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Plant Bio-regulators for Enhancing Grain Yield and Quality of Legumes: A Review

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ABSTRACT

Plant bio-regulators (PBRs) are hormone-like natural or synthetic compounds that are able to increase the yields, alter growth patterns, nutritional qualities and provide resistance to various kinds of stresses (cold, heat, drought, insect, diseases, salinity) when applied at very low concentration to the plants. PBRs are known to enhance source-sink relationship and help in stimulating the photosyntheates thereby reduce flower drop, helps in fruit and seed development in better way ultimately improving the yield of the crops. Nowadays, abiotic stresses are major factors limiting crop productivity and sustainability throughout the globe. Bio-regulators application on crops induces long-term thermo tolerance (heat and cold) in plants as these can modulate plant responses to stresses at the cellular, tissue or organ level and hence are able to enhance the yield, quality and nutritional aspects of the food and forage legumes in the changing climatic scenario. Application of bio-regulators on crops is farmer friendly, convenient and effective approach for improving the productivity and quality of legume crops. The influence of some of the important plant bioregulators on growth, yield, quality and stress management in important food and forage legume crops are reviewed in this article.

Key words: Bio-regulators, Legumes, Quality, Stress Management, Yield.

Legumes rank third in the world crop production, after cereals and oilseeds and are important source of food, feed and fodder in the temperate as well as tropical climate (Sita *et al*., 2017). Grain legumes contribute 33% of human protein in the diet and are able to fix atmospheric nitrogen (N) in the soil thereby helping the succeeding crop by meeting its N requirement. Various agronomic factors, edaphic conditions, biotic and abiotic stresses affect the growth of legumes at different growth stages and cause crop losses worldwide (Kumar *et al*., 2014; Sita *et al*., 2017). Abiotic factors are major yield-limiting factors for the crop plants. About 90% of arable lands are prone to various kinds of stresses which cause up to 70% yield losses in food crops (Waqas *et al*., 2019). Very little attention has been given to the physiological processes which limit the crop productivity. The bio-regulators are known to improve the physiological efficiency offering a significant role for realizing the higher yield of the crops. The effective translocation of photo-assimilates from source to sink is very important for the good development of economic part in legume crops.

Bio-regulators are endogenous or synthetically produced substances that can control specific biochemical and physiological functions of many species probably by their influence on gene and enzyme interactions (Olaiya, 2010; Kumar *et al*., 2013). Bio-regulators influence growth and development at very low concentrations but inhibit plant growth and development at high concentrations (Kumar *et al*., 2013; Wahid *et al*., 2007; Khan *et al*., 2020). Manipulating plant responses using natural and synthetic bio-regulators is an emerging field of modern plant biotechnology with the potential of enhancing yield and phytonutrients in food crops (Cowan, 2009; Kumar *et al*., 2014). Bio-regulators also play an important role in plant responses to environmental factors and in forming plant tolerance to extreme conditions. It has been suggested that bioregulators could be used to manipulate gene expression in transgenic plants (Olaiya *et al*., 2013; Khan *et al*., 2020), a possibility that allows grower flexibility under conditions of environmental fluctuation. This is of importance in the envisaged genetic engineering techniques of manipulating crop growth. Different legumes differ in their requirement of hormones for reducing flower abscission and increasing biomass production and seed yield (Patil *et al.,* 2005). Foliar application of bio-regulators at the flowering stage may improve the physiological efficiency and may play a significant role in raising the productivity of legumes, however, its effects depend on the application rate, time of treatment and the developmental stage of the plant (Olaiya *et al*., 2013; Khan *et al*., 2020).

