rapid urbanization and growing export markets, the demand for farm products is increasing and is likely to increase in the future.

India is the vast country with varied climate, soils and ecological conditions. In addition to this, individual farmer is having his own set up of resources and socio-economic condition. Besides, diseases, insects, weeds, and adverse climatic and soil conditions are the major environmental impacts limiting crop productivity. According to Boyer (1982) the average crop yield in USA was reduced as compared with the maximum yield that can be achieved under non-stressful environment to develop crop plant. So the government policy must aimed to increasing agricultural production under varied environmental stresses and it is crucial to develop crop plants, with increased tolerance to varied environmental stresses (Kasukabe et. al., 2006).
An important ray of hope, which one can notice in this complex changing scenario of agriculture, is that adopting some eco-friendly pathways, use of organic matter i.e. 'chemical messengers', which regulate the crop production to the maximum. Nowadays, many of the hard work and high inputs, instead nature should be allowed to do more work in the farming system, which it has been designated to do anyway.

Plants possess the ability to biosynthesize a large variety of steroids, whose function as hormones has frequent been postulated. However, it was not until 1979 that the presence of steroidal hormones was confirmed in plants. In that year, Americans scientists published data on a new steroidal lactone called brassinolide (BL), which was isolated from pollen of *B.napus* L. that had been collected by bees (Grove *et al.*, 1979). Three years later, castasterone, a biosynthetic precursor of BL, was isolated from insect galls of *Castanea crenata* (Yokota *et al.*, 1982). As the first steroidal hormone with growth promoting nature was obtained from *B.napus*, the name brassinosteroids (BRs), was given to this new class of substance. BRs are considered as hormones with pleiotropic effects, as they influence varied developmental processes like growth, germination of seeds, rhizogenesis, flowering, and senescence. BRs also confer to resistance to plants against various abiotic stresses. Natural BRs so far identify, 24-epibrassinolide (EBL) has a common 5 α-cholestan skeleton, and their structural variations come from the kind and orientation of functionalities on the skeleton (Fujioka and Sakurai, 1997). Today, more than 44 structurally and functionally related steroids have been identified from natural sources. BRs are presents in plants at extremely low concentration (nano-gram levels). Levels of endogenous BRs vary among plant tissues. Young growing tissues contain higher levels of BRs than mature tissues (Yokota and Takahashi, 1986). Pollen and immature seeds are the richest sources (Takatsuto, 1994). Stimulation of growth is considered as the important physiological role of BRs in plants. BRs are highly effective in stimulating growth in
young vegetative tissues (Sasse, 1991). BRs are also found to stimulate the growth of algae and fungi. A 2-3 fold increase in the growth of *Chlorella vulgaris* by BL and 24-EBL was observed (Bajguz and Czerpak, 1996). The promotion of growth by BRs is due to both cell division and cell elongation. BRs induced changes in the growth and development are the result of a cascade of biochemical reactions, which can be initiated via direct action of BRs on the genome or by an extra genetic route.

Experiments to investigate the potential of BRs for use in agriculture began in the 1970s in the USA and showed beneficial effects (Maugh, 1981; Mandava, 1991). Since then, numerous reports from all over the world have appeared and many potential practical uses have been patented growth alteration.

BL, 24-EBL and 28-HBL and some other BRs have been tested in the field trials to determine their influence on plant growth and development and on crop yield in natural conditions. Under field condition, better results obtained with 24-EBL and 28-HBL, even though the activity of BL is usually higher in bioassays. One reason for this effect could be the lower stability of BL compared to 24-EBL and 28-HBL under field conditions. This, together with the fact that 24-EBL and 28-homobrassinolide (28-HBL) can be synthesized more cheaply than BL, has promoted use of these two natural hormones in agricultural applications. Nevertheless rapid development of the application of 28-HBL in agriculture might be expected because of its specific action in plants. An important feature of BRs is their ability to increase not only the yield, but also improve the quality of crops (Prusakova et al., 1999). Another new aspect of the influence of BRs is their ability to regulate the uptake of ions into the plant cell. BRs can be used to reduce the accumulation of heavy metals and radioactive elements, when plants are grown in areas that are polluted by these contaminants (Khripach et al., 1996). The metabolism of BRs in mammals has not yet been investigated. It may be speculated, however, that a normal catabolism of the
steroidal skeleton will take place. Being normal constituents of practically all plants, BRs have been, and are, consumed by mammals, and so additional harmful effects are not likely from their use in agriculture. This assumption is an important prerequisite for considering BRs as ecologically safe, non toxic chemicals for agriculture.

