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# Plant Growth Regulators in Mulberry

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### Authors' contributions

This work was carried out in collaboration between both authors. Author TG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NM managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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## ABSTRACT

Plant growth regulators are organic compounds synthesized in specified plant parts in small quantity and are transported to the place of requirement leading to a change in physiological responses. Plant growth regulators can be classified into growth promoters and growth retardants. Plant growth regulators are auxins, gibberellin, cytokinin and growth retardants are Absciscic acid and ethylene. The latest one added to the growth promoter is Brassinosteroid, used to translocate the nitrogen and phosphorus. Triacontanol is one of the commercial formulations and used to increase the moisture and protein content of leaves, which ultimately built the disease resistance in silkworm. Plant growth promoting Rhizobacteria stimulates the plant growth regulators like auxins, gibberellins etc., and help in better nutrient uptake and increase tolerance. Vermicompost also contains some plant growth regulators. The combined effect of different plant growth regulators will give positive result in mulberry growth.

**Keywords:** Mulberry; silkworm; auxin; gibberellins; cytokinin; ethylene.

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## 1. INTRODUCTION

Plant growth regulators are organic compounds synthesized in specified plant parts in small quantity and are transported to the place of requirement leading to a change in physiological responses. The plant growth regulators play a vital role in mulberry leaf yield which leads to increase the cocoon yield.

The commercially available plant growth regulators are auxins, gibberellins, cytokinins, abscisic acid and ethylene. The newly included plant growth hormone as promoter is Brassinosteroid. Vermicompost and bio-fertilizers like Plant growth-promoting rhizobacteria (PGPR) are also having plant growth-promoting activity to increase the leaf yield and quality and also inducing the systemic resistance against the pathogen. Mulberry is the perennial crop and it is the sole food crop for silkworm, *Bombyx mori* L. Thus, the usage of plant growth regulators is also somewhat different. Under favorable conditions, plant growth promoters are increasing the sprouting of plants and enhance the leaf yield. Under unfavorable conditions, plant growth retardants are used to reduce the yield losses by reducing the evaporation and transpiration.

## 2. PLANT GROWTH REGULATORS

### 2.1 Auxins

Auxin is synthesis by Indole-3pyruvic acid (IPA) pathway by using the precursor Tryptophan. Plants largely produce auxin in shoot tips and translocate to roots. The primary auxin in plants is indole-3-acetic acid. The synthetic auxins are  $\alpha$ -naphthaleneacetic acid (NAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D).

Auxin, mainly used to induce the apical dominance, fruit development and lateral root formation. Generally, auxin and auxin like compounds are inducing root formation, increase in root number and length. IAA at 500 ppm were increased the leaf yield by 67% [1]. IAA (Indole-3-acetic acid) at 1000 ppm, improves leaf lobation and induces femaleness [2].

Das et al. [3] have shown that the leaf yield can be increased by 29% by spraying 250 ppm of NAA in cultivar S1 and also in case of local variety, which also gave net profit of Rs.1870/- per hectare per crop. The application of NAA at 500 ppm were reduced the lateral growth of plant [4]. NAA and IBA are more effective in inducing the rooting of plant. In soaking method, the

concentration of auxin is about 50 – 150 ppm (2 cm of basal portion is immersed for more than 16 hours) and 4000-7000 ppm in dip method (dip the 2 cm of basal portion for 2-3 seconds before planting in the field) [5].

Supplementation of sucrose at 2% with IAA or NAA or 2,4-D or IBA was also more effective in inducing rooting in Kosen variety [6]. Mukherjee and Sikdar [7] showed that in addition of vitamins (Thiamine) with sucrose at 2% and IAA or NAA or 2,4-D or IBA also induce rooting. IAA spray at later stages, considerably decreases the leaf senescence and leaf abscission, because auxin closely linked to the regulation of protein and RNA synthesis [8]. Application of TIBA (auxin transport inhibitor) to the shoot apex, the movement of auxin down to laterals inhibited and thus induces more growth of the laterals [9].

Auxin favors root formation, in tissue culture. After 30 days of growth shoot apices in MS medium, the shoots were transferred to MS medium containing 2.6  $\mu$ M NAA, 30 mg  $l^{-1}$  sucrose and 0.2% NaCl, for screening the salinity tolerance under in-vitro condition for root-related studies [10]. Successful root formation from calli in the presence of 1  $\mu$ M each of IAA, IBA and IPA supplemented individually [11].

Frequency of callus initiation was high on MS modified medium incorporated with 2.0 mg  $l^{-1}$  of 2,4-D, 100 mg  $l^{-1}$  of casein acid hydrolysate and 150 ml  $l^{-1}$  of coconut water.

Regeneration through organogenesis was achieved in six genotypes indicating genotypic specificity [12].