Bio-regulators role in the plants include seed germination and dormancy, plant development, embryogenesis, growth, flowering, fruit development and senescence (Raskin, 1992a and b; Wahid *et al.,* 2007; Kumar *et al.,* 2013; Kumar and Sarlach, 2020); plant defense mechanisms; abiotic stress

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management due to heat/cold/salinity (Mandavia *et al.,* 2010; Kumar and Sarlach, 2020; Khan *et al.,* 2020), growth hormone biosynthesis and signal transduction (Olaiya *et al.,* 2013; Waqas *et al.,* 2019), quality improvement of the grains (Field *et al.,* 1989; Upadhyay, 1994; Kumar and Sarlach, 2015) and enhancement of the yield of the crops (Garg *et al.,* 2006; Jeyakumar *et al.,* 2008; Kumar *et al.,* 2013; Meena *et al.,* 2016). In the last 30-years much of the work has been done on the application of bio-regulators for improving the vegetative growth and yield of the crops including legumes. For example, spraying of IAA, gibberellic acid and cytokinins enhanced the plant growth, yield attributes and yield and improved the quality in *Vicia faba*, soybean and cowpea (Kannan *et al.,* 2003; Ibrahim *et al.,* 2010; Kumar *et al.,* 2013). Salicylic acid, sitosterol and putrescine were used to enhance the biochemical constituents and phytonutrients in edible legume crops (Kumar *et al.,* 2014). Foliar application of salicylic acid, IAA and polyamines have been found to be useful in tolerance to plants against salinity, drought, water logging and heat stress in crops (Olaiya *et al.,* 2013; Kumar *et al.,* 2013; Kumar *et al.,* 2014; Khan *et al.,* 2020).

Osmo-protectants (such as KNO_3 and $CaCl_2$) affects the crop growth positively through their effects on water uptake, root growth, stabilization of proteins and membranes, stimulation of photosynthetic machinery and cell division (Bardhan *et al.,* 2007; Abou, 2011) and hence affect the yield and quality of legume crops. Bio-regulators application on legumes induce long term thermo tolerance in plants and are helpful in producing good quality seed under heat stress conditions (Wahid *et al.,* 2007; Khan *et al.,* 2020). So application of bio-regulators serves as farmer friendly, convenient and effective approach for improving the productivity and quality legume crops. So, it can be said that plant chemical bioregulation research is rapidly advancing and it is a powerful tool that will definitely lead to important long term implications in the agriculture. In this review the work related to different types of bio-regulators for improving the yield and quality of legume will be discussed.

Bio-regulators and their mode of action

In the plants, endogenously produced organic compounds act at cellular level and affect the growth and development processes and carry out coordinated role in plant system. They are known as phytohormones such as auxins, cytokinins, gibberellins, absicissic acid and ethylene. Recently many several other compounds that can regulate many facets of plant growth and development have been described. These new compounds are brassinosteoriods, jasmonates, polyamines, salicylates, juglones, peptide hormones, phospholipids and vitamins (Olaiya *et al.,* 2013; Janeczko *et al.,* 2015; Kumar *et al.,* 2013; Sita *et al.,* 2017; Waqas *et al.,* 2019). These bio-regulators act at very low concentration and any exogenous application of these influences the processes of growth and development which are being coordinated by the phytohormones in plants. For example, auxins cause cells to elongate and promote root growth in plants (Jeyakumar *et al.,* 2008); gibberllins helps in cell elongation, cell division, stimulation of seed germination, seedling growth, development of flowers and increase in size of fruits (Ngatia *et al.,* 2004); cytokinins stimulates cell division, morphogenesis, delay senescence and has role in stomata opening (Raskin 1992a); abscissic acid a growth inhibiting hormone and has role in seed dormancy, stomata closing and opening during drought *etc.*, while ethylene is associated with senescence and abscission (Olaiya *et al.,* 2013; Kumar *et al.,* 2013; Khan and Mazid, 2018).