The ability to combine BRs treatments of plants with other pesticides, for example with fungicides for potato treatments, allows BRs to be used with existing technologies for plant protection, with no significant additional expense. A new variant of BRs application has been developed recently (Pirogovskaya et al., 1996), based on the use of combinations of BRs with mineral fertilizers. The potential applications of BRs in agriculture and horticulture are based not only on their ability to increase crop yield, but also to stimulate other physiological processes. As a result, it may become feasible to grow crops under unfavorable (stressful) conditions, such as high salinity, drought or insufficient nutrients (Prusakova et al., 1999). Recent data (Savel’eva et al., 1999) confirmed the efficiency of BRs application in the reduction of Phytophthora infection (up to a maximal 40%). A newly discovered aspect of the protective action of BRs on plants is related to their ability to stimulate resistance to viral infection (Bobrik et al., 1998). BRs, open ups new approaches for plant protection, based on the employment of very small amounts of environmentally-friendly natural substances instead of traditional pesticides, which are often environmental-unfriendly.

Data obtained by Americans investigators on the application of BRs resulted in the conclusion in 1981 that: ‘New chemicals promise larger crops’ (Maugh, 1981). Now, almost 30 years later, all the collected data lead us to the conclusion that the use of BRs can produce larger and better quality crops. Research suggests that BRs can be developed to be a new generation of agricultural chemicals, which do not interfere with the environment, act in natural doses and in a natural way. For these reasons, BRs have good prospects for
enhancing plant production and protection in the near future. This allows a more environmentally-friendly type of agriculture, which may become an issue in the near future.

**CELL DIVISION:**

Reports of productive effects on cell division in whole plants (Mandava, 1988) proved hard to confirm in model systems with elongation (Sala and Sala, 1985) or inhibition (Gaudinova et al., 1995; Roth and Bach; Wilen et al., 1995) noted instead. Furthermore, microscopic examination of BR-deficient and BR-insensitive mutants in Arabidopsis showed that dwarf phenotype was due to reduced cell size, not cell number (Kauschmann et al., 1996). However, in cultured parenchyma cells of *Helianthus tuberosus*, application of nanomolar concentrations of BR-stimulated cell division by at least 50% in the presence of auxin, and cytokinin (Clouse and Zurek, 1991). In Chinese cabbage protoplast, 24-EBL, when applied with 2,4-D and kinetin, promoted cell division in a dose-dependent manner and enhanced cluster and colony formation. The data suggested that dedifferentiation of protoplast was enhanced and that BR promoted or accelerated the necessary regeneration of cell division in *Petunia hybrida* (Oh and Clouse, 1998; Oh, 2003) protoplast in the presence of auxin and cytokinin but could not take the place of either hormone.

Experiments using cell culture requires are complicated by the fact that cell division in culture requires both the optimal concentration and combination of hormones, and that BRs may crosstalk with other plant hormones. If BRs affect the kinetics of cell division, but not the overall number of division, this may be obscured by a limited number of time points. Overall the role of BRs in regulating cell division is far from resolved. Further studies could also consider the role of BRs in leaf organogenesis and in developing competency to respond to cell division.
CELL EXPANSION:

Plant growth via cell elongation requires the coordination of several processes, some of which appear to be influenced by BRs. Plasticity of the cell wall is increased when proton extrusion by H^+ -ATPase acidifies the apoplast, thereby activating cell wall-loosening enzymes. Turgors pressure then drives cell expansion as new cell wall and membrane materials are synthesized and secreted. A few studies have reported that treatment with BRs increases ATPase activity in Azuki bean epicotyls and maize roots, leading to proton extrusion (Cerana et al., 1983, 1984). and that addition of BL increase cell wall relaxation (Wang, 1993; Congrove and Arteca, 1993; Tominaga, 1994; Sakurai and Kuraishi, 1994). Cell elongation also depends on an adequate supply of new wall component and on the orientation of microfibrils (correlated with the orientation of microtubules within the cell). BRs application at nM to µM levels causes pronounced elongation of hypocotyls, epicotyls, and peduncles of dicots, as well as coleoptiles and mesocotyls of monocots (Clouse, 1996; Mandava, 1991; Sasse, 1991) Young vegetative tissues is particularly responsive to BRs, and, if endogenous BRs are directly involved in the control of cell expansion, they must be present in such tissue. While both BR and auxin promote elongation, their kinetics are quite different. Auxin generally shows a very short lag time of 10-15 min between application and the onset of elongation, with maximum rates of elongation reached within 30-45 min (Taize, 1984). In contrast, BR has lag times of at least 45 min with elongation rates continuing to increase for several hours (Clouse et al., 1992; Katsumi, 1991; Mayumi and Shibaoka, 1996). Microarray analysis has also shown that BL treatment up-regulates several additional genes related to cell expansion and cell wall organization, including putative expansions, extensions, arabinogalactan proteins and pectin-modifying enzymes (Goda et al., 2002). One study employing cell swelling and shrinking assays on Arabidopsis wild-type, has proposed that BRs impact turgor-driven cell
expansion by affecting the activity of aquaporins, water channels that help the plant osm-regulate (Morillon et al., 2001). BL-induced expansion is accompanied by proton extrusion and hyperpolarization of cell membranes and these effects have also been observed in the asymmetric expansion of the joint pulvini of rice lamina (Cao and Chen, 1995) and in an alga (Bajguz and Czerpak, 1996) where BRs at concentrations from $10^{-15}$-10$^{-8}$ M markedly stimulated and accelerated the growth cycle. However, some authors found little effect in vivo of 24-EBL on plasmalemma ATPases of wheat roots (Xu et al., 1995) and decreased activity in diploid and tetraploid buckwheat (Deeva et al., 1996). Indirect modulation of ATPase activity had also been invoked to explain BR-induced effects on sucrose transport (Adam, 1994; Petzold et al., 1992). In general, exogenous application of BRs inhibits primary root extension and lateral root formation, with occasional promotion of elongation or adventitious rooting seen with <pM concentrations (Clouse et al., 1993; Clouse et al., 1996; Roddick and Guan, 1991; Saase and Sasse, 1994). However, there is some evidence for involvement of endogenous BRs in the control of lateral root initiation; unicozale treatment produced many stunted lateral roots in Lotus, but concomitant BL treatment reduced the number the control value (Kawaguchi et al., 1996). It is not clear what role endogenous BRs play in the early stages of germination, but changes in levels of castasterone and BL were seen after radish seeds germinated (Schmidt et al., 1990).

**SENESCENCE:**

It is the process, which refers to, endogenously regulated deteriorative, changes that become the natural cause of death of cells, tissues, organs or organism (Arteca, 1997), other hormones (Rao et al., 2002), BRs also play a crucial role in regulating the processes leading to senescence. The BL promotes senescence in Xanthium and Rumex explants (Mandava et al., 1981). In addition to it, BRs also accelerate senescence in the detached...
cotyledons of cucumber seedlings (Zhao et al., 1990) and leaves of mungbean seedlings (He et al., 1996). Similarly, the senescence of the mungbean and mustard delayed, if supplied with 28-HBL at early stage of growth (Fariduddin, 2002). Altered activities of peroxidase, superoxide dismutase and catalase and a marked increased in the level of malondialdehyde were observed, and the authors suggested BRs might regulate these effects via “activated oxygen”. Delayed senescence in Arabidopsis BR mutants would tend to support the role of BRs in accelerating senescence in normal plants (Clouse et al., 1996; Kauschmann et al., 1996; Li et al., 1996; Szekeres et al., 1996). However, work concerned with lipid peroxidation suggests 24-EBL inhibits oxidative degradation, decreases malondialdehyde levels (Ershova and Khripach, 1996), and act as a membrane protectant, thus delaying senescence. Examination of the effect of BR application on senescence associated mutants of Arabidopsis and study of the expansion of senescence associated genes in the BR mutants will be necessary to help clarify the role of BRs in this process.

FLOWERING:

There has been very limited use of steroids in regulating flowering. The number of flowers in strawberry increase by the application of BRs at the foliage (Pipattanawong et al., 1996). However, in case of grapes, the application of BRs in autumn improved the number of flowers but inhibited if the time of application is delayed to late winter (Rao et al., 2002). Exogenous application of 24-EBL also caused significant decrease in node position of the first female flower on monoecious cucumber plant and significant increase in female flowers (Papadopoulou and Grumet, 2005). Earlier and increased flower production might be due to increase in ethylene production. BRs have been reported to cause increased ethylene biosynthesis in excised mungbean hypocotyls (Arteca et al., 1988), Arabidopsis seedlings (Vardhini and Rao, 2002), and tomato pericarp discs (Woeste et al., 1999). The
spraying of BRs at leaf stage significantly influenced the flowering in watermelon 
*(*Citrullus lanatus*) *(Sushila et al., 2010)*.