Incubation of the immobilized Subong cells in a full strength MS liquid medium containing 1 mg  $l^{-1}$  of 2,4-D, produced 4.2  $\mu$ g secreted rutin  $g^{-1}$  callus cells, which is the sum of 0.7  $\mu$ g endogenous rutin and 3.5  $\mu$ g secreted rutin  $g^{-1}$  of callus [13]. Treatment of cuttings with chemical formulations of 0.02% IBA + 0.2% p-HBA + 2% sucrose + 5% captan maximized rooting (96.05%), number of leaves per cutting (7.46), primary root number (12.56), primary root length (7.91 cm), fresh root length (8.62 g) and dry root weight (5.64 g).

IBA at 4000 ppm showed highest percentage of rooting, number of primary roots, length of longest primary root and length of basal portion of cuttings showing roots. IBA translocates poorly and remains near the site of application, and so it

was found to be one of the best rooting stimulator. NAA at 4000 ppm also noted similar differences and combination of IBA and NAA where 4000 ppm was also more effective and synergetically promoted secondary root to form profuse network). However, high level of auxins associated with comparatively reduced root growth would have retarded the carbohydrate metabolism and caused nutritional imbalance [14].

NAA applied as a spray reduced the number of lateral buds sprouting from both defoliated, decapitated erect shoots and intact horizontally trained shoots in the 10 yrs old plant [4].

IAA at 150 ppm recorded highest rooting (100%), length of the longest root per plant (19.20 cm), average number of roots per plant (10.60), survival (74%), number of leaves per plant (14.30), fresh weight of shoot (10.02 g/plant) and weight of root (1.34 g/plant) in S36. IAA at 200 ppm resulted in higher rooting (100%), length of the longest root per plant (21.16 cm), average number of roots (10.20), survival (86%), number of leaves per plant (13.50), fresh weight of shoot (9.94 g/plant) and fresh weight of root (1.66 g/plant) [15].

## 2.2 Gibberellins – Regulators of Plant Height

Gibberellins were isolated from the fungus *Gibberella fujikuroi*. Gibberellins are synthesized in apical portion of stems and roots. The main function is to stimulate stem growth through cell elongation and cell division, to promote seed germination and break the dormancy in seeds and buds. It slows the process of senescence (biological aging) by preventing the breakdown of chlorophyll in leaves.

Suzuki and Kitano [16] reported that GA stimulates shoot elongation but depresses the leaf enlargement and decrease the senescence. But GA found more effective under limited moisture conditions [17]. Jaiswal and Kumar [18] found that 100 or 300 ppm of GA induced male inflorescence. Kumar et al. [19] have shown that GA at 2.5 ppm in pollen germination medium containing 15% sucrose and 50 ppm boron, gave highest pollen germination and pollen tube growth. Saito and Morhashi, [20] showed that irradiation by red light enhances the seed germination by increased GA synthesis and with depression of endogenous auxin.

Pre-treatment with GA<sub>3</sub> significantly enhanced germination of non-stratified black mulberry

seeds ( $p < 0.01$ ). GA<sub>3</sub> treatments at 1000 and 2000 mg l<sup>-1</sup> concentrations yielded the highest germination percentage (60-67%). Seeds stratified for 100 days showed 88% germination. The combined treatment of 250 mg l<sup>-1</sup> GA<sub>3</sub> and 100 days of stratification yielded 96% germination of seeds [21].

The height of plants increased and the number of days increased with increasing concentration of GA<sub>3</sub>. GA<sub>3</sub> at 100, 200 and 400ppm concentrations decreased the production of female and increasing of male and mixed flowers respectively. The pollen viability is moderate and seed set percentage decreased with increasing concentrations of GA<sub>3</sub>.

Ponnaiyan and Vezhavendan [22] reported that the seeds were nicked and then treated with Gibberilic acid (GA) at 1000 ppm for a period of 24 hr was recorded the highest germination of 27% and 81% at the end of 3rd and 9th week after sowing respectively, in Indian mulberry (*Morinda citrifolia* L.)

Singh and Rai [23] showed that GA at 800 ppm followed by 1000 and 1200 ppm induced significantly higher percentage of germination (91.06, 87.9 and 80.4% respectively) against water treatment. Soaking for 24 h was best in this respect. Every increase in concentration of GA significantly decreased the time taken for germination (58.3 to 9.0 days), increased the height of seedlings (16.05 to 19.23 cm) and number of leaves per plant (9.24 to 13.10).

Foliar spray of 100 ppm GA<sub>3</sub>, in S-146 variety of *M. alba* recorded an increase of 29.75% in leaf area, 5.86% increase in leaf moisture content and 11.92% increase in foliage moisture retention capability over control in Doon valley condition [15].

The effect of foliar application of 10 ppm GA<sub>3</sub> were resulted in increased on leaf yield and leaf attributes on different genotypes S54 (797.4 g/plant), S799 (537.4 g/plant), S36 (611.8 g/plant), K2 (625.9 g/plant) and local (458.9 g/plant) [15].