Brassinosteroids (BRs) are group of some 40 different steroids that are synthesized by the plants and has broad spectrum roles in cellular and physiological processes, including seed germination, stem enlargement, apical dominance, leaf senescence and production of ethylene, inhibition of root growth, increase in resistance to freezing, proton pump activity, xylem differentiation, photosynthesis, prevention of premature abscission of fruit and improvement in the total proteins and enzymes (Wahid *et al.,* 2007; Olaiya *et al.,* 2013; Sita *et al.,* 2017). Brassinosteroids also induce plant tolerance to various kinds of abiotic stresses such as heat and cold stress as well as drought and salinity stress.

Salicylic acid (SA) regulates many physiological processes along with its protective effects against abiotic stresses in plants (Kumar *et al.,* 2013; Kumar *et al.,* 2014; Khan *et al.,* 2020). SA inhibits ethylene production, helps to increase chlorophyll content and photosynthetic activity in plants and reduces damage or injury to the cells by reduction in respiration activity during stress which lead to induction of long term tolerance during heat stress (Kumar *et al.,* 2014; Kumar and Sarlach, 2015; Khan *et al.,* 2020).

Polyamines (PAs) are organic compounds having two or more primary amino groups -NH2, having low molecular weight found in all living organisms that play a critical role in growth, development and stress responses. The common polyamines in plants are spermidine, spermine and their diamine precursor, putrescine. Juglone also called 5-hydroxy-1,4-naphthalenedione or 5-hydroxynaphthoquinone, is an organic compound with the molecular formula $C_{10}H_6O_3$ (Zaghlool, 2002). Juglone occurs naturally in the leaves, roots, husks, fruit and bark of plants in the Juglandaceae family, particularly the black walnut (*Juglans nigra*) and is toxic or growth-stunting to many types of plants.

Jasmonates are cyclopentanone compound or plant hormones derived from α-linolenic acid. It includes group of oxygenated fatty acids collectively called oxylipins and jasmonic acid is main precursor to different compound to this groups. Methyl jasmonate was first isolated from the essential oil of *Jasminum grandiflorum*. They are ubiquitous in plant kingdom and are also produced by certain fungi. Jasmonates promote the ripening of fruit, required for production of viable pollen, drive the coiling of tendrils, inhibit root growth, affect seed germination, development and opening of flowers and promote the secretion of nectar in flowers (Wang *et al.,* 2020). Currently, the mode of action most of bio-regulators is not fully understood, however they

are believed to be interfering with plants hormone system thereby modifying the hormonal status of plant (Olaiya *et al.,* 2013). This interference can happen by application of these substances to the seed, soil or leaf so that essentially these can be absorbed so that they can exercise their activity. Evidence of mode of action of substituted tertiary amines (STA) bio-regulators that these affect the gene expression including induction and regulation of pigment synthesis in the genotypes that do not normally synthesize the pigments had been elucidated by Van Pelt and Popham (2006). More studies are needed to fully understand the cellular and molecular mechanisms of action of most bio-regulators particularly in response to various kinds of stress and environmental conditions.

Importance and benefits of bio-regulators

In present day scenario, use of bio-regulators in agriculture and horticultural crops is highly imperative as they act in multi-facets way in these crops. The chemical modification of plants by bio-regulator application at very low concentrations has the potential to increase food production much more quickly than plant breeding techniques (Kumar and Sarlach, 2015). The beneficial effects of a commercially viable chemical are also usually not restricted to a single species and the time-scale involved to achieve a commercially desirable result is often shorter. Some benefits of the use of bio-regulators for food production include better weather resistance, lodging control in pea, rice, mungbean, berseem, clusterbean, lentil, sugarcane; maturation and inhibition of natural breakdown in sugar contents of sugarcane and sugar beet after ripening, strawberry runner control, promoting biomass formation, inhibition of transpiration and increased efficiency of water utilization, enhanced yield and of many food and forage legumes (Wahid *et al*., 2007; Kumar *et al*., 2013; Sita *et al.,* 2017; Kumar and Sarlach, 2020). Bio-regulators can be used for wide variety of actions in many plants (Table 1).