**VASCULAR DIFFERENTIATION:**

Auxin and cytokinin are required for the initiation of xylem development both *in vivo* and *in vitro* *(Fukuda, 1997)*. However, evidence continues to mount that BRs may also play a significant role in vascular differentiation. In *H. tuberosus* explants, one of the major *in vitro* systems for studying xylem differentiation, Clouse and Zurek *(1991)* found that nM concentrations of exogenous BL increased differentiation of tracheary elements 10-fold after only 24h. Normally, tracheary elements differentiation requires 72h in this system. In isolated mesophyll cells of *Zinnia elegans*, a second widely used model system for xylem differentiation, tracheary elements formation has been divided into three stages *(Fukuda, 1997)*. In stage I, the mesophyll cells dedifferentiate after induction by auxin, cytokinin, and wounding, and specific transcripts are induced including the phenylpropanoid pathway members phenylalanine ammonias-lyase and cinamate hydroxylase. During stage II, phenylpropanoid pathway gene expression abates and three-dimensional networks of actin filaments form. In stage III, phenylalanine ammonia-lyase and cinnamate hydroxylase gene expression again increases, the highly lignified secondary wall is formed, and programmed cell death ensues. Current evidence suggests that endogenous BRs are required for entry into stage III *(Fukuda, 1997)*. Uniconazole, an inhibitor of both gibberellins and BR biosynthesis, prevented differentiation of *Z. elegans* mesophyll cells into tracheary elements, and this inhibition was overcome by BR but not by gibberellins application *(Iwasaki and Shibaoka, 1991)*. Uniconazole also inhibited stage III-specific genes but not those specific to stage I or II. Moreover, expression of phenylalanine ammonia-lyase and cinnamate hydroxylase was inhibited by uniconazole during stage III but not stage I, and this inhibition was overcome by several BL and several BR biosynthetic intermediates.
The spatial expression of \textit{BRU1}, a BR-regulated gene encoding a xyloglucan endotransglycosylase (XET) in soyabean \cite{Zurek1994} also points to a role of BRs in xylem differentiation. XETs are thought to be involved in processes requiring cell wall modification, including expansion, vascular differentiation, and fruit ripening \cite{Fry1992}. In cross-section of elongating soyabean epicotyls, \textit{BRU1} expression was most intense in paratracheary cells surrounding vessels elements \cite{Oh1998} suggesting a role for BRs and XETs in xylem formation. The modification of cambial division seen in a BR- deficient mutant \cite{Kauschmann1996, Szekeres1996} also suggests involvement of endogenous BRs in xylem differentiation in vivo, and it is revelant that BRs have been identified in cambial scrapings of \textit{Pinus silvestris} \cite{Kim1990}.

\textbf{STOMATA:}

BRs, regulate stomatal development and function, and provide another example of a hormone that cross talk with ABA during stomatal regulation. Five to six times more leaf stomata, stomata density has been observed in \textit{Arabidopsis} BR biosynthetic mutant \textit{bul1/dwf7} in comparison to wild type plants \cite{Catterou2001}. Jackpine seedlings pretreated with HBL show delayed stomatal closure in response to water stress \cite{Rajasekaran1999}. In contrast, the specific BR, brassinolide, promotes stomatal closure and inhibits stomatal opening in epidermal peels of \textit{Vicia faba} \cite{Haubrick2006}. In addition, the role of BL has studied in ion channel regulation in guard cell protoplasts of \textit{Vicia faba}, where it has been shown to inhibit the inwardly rectifying K$^+$ channels that mediated K$^+$ uptake during stomatal opening. In \textit{Vicia faba}, co-application of BL did not alter the extent of stomatal response to a given concentration of ABA, while in whole-plant physiological observation in sorghum, co-application of EBL and ABA resulted in an increase drought protective effect over that observed with ABA alone \cite{Xu2008}.
al., 1994 a,b). Conversely, a BR-deficient mutant of *Arabidopsis*, *saxl*, was reported to show enhanced stomatal closure in response to ABA (Ephritikhine et al., 1999). In Arabidopsis, early induction of drought-inducible genes *RD29A*, *ERD10* and *RD22* in response to EBL has been shown (Kagale et al., 2007). Three of the BR-related mutants are affected in stomatal function; *sax*, *det3*, and *gpal*. The *det3* mutant produced typical Ca$^{2+}$ oscillation in response to ABA and cold, leading to stomatal closure (Allen et al., 2000). Finally, Wang et al., demonstrated that protein-null mutants of *gpal* lack ABA inhibition of stomatal opening and inward K$^+$ currents (Wang et al., 2001). Mutation results in decreased BL sensitivity in root growth inhibition and abaxial leaf surfaces (Perez-Perez et al., 2004). This suggests crosstalk between ABA and BR signaling. Taken together, these observation suggest that interaction between BRs, ABA and guard cell output may occur in a species specific manner (Haubrick and Assmann, 2006; Haubrick et al., 2006). There may also be interactions between BRs and ethylene. An inductive effect of BRs on expression of ACC synthase in mungbean and *Arabidopsis* has been reported (Vert et al., 2005; Yi et al., 1999). Overall, it has been seen that BRs are positive regulators of stomatal closure. In addition, BRs also modulated expression of drought-related genes (Huang et al., 2008; Nemhauser et al., 2006).