Role of foliar bio-regulators in legumes

Different legumes differ in their requirement of bio-regulators for reducing flower abscission, increasing biomass production, seed yield, seed quality and protective response under high temperature stress conditions (Kumar *et al.,* 2013; Sita *et al.,* 2017; Khan *et al.,* 2020). The foliar application of growth hormones has resulted in significant increase in yield of food and fodder legumes (Wahid *et al.,* 2007; Kumar *et al.,* 2014; Sita *et al.,* 2017). Plant phenolics which have hitherto been considered inhibitory, have shown to modify plant growth and development and enhanced yield potential of many crop plants (Kumar *et al.,* 2013).

Effect of bio-regulators on growth parameters of legumes

Foliar application of bio-regulators at various growth and reproductive stages resulted in improved growth and development in many legume species (Kumar *et al.,* 2013; Waqas *et al.,* 2019). Increase in plant height as a result of salicylic acid application may be due to increased cell enlargement, endoreduplication and/or cell division (Kumar and Sarlach, 2015). Amanullah *et al.* (2010) reviewed increase in plant height, root growth, number of leaves plant^{-1}, leaf area, crop growth rate and total dry matter production when 100 ppm of salicylic acid was sprayed on green gram at 75 DAS. Govindan and Thirumurugan (2000) revealed that growth parameters like plant height, leaf area index and dry matter production were significantly higher in green gram with treatments which received foliar spray of KNO_3 1% as compared to control. Upadhyay (1994) observed increase

Table 1: Types of bio-regulator action in various crops.

Type of action	Aim of treatment	Crop/ tree
Termination of dormancy	Improvement of bud burst and growth	Fruit trees, sugar cane *etc.*
Promotion of development	Germination, bud burst	Seed, cuttings e.g. cereal, sugar beet and sugarcane, potatoes, vines, shrubs *etc.*
Promotion of growth	Increased biomass production	Plants for alcohol or feed production *e.g.* sugarcane, euphorbia, grasses for grazing
Promotion of resistance to	Frost, heat, water, stress, salinity, pathogens and pests.	Cereals, legume, sugar beet, citrus, coffee and cotton.
Speeding up of senescence	Abscission of leaves and fruits	Cotton, shrubs, potato, canning fruits, olive *etc.*
Improvement of quality	Grains, beans, nuts, fruit, plants	Cereals, soybean, green gram, groundnut, fruit, ornamentals, *etc.*
Promotion of lodging resistance	Avoidance of yield loss	Cereals, soybean, rape, sunflower *etc.*
Increasing yield	Improvement of fruit set and development	Cereals, cotton, soybean, berseem, rape, fruit, groundnuts and potato.
	Improvement of quantity and quality of contents	Carbohydrates (sugar beet, sugarcane, potato, brewing barley; proteins (soybean, rape, sunflower, groundnut, oil palm); latex (Hevea, Euphorbia); resins (pines); tannins (cotton)
Inhibition of growth	Reduction of undesired growth	Cotton, turf, fruit and ornamental shrubs

in plant height, number of branches, vegetative growth in chickpea due to foliar application of NAA (10, 20, 30 ppm) and KNO_3 (100, 200 and 300 ppm) over control. Kannan *et al.* (2003) reported that application of NAA (30 ppm) to blackgram (*Vigna mungo)* increased leaf area index, leaf chlorophyll content, photosynthetic rate. Khan *et al.* (2003) reported that foliar sprays of salicylic acid, acetyl salicylic acid and geninic acid enhanced the photosynthesis, stomatal conductance and transpiration rate in soybean. Jeyakumar *et al.* (2008) reported that foliar application of salicylic acid (125 ppm) increased the dry matter production in black gram. In soybean, cowpea and berseem plants, treatment with salicylic acid, increased chlorophyll pigments content as well as the rate of photosynthesis (Kumar *et al.,* 2014; Kumar *et al.,* 2013). Kumar and Sarlach (2015) also recorded improvement in growth parameters such as plant height and fresh weight per plant with foliar application of ethylene production inhibitors and osmo-protectants in cowpea. Kumar and Sarlach (2020) recorded better growth in terms of higher plant height and tiller per plant of berseem crop with the foliar application of thiourea at different crop stages.