**POLLEN AND REPRODUCTIVITY BIOLOGY:**

Pollen is a rich source of endogenous BRs and in vitro studies have suggested that pollen tube elongation could depend in part on BRs (Hewitt et al., 1985). Male sterility of BR-insensitive mutants would support this (Clouse et al., 1996; Kauschmann et al., 1996; Li and Chory, 1997), but the failure of the filament to elongate such that the pollen, although viable, cannot reach the stigma was suggested as an alternative mechanism of male sterility for the BR-deficient *dwf4* mutant. However, the *cpd* mutant was reported to be male sterile because the pollen itself failed to elongate during germination (Szekeres et al., 1996). In
addition, pollination is often the initial step for the genesis of haploid plants, and in *A. thaliana* and *B. juncea*, treatment with BL induced the formation of haploid seeds that developed into stable plants (*Kitani, 1994*). Subcellular localization of BRs was explored in pollen of *B.napus* and *Lolium temulentum*, using polyclonal antibodies generated against castasterone, and the data suggested BRs could be stored in developing starch granules and be released on imbibition (*Sasse et al., 1992; Taylor et al., 1993*). The relative distribution of BRs in maturing pollen has also been explored chemically (*Asakawa et al., 1996*), and conjugated teasterone was present at the microspore stage. Its level decreased as the pollen developed, and levels of free BRs increased. Taken together, these data suggest that BRs have important physiological roles in the fertilization of plants.

With respect to the general effect of BRs on sex differentiation in plants, *Suge (1986)* found that direct application of BL to the staminate inflorescence of *Luffa cylindrical* induced bisexual and pistillate flowers. Numerous model systems of sexual morphogenesis in plants are currently available, and application of BRs to these systems could be a profitable exercise.

**NODULES**

Leguminous plants form nitrogen-fixing root nodules and number of nodules is controlled by a self-regulating mechanism called auto-regulation. However, signaling substances involved in nodules regulation have not been identified.

Exogenous application of BRs resulted in an increase in nodulation. Among the three BRs, 24-EBL was to be most effective in enhancing the number of nodules and weight of nodulated roots in groundnut (*Vardhini and Rao, 1999*). Although, similar work was performed in nodulated roots of the *Phaseolus vulgaris* L. shows increase of root nodulation and pod yield under water stress (*Upreti and Muriti, 2004*) with application of EBL and HBL. Foliar application or direct injection of BL into the root base inhibited
nodule formation and root development in the super-nodulating mutant of soybean. The internodes in the plants subjected to foliar application were significantly longer than those in the untreated plants, which show that BRs may regulate the nodule number in soybean plants (Junko et al., 2006). Moreover, the nodule number in Lens culinaris (Hayat and Ahmad, 2003) was reduced by EBL and HBL, respectively.

SEED GERMINATION:

Seed germination is affected by many hormonal and environmental factors (Kucera et al., 2005). It is well documented that BRs promote seed germination, like other hormones. The treatment of the seeds of Lepidium sativae (Jones-Held et al., 1996) and Eucalyptus camaldulensis (Sasse et al., 1995) with BL improved per cent germination. Similarly, BRs promoted seed germination in case B.napus (Chang and Cai, 1998), rice (Dong et al., 1989), wheat (Sairam et al., 1996; Hayat et al., 2003), tomato (Vardhini and Rao, 2000), Orabanahal minor (Takeuchi et al., 1995), mungbean (Kshitij et al., 2011) and tobacco (Leubner-Metzer, 2001). Moreover, BL, 24EBL and HBL promoted seed germination in groundnut seeds (Vardhini and Rao, 1996) and Pisum sativum (Takahito et al., 2007). Treating the seeds of Coffea Arabica with biobras-16 (Soto et al., 1997), Oryza sativa with BRs (Wang, 1997), Cicer arietinum (Fariddudin et al., 2000) and Triticum aestivum (Hayat et al., 2001) with HBL resulted in the production of tall plants with higher fresh and dry mass. Moreover, the ability of BRs to confer resistance to plant against abiotic stresses is gaining much attention. Available data indicate that exogenous application of BRs is effective during stress, rather than under optimal conditions (Sasse, 1997). The ability of BRs to alleviate chilling stress in seedlings of maize (He et al., 1991), cucumber (Katsumi, 1994) and rice (Wang and Zang, 1993) has been reported. Earlier, BRs were found to ameliorate the impact of drought stress on germination and seedlings growth in spring wheat (Puroska et al., 2000), water stress in tomato (Shagun et al., 2011), salinity
stress in rice (Anuradha and Rao 2001), water stress in sorghum (Vardhini and Rao, 2005). The BRs induced tolerance to high temperature stress in brome grass (Wilen et al., 1995) and moisture stress in wheat (Sairam, 1994). For instance, exogenous supply of BRs rescues growth of A. thaliana and B. napus (Kagale et al., 2007) under salt stress. In addition, BRs and ethylene play crucial role in germination of cucumber seeds exposed to salt stress (Wang et al., 2011).

FRUITING:

Fruit ripening is a unique plant development process with direct implications for our food supply, nutrition, and health. The decision to set fruit is on the successful completion of pollination and fertilization. It has been found previously that parthenocarpic growth could be induced by auxins, GAs and cytokinins (Schwabe and Mills, 1981; Li et al., 2003). Meanwhile, BRs have been found to play a role in fruit maturity of tomato and grape fruits (Vardhini and Rao, 2002; Symons et al., 2006). The induction of parthenocarpic growth by BRs in cucumber provides strong evidence for a critical role of BRs in early growth of fruits (Qing et al., 2008). Recently several studies have showed that biosynthesis of BRs is enhanced in developing of seeds and fruits of tomato, pea and Arabidopsis (Shimada et al., 2003; Montoya et al., 2005; Nomura et al., 2007). To our knowledge, however, BRs have not been implicated in the regulation of early development of fruits, although some have observed that exogenous BRs can increase fruit set (Kamuro and Takatsuto, 1999). The increase in endogenous BRs levels are associated with ripening of grapes (Gregory et al., 2006). Foliar application of BRs during the period of reproductive development can increase the number of fruits per plant in Passiflora edulis (Gomes et al., 2006).
BIOCHEMICAL EFFECTS

PROTEIN SYNTHESIS

Foliar spray of EBL limits the loss of the transitional apparatus during prolonged heat stress, and increases the level of expression of some of the components of the transitional machinery during recovery, which correlates with a more rapid resumption of cellular protein synthesis following heat stress and a higher survival rate (Dhaubhadel et al., 2002). In wheat leaves under heat shock (40°C) condition HBL and EBL activated total protein synthesis, which results in chloroplast degradation (Kulaeva et al., 1991). An increase in the activity of CA (second most abundant soluble protein in C3-chloroplast) in the leaves was attained by the application of HBL (Hayat et al., 2000, 2001) to the shoot of the B. juncea.

STRESS RESPONSE:

Plant respond to abiotic and biotic factors in environment. These include heavy metals action, wounding, drought, high salt, and changes in temperature and light, and pathogen and pest attack, which may leads to a morphological, physiological, bio-chemical and molecular changes and cellular damages.

Response to Saline Stress: The application of EBL resulted in substantial improvement in the seed germination and seedling growth of E. camaldulensis under saline stress. Seed germination in the presence of 150 mM NaCl was enhanced by EBL, but when seedlings were grown hydroponically in salt, uptake of EBL through roots caused more damage (Sasse et al., 1995). BRs removed the salinity-induced inhibition of seed germination and seedling growth in case of rice. BRs also restored the level of chlorophylls and increased nitrate reductase activity under salt stress. The seed soaking of HBL enhanced the nitrogen fixing capability in chickpea plants pre-treated with NaCl (Ali et al., 2007). The plants also
showed increased dry matter accumulation, together with an increase in the activity of nitrate reductase and carbonic anhydrase activities. The HBL treated plants also possessed higher yield in comparison to the plants subjected to NaCl stress, at harvest. Similarly the spray of HBL to the foliage or supply through roots of *B. juncea* plants generated from the seeds soaked in NaCl enhanced the growth, nucleic acid content, ethylene and seed yield *(Hayat et al 2006; Hayat et al., 2007; Hayat et al, 2007)*.