Effect of bio-regulators on yield and yield attributes of legumes

Foliar application of bio-regulators has shown significant effect in improving the yield attributes and yield of the many legume crops. Upadhyay (1994) observed increase in number of flower buds, number of flowers, pods, seeds per pod and yield in chickpea due to foliar application of NAA and KNO_3 over control. Shukla *et al.* (1997) found that two foliar sprays of NAA significantly increased total biomass yield in soybean. Zaghlool (2002) observed increase in number of pods in mungbean when salicylic acid was sprayed exogenously over the crop. Sharma and Kaur (2003) observed that application of 50 ppm salicylic acid as spray on the soybean crop increased the number of pods significantly as compared to control. Patil *et al.* (2005) also reported that application of 25 ppm of NAA gave 31% more pod setting and 55.0% more pods plant^{-1} as compared to control in mungbean. Amanullah *et al.* (2010) reported that foliar application of 100 ppm of salicylic acid on green gram crop at 75 days after sowing recorded significantly more 100-seed weight and yield as compared to control. Jeyakumar *et al.* (2008) reported that application of salicylic acid (125 ppm) increased the dry matter production and seed yield in black gram. Singh and Kaur (1981) found that salicylic acid sprayed on mung bean significantly increased the pods plant^{-1} and seed yield. Bardhan *et al.* (2007) also recorded that foliar application of KNO_3 @ 100 and 200 ppm at 40 and 60 days after sowing on chickpea resulted in significantly more 100-seed weight, biomass plant^{-1} and grain yield as compared to un-irrigated control. Mandavia *et al.* (2010) found that application of 50 and 100 ppm of salicylic acid as foliar spray at vegetative (40 DAS) and reproductive stage (55 DAS) on chickpea had significantly increased the seed yield as compared to control. Obvious effects on yield of various legume crop species have been achieved following exogenous application of salicylic acid, NAA, gibberelic acid and other osmo-protectants; an increase in seed yield and number of pods had been observed in mungbean (Singh and Kaur, 1981; Zhaglool, 2002), *Phaseolus vulgaris* (Khodary, 2004; DeGuang *et al.,* 2001), blackgram (Jeyakumar *et al.,* 2008), soybean (Kumar *et al.* 2000; Sharma and Kaur, 2003), cowpea (Kumar *et al.,* 2014; Kumar and Sarlach, 2014) and berseem crop (Kumar *et al.,* 2013).

Swarna Ramesh (2002) reported that application of NAA (20 and 40 ppm) in mothbean significantly increased the number of seeds plant^{-1}, number of pods plant^{-1}, pod weight, pod length and grain yield. Foliar application of cowpea plant with 15 mg per litre of naphthaleneacetic acid (NAA) at 15, 30 and 45 days after sowing increased fruit set and grain yield (Resmi and Gopalakrishnan, 2004). Kumar *et al.* (2013) and Kumar *et al.* (2014) reported that foliar spray of sodium benzoate inhibited the ethylene synthesis and resulted in longer grain filling period, increased grain weight and protein content in berseem and cowpea crop. Hydroxyl scavenger (sodium benzoate) application resulted in a significant reduction in the transpiration rate compared to the control, proving that the sodium benzoate application stimulated water conservation via stomatal movements and/or decreasing water loss which has positive effect on the legumes (Kumar *et al.,* 2014).

Osmo-protectants ($CaCl_2$ and KNO_3) improved yield and yield attributes in cowpea and berseem (Kumar *et al.,* 2013; Kumar *et al.,* 2014). Three foliar sprays of 0.5% and 1.0% $CaCl_2$ starting from flower initiation at weekly interval recorded 17.3-18.9% higher yield than the control in berseem (Kumar *et al.,* 2013). Calcium ions play an important role in many biochemical processes, delaying senescence and controlling physiological disorders in legume (Kumar *et al.,* 2013; Kumar and Sarlach, 2014). Abou (2011) also reported that foliar application of 500 ppm chelated Ca to *Phaselous vulgaris* at 40 and 60 days after sowing recorded significantly more number of fruit set and seed yield as compared to control. Jayaramireddy *et al.* (1996) reported that foliar spray of NAA (20 ppm) + KNO_3 (0.5%) recorded the maximum seed yield (8.57 q ha^{-1}) in pigeon pea over control (7.35 q ha^{-1}). Kumar and Sarlach (2014 and 2020) recorded higher yield, quality and net returns with foliar sprays of salicylic acid and thiourea in cowpea and berseem. So, the increase in seed yield of legume by application of foliar bio-regulators is due to increase in chlorophyll content, more photosynthetic rate and reduction in ethylene production.

Effect of bio-regulators on quality parameters of legumes

Bio-regulators use is an emerging modern plant biotechnology approach which can modify plant gene expression, can affect levels of DNA, RNA, enzymes and finally their products such as protein, carbohydrates, lipids, allelochemicals for enhancing yield and phytonutrients in food crops (Olaiya *et al.,* 2013; Kumar *et al.,* 2014). Bio-regulators act at the gene level of plant influencing the

translational and transcriptional mechanisms and on the transaminase enzyme level in the plant (Kumar *et al.,* 2014) for N and protein synthesis. Bio-regulators application on foliage can improve the nutrition of the plant and seed or grain (Wahid *et al.,* 2007; Kumar *et al.,* 2013; Kumar *et al.,* 2014). Sodium benzoate application in plants inhibit ethylene production and delay senescence which results in more accumulation of photo assimilates and hence increase the quality of legume crops under heat stress condition (Kumar *et al.,* 2013; Kumar *et al.,* 2014). Mandavia *et al.* (2006) reported that exogenously applied salicylic acid enhanced nitrogen and crude protein content of soybean plant and seed due to enhanced activity of nitrate reductase enzyme. Salicylic acid might be involved in mobilization of internal tissue NO_3^- and chlorophyll biosynthesis to increase the functional state of the photosynthetic machinery in plants (Wahid *et al.,* 2007; Kumar *et al.,* 2014), or may induce accumulation of α-amino levulinic acid (α-ALA) in cotyledons. Karim *et al.,* (2006) also found lowest values of ether extract (7.94%) and crude fiber (4.74%) content of chickpea seed in treatment where foliar application of 20 µg ml^{-1} NAA was applied on the crop at 45 days after sowing.

Foliar application of bio-regulators on plant can enhance nutritive quality of grain due to enhanced translocation of photosynthates in the legumes (Wahid *et al.,* 2007; Olaiya *et al.,* 2013). Also application of bio-regulators encourages the uptake of nitrogen from the soil and may activate transaminase enzyme which in turn increases the quality of grain (Karim *et al.,* 2006). Kumar *et al.* (2014) also reported that foliar application of sodium benzoate, salicylic acid and osmo-protectants enhanced the N, P, K, Zn, Cu, Fe content in cowpea grains. They further reported reduction in ash content and enhancement of crude protein and organic matter content of cowpea grains. Jeyakumar *et al.* (2008) reported that highest seed protein content in blackgram was recorded with foliar application of salicylic acid. Increment in the protein content of berseem+ oat fodder by 6.3% was reported with the foliar sprays of 0.05% TU over control by Kumar and Sarlach (2020).