**Response to Thermal Stress:** Treatments of BRs enhanced the germination rate of seeds and the growth after direct sowing in the submerged paddy pots at low temperature. Furthermore, leaf spraying of BRs on the rice seedlings at the 4th leaves increased plant height and the fresh weights of tops and roots under chilling stress. BR sprayed on the completely expanded 4th leaves did not increase their blade length, but that sprayed on expanding 5th and 6th day leaves strikingly increased their blade length. These results are the evidence that BRs were more effective on the protective of old rice leaves against cold stress *(Fuji and Saka, 2001; Hotta et al., 1998)*. The similar activity of BRs to improve seed germination and seedling growth of maize *(He et al., 1991)* and cucumber *(Khripach and Zhabinsakii 1999)* under chilling stress has been early reported. Extreme temperature *(Arteca and Tsai 1993)* (34°C) increased stress symptoms that necrotic areas on the leaves of bananas. However, in plants treated with a trihydroxylated spiritane an analogue of BR, the effects of thermal stresses were significantly reduction in there number, but application of BR an analogue was effective only in the plants exposed to the warmer temperature *(Olmedo et al., 2006)*. Application of EBL minimally increased freezing tolerance of brome grass of cells by 3-5°C, but markedly enhanced cells viability following exposure to high (40-45°C) temperature stress. The net effect on hardening as less than that obtained with ABA. Treatments of cells with EBL increased the accumulation of subset of ABA-
inducible heat-stable proteins. Treatments of *B. napus* and tomato seedlings with EBL leads to an increase in the basic thermo-tolerance this suggest EBL treatments limits the loss of some of the components of the translation apparatus during a prolonged heat stress and increased the level of expression of some of the components of the translation machinery during recovery (*Dhaubhadel et al., 2002; Dhaubhadel and Choudhary, 1999*). Significantly higher in vitro pollen generation in enhanced pollen tube growth and lower pollen bursting have been observed in the presence of EBL at 35°C, a temperature high enough to introduce heat-stressed symptoms in tomato, indicating a possible role of BR during plant growth and reproduction. The beneficial effect of BR application was also observed in fruit yield, which was increased during heat-stress conditioned. This increase in fruit yield was mainly due to increased in fruit number by EBL application (*Singh and Shono, 2005*). The effects of high-temperature stress in BR-treated and untreated wheat leaves were examined at the level of total protein synthesis and leaf cell ultrastructure. Protein synthesis was maintained in BR-treated leaves at 43°C at levels similar to those at 23°C, whereas untreated leaves decreased 2.5-fold at 43°C as compared to samples at the control temperature (*Kuleva et al., 1991*). BR not only significantly promoted epicotyls elongation of mungbean, but also allowed the plant to partly recover from the growth-inhibiting effects of chilling. Using proteomics, it has been identified some down-regulated proteins that were re-up-regulated after BR treatment under chilling condition. Most of these proteins were predicted to be involved in cell growth, wall formation, ATP production, the stress response, and methionine assimilation. However, the crosstalk’s between BRs actions and methionine’s minimals. Therefore, the possible involvement of methionine assimilation down-stream of BRs action may be considered a novel project for future search (*Huang et al., 2006*).
Response to Heavy metals stress: Plants have a remarkable ability to take up and accumulate heavy metals from their external. High concentrations of all metals including those essential for growth and metabolism exert toxic effects on the metabolic pathways of plants. Toxicity mechanisms include the blocking of functional groups of important molecules (Smirroff, 1995). Heavy metals toxicity can elicit a variety of adaptive response in plants. A ubiquitous mechanism for heavy metals detoxification is the chelation of the metal ion by a ligand. Such ligands include organic acid, amino acid, peptides acid and polypeptides. The culture of a Cholera vulgaris treated with BRs and heavy metals show lower bioaccumulation of heavy metals than the culture treated with metals alone. The inhibitory effects of BRs mixed with different heavy metals on their accumulation was arrange in following order: zinc>cadmium>lead>copper. A stimulatory effect of BRs after blocking the accumulation of heavy metals o the growth and development of C. vulgaris occurs. Application of BRs to C. vulgaris cultures reduced the impact of heavy metals stress on growth, prevented chlorophyll, sugar and protein loss. Concentration-dependent stimulation was observed with increasing concentration of BR and decreasing concentration of heavy metals (Bajguz, 2000; Bajguz, 2002). BRs also reduced the content of cadmium in the seedlings of winter rape (Bneczko et al., 2006) and copper in Indian mustard (Sharma and Bhardwaj, 2007). BR eliminates the toxic effect of cadmium on photochemical pathways in rape cotyledons, mainly by diminishing the damage in reaction centres. Moreover, Bilkisu et al., (2003) reported that BL during aluminium-related stress stimulated growth in Phaseolus aureus. It was shown that changes in the ions/metals content were influenced by EBL and dependent o the stage of plant development when the seeds were treated. The application of BRs also improved the performance of mustard (Hayat et al., 2007), chickpea (Hasan et al., 2008), subjected to cadmium stress and also of mungbean (Ali et al., 2008) and mustard (Alam et al., 2007) to aluminum and nickel,
respectively. Hasan et al., (2008) reported that BRs enhanced the antioxidant (catalase, peroxidase, and superoxidaes) and proline content. The activities of the enzymes catalase, peroxidase carbonic anhydrase and nitrate reductase also exhibited a significant enhancement in mustard plants grown under nickel stress when sprayed with BRs (Alam et al., 2007). These plants also exhibited an elevation in the water content and photosynthetic performance. Ali et al., (2008) also reported EBL enhanced the level of antioxidant systems. After the treatment of BRs the decrease in heavy metal (copper, lead, zinc, cadmium) contents was observed during the fruiting stage of spring wheat (Kroutil et al., 2010). The effect of EBL and HBL were studied on seed germination and seedling growth in radish under cadmium effect shows that HBL was found to be more effective than EBL in stress alleviation (Anuradha and Rao, 2007).