Effect of bio-regulators on stress management

Rapidly changing climate dynamics makes heat and cold stress serious threat to food production system. For example, a 30% decrease in the yield of legume crops by 2050 is expected for spring sown crops in southern Europe due to drought stress (Waqas *et al.,* 2019). Exogenous application of salicylic acid to the plants can increase total antioxidant capacities, under high night temperature, thereby preventing damage to membranes, hence increasing yield in the crops (Wahid *et al.,* 2007; Kumar *et al.,* 2013; Kumar *et al.,* 2014). Salicylic acid increases photosynthetic rates and decrease respiration rates and injury to the membranes (Farooq *et al.,* 2008; Kumar and Sarlach, 2015). It also promotes pathogenesis-related (PR) gene expression and induces systemic acquired resistance (SAR) in plants (Wahid *et al.,* 2007). Plants pre-treated with salicylic acid have shown increased thermo tolerance (Kumar *et al.,* 2013) and reduction in ethylene production (Khodary, 2004), thereby preventing oxidative damage and protecting the membranes and enzymes by detoxifying superoxide radicals (Kumar *et al.,* 2013). Exogenous applications of salicylic acid also act as anti transpirant in leaves of crop and also increase the stomatal resistance for reducing the transpiration (Larque Saavedra, 1978). The increment of yield under drought stress by foliar application of KNO_3 was also reported by Purcell and King (1996) in soybean and Bardhan *et al.* (2007) in chickpea.

Thiourea (TU) a synthetic compound containing nitrogen and sulfur is an important plant bio-regulator which influences plant growth particularly under stress conditions (Garg *et al.,* 2006). Exogenous application of TU as foliar sprays stimulates defense mechanism in plants under abiotic stress and modulates key physiological events and mechanisms including photosynthesis, nitrogen metabolism and plant water relation during different plant developmental stages (Waqas *et al.,* 2019; Singh and Singh, 2017; Kumar and Sarlach, 2020). Foliar application of TU (1000 ppm) at the pre-flowering stage of mung bean increased seed yield by 24% by improving photosynthetic efficiency and plant metabolic functioning under water stress conditions (Mathur *et al.,* 2006). Similar improvement in water use efficiency, growth and economic yield of cluster bean due to improvement in photosynthetic efficiency and nitrate reductase activity has been reported under rainfed conditions by Garg *et al.* (2006). TU also improves the mineral nutrition of legume under stress conditions. TU seed treatment and foliar application (500 ppm) improved nitrogen (15%) and phosphorus (21%) uptake in cluster bean which in turn improves the yield and quality of grain in drought conditions (Meena *et al.,* 2016). In fodder legume berseem, Kumar and Sarlach (2020) reported that TU sprays on the foliage can mitigate cold stress and frost injury, thereby increasing its yield and quality. The information pertaining to the use of different bio-regulators use in food and forage legume is given in Table 2.

Precaution for use for bio-regulators

For using the bio-regulators in the field crops, a few precautions needed to be taken care off. Preferably, bio-regulators should be sprayed in the afternoon on the crop surface when the wind is calm. Spray of bio-regulator should be uniform and proper wetting of leaf surface must be ensured. Bio-regulator should properly stick to the leaf surface, or that surfactant or adhesive material like Teepol or Tween-20 (0.5-1.0 ml/l) should be used in conjunction with the bioregulator. Bioregulators are effective when their spray is done at specific stages of crop growth, so select the proper stage in the crop while spraying the solution. Solution of bioregulator should always be prepared in distilled water for spraying over the crop and repeat the spray within 7-8 hours if chemical is washed off due to rain.

Constraints in use of bio-regulators

The difference in sensitivity of each plant species or even

Table 2: Rate and method of application of different bio-regulators use in food and forage legumes.

Bio-regulator	Conc. (mg/L or ppm)	Method of application	Crops	Attributes affected	References
Indole acetic acid (IAA)	10-25	Foliar spray	Legumes like cowpea, soybean and mung bean, pea, black gram	Seed germination, fruit set and yield	Kannan *et al.* (2003); Jeyakumar *et al.* (2008); El-Shraiy and Hegazi (2009); Khan and Mazid (2018)
Naphthalene acetic acid	10-25	Seed and Foliar	Legumes like black gram, cowpea, moth bean, soybean,pigeon pea and berseem	Flower drop, fruit set and yield	Resmi and Gopalakrishnan, (2004); Kannan *et al.* (2003); Jayaramireddy *et al.* (1996); Jeyakumar *et al.* (2008) Kumar *et al.* (2013)
Gibberalic acid	20-100	Foliar spray	Berseem, pea, soybean, pigeon pea, broad bean, common bean, kidney bean	Sex expression, fruiting and yield improvement	El-Fouly *et al.* (1988); Zaghlool (2002); *Ngatia et al.* (2004); El-Shraiy and Hegazi (2009); Khan and Mazid (2018)
Triacontanol	2-100	Foliar spray	Peas, black gram, soybean	Fruit set, yield and quality	Soureche *et al.* (2000); Jeyakumar *et al.* (2008)
Salicylic acid	50-250	Foliar spray	cowpea, chickpea, soybean, berseem and black gram	Flower, induction, seed yield, heat stress tolerance	Raskin (1992a and b); El-Shraiy and Hegazi (2009); Mandavia *et al.* (2010) Kumar *et al.* (2013); Kumar *et al.* (2014); Sharma and Kaur (2003); Kumar and Sarlach, (2015); Khan *et al.* (2020)
Brassinosteriods	0.1-0.5	Foliar spray	Green gram, chick pea and French bean	Quality improvement, yield enhancement	Jeyakumar *et al.* (2008); Mandavia *et al.* (2010); Sita *et al.* (2017); Khan and Mazid (2018)
Ethrel	200	Foliar spay	Soybean, cowpea, pigeon pea, mung bean	Pod and yield improvement	Bora and Bohra (1989); Kaur *et al.* (2015)
Paclobutrazole	10-20	Foliar spray	Field bean, lentil, soybean and broad bean	Reduction of stem height, reduce lodging problem, pod and yield, enhancement	Field *et al.* (1989); El-Aal and Eid (2017)
Thiourea	500-1000	Foliar spray	Lentil, mung bean, cluster bean and berseem	Growth, yield and quality improvement, stress (heat and cold) management	Garg *et al.* (2006); Mathur *et al.* (2006); Meena *et al.* (2016); Singh and Singh (2017); Waqas *et al.* (2019); Kumar and Sarlach (2020)

cultivars to given chemical treatment restricts the use of bioregulators. The cost of developing new plant bio-regulator is very high due to which they are costly. Furthermore, screening for plant growth regulatory activities entails high costs for the use of PBRs. Some of synthetic bio-regulators cause human health hazards *e.g.* dominozide. There is lack of basic knowledge of toxicity and mechanism of action of the bioregulators. At field level, growers face difficulty in identification of proper stage of crop at which the growth regulators should be applied. Restricted penetration of the cuticle and entry into the plant cells has been another problem with some of the PBRs such as indole acetic acid and cytokinins. However, PBRs have not come into wider usage on field crops due to inconsistencies in yield and quality improvement. Also, currently PBRs have inadequate market potential.

CONCLUSIONS AND FUTURE THRUST

The present review suggests that foliar application of bio-regulators and nutrients can enhance seed yield and quality of many legume crops by affecting the physiological processes. Application of bio-regulators is also cost effective and can increase the yield in a shorter span. Being stress alleviating compounds, application of foliar bio-regulators will be beneficial for the farmers in increasing the production of legumes in the changing climatic scenario. Furthermore, most of the biological processes associated are polygenic so gene transfer may be difficult in the crops and hence the use of bio-regulators in legume may be beneficial for short imperatives. Bio-regulators provide an immediate impact on crop improvement programmes and are less time consuming. However, bio-regulators must be specific in their action and toxicologically and environmentally safe. There is need to evolve some bio-regulators which can inhibit photorespiration in plants and could be more beneficial in increasing the crop production. In addition, more research is needed to develop simple, economical and technical viable production systems for bio-regulators.

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