**Response to Pesticides application:** Pesticides may be a chemical substance or biological agent (such as virus and bacteria) used against pests that compete for human for food, destroy property, spread diseases or a nuisance. Many pesticides have been developed to target the specific biochemical reactions within their target organism. However, it is of great concern to understand their effects on non-targeted crops, they are meant to protect (Matthews, 2006).

BRs are effective in reducing the damages caused by the pesticides. Effects on herbicides safening, where BL treatment reduced the damage from the treatment of rice from the simazine, and butachlor have been confirmed (Sasse, 2003). Plant treated with paraquat showed severest phytotoxic symptoms with the highest reduction in the net photosynthetic rate ($P_n$) while other pesticides inhibit the $P_n$ to various degrees. For other pesticides inhibition of $P_n$ was accompanied by reduction in stomatal conduction. Interestingly inhibitions of $P_n$ were alleviated by EBL, pre-treatment as for the pesticides. Pesticides
impairs the photosynthesis of cucumber seedlings, while EBL pre-treatment can increase the resistance of plants to pesticides, which might be mediated by enhanced activities of CO$_2$ assimilation (Bajguz and Hayat, 2009).

**Response to Pathogen stress:** Plant immunity is based on complex response that is highly flexible in its capacity to recognize and counteract different invaders. To combat the invasion by microbial pathogen and herbivorous insects effectively, plants make use of pre-existing physical and chemical barriers that become activated upon attack. Apart from reacting locally, plant can mount a systematic response that enhanced defensive capacity in tissues distant from attack. Induced defence responses are regulated by a network of interconnecting signal transduction pathways in which hormonal signal can be involved (Dong, 1998).

All known phytohormones are involved in regulation of potato dormancy. BR stimulated ethylene synthesis in intact potato tubers, and ABA synthesis in cells of tuber apices. BR-induced changes correlates with the duration of deep dormancy that also increases. The most sensitive to BR was the rib meristem, which play leading part in the initial stages of growth of potato tuber apices (Korableva et al., 2002). Application of low concentrations of BR-containing extract of *Lychnis viscaria* seeds yielded an increased resistance of tobacco, cucumber and tomato to viral and fungal pathogens. It has been also reported that BR biosynthetic mutant *Arabidopsis* mutant *cpd*, showed a low expressions of pathogenesis related protein genes (Szekeres et al., 1996). The immune-inhibiting effect of BRs is a systematic and long term character, which was observed for at least 4 months after treatments of whole potato tubers with BRs (Vasyukova et al., 1994). Field application of EBL to barley plants significantly decreased the extent of leaf diseases induced by mixed fungal infection, along with an increase in crop yield. A protective effect of EBL was also established in the field trials with cucumber (Korableva et al., 2002). In tobacco, BL
enhanced the resistance to the viral pathogen tobacco mosaic virus (TMV), the bacterial pathogen *Pseudomonas syringae* pv. *tabaci*, and the fungal pathogen *Oidium* sp. In rice plants, BL enhances resistance to the fungal pathogen and the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (Bajguz and Hayat, 2009)