## 2.3 Cytokinin – Regulators of Cell Division

Cytokinin, promote cell division, or cytokinesis, in plant roots and shoots. They are involved primarily in cell growth and differentiation, but also affect apical dominance, axillary bud growth, and leaf senescence. It largely produces in roots and translocates to shoot tip. Cytokinins

represented by kinetin, zeatin and 6-Benzylaminopurine (BAP). Kinetin was the first cytokinin discovered and so named because of the compounds ability to promote cytokinesis (cell division). Though it is a natural compound, It is not made in plants, and is therefore usually considered a "synthetic" cytokinin (meaning that plant hormone is synthesized somewhere other than in a plant). The most common form of naturally occurring cytokinin in plants today is called zeatin, which was isolated from corn (*Zea mays*).

Kinetin stimulates the root growth of mulberry cuttings and at 100 ppm increase the growth of axillary buds [24]. A decrease in strength of MS medium to 1/4 with 10 ppm of 6-benzyladenine (BA), resulted in more adventitious bud induction in isolated mulberry leaf taken from winter season [25]. BAP at 1.0 mg/l also induce adventitious buds from immature leaf cuttings on MS medium. Mulberry shoots with winter buds, when exposed to low temperature of 10°C for various length of time, it was found that an increase in duration decreased the ABA content and increased the sprouting [26]. Treating resting buds with BA at 50 or 100 ppm promoted sprouting. These treatments however, do not accelerate during the quiescent period of dormant buds [27]. BA at 8.8 µM, with the combination of 30 mg l<sup>-1</sup> sucrose and 1.0% NaCl in MS medium are used for screening the salinity tolerance under in-vitro condition for shoot related studies [10].

Dennis [11] showed that an MS medium supplemented with 2.5 µM BA was optimum for in vitro-raised seedlings of *Morus alba*. Rapid clonal propagation through in vitro culture of apical shoot buds and nodal explants from mature leaves in MS medium was supplemented with 2.2 – 4.4 µM BAP, in an attempt to induce bud break and multiple shoot formation. And also Dennis showed an efficient in vitro culturing method for germplasm preservation on a modified MS medium supplemented with 4.4 µM BA. However fructose was added along with that media somewhat soft so that the cultured buds were prone to sinking. This new culture method was most successful when the media were plant hormone free. Rooting of explants was also observed on the medium without supplemental regulators.

Female flower formation from immature leaves that were cultured on MS medium supplemented with 4.4 µMBA, 20 g l<sup>-1</sup> fructose and 4.0 g l<sup>-1</sup>

gelrite. The highest percentages of flower-bud formation were observed in genotypes Kanadasanso (6.1%) and Shin-ichinoso (2.4%). The ovaries of these flowers were swollen 70 days after culture initiation. On MS media supplemented with combination of 4.4 µM BAP and 4.6 µM Kinetin, were able to produce gynogenic plants from ovary culture of *M. indica*. Four plants developed from a single ovary within three weeks of culture [11].

Using an MS medium supplemented with 2.2 – 22.0 µM BA was able to produce multiple shoots from nodal plants of a 10- year old *M. laevigata*. Shoot proliferation was higher when the BA level was raised to as much as 11 µM, but further increases suppressed development [28].

Shoot apex and axillary bud explants of mulberry were cultured in liquid MS medium containing N-(2-chloro-4-pyridyl)-N'-phenylurea (a urea-type cytokinin) at 0.5-2.0 mg l<sup>-1</sup>. a single-step liquid medium which induces consecutive development of shoots and roots in a same medium and which yielded a high frequency of multiple bud bodies is useful for effective multiplication of mulberry plantlets with reduced labor and cost [29].

Bhau and Wakhlu [30] showed MS medium containing BAP at 1.5 mg dm<sup>3</sup> with sucrose resulted in increased in shoot multiplication. BAP was the most effective cytokinin for shoot induction. Sucrose was the most suitable carbon source examined for shoot multiplication. Nodal explants rooted on an auxin-supplemented medium. The acclimatized plants were successfully transplanted in the field.

Single node explants were excised and cultured on MS medium containing cytokinins. The number of shoots per explant increased with the increase of cytokinins up to 10 µM and declined in 15 µM. Maximum number of shoots was achieved in 10 µM BA followed by 10 µM kinetin. And maximum shoot length was also recorded in 5 µM BA [31]. Highest percentage of shoot regeneration (80 ± 6%) was obtained with genotype S799 on medium containing glucose and 8.9 µM BA [32].

The foliar spray of kinetin based PGRs (Biozyme) spray to mulberry plant before onset of water logging, showed that biozyme partially compensated the water logging effect and increased the leaf yield by 30% and improved the chlorophyll, sugar content and photosynthetic rate significantly [33].

## 2.4 Abscisic Acid–Stress Hormone

### 2.4.1 ABA synthesis from carotenoid intermediate

The pathway for synthesis of ABA is Terpenoid pathway. It induce seed dormancy and desiccation tolerance activate the closing of stomata. Mulberry is hypostomata type, which is having stomata on lower surface and inhibits growth of plant. The use of ABA in tissue culture is limited because ABA suppresses the metabolism of callus [17]. But it is useful for preservation of callus up to 20 weeks at 28°C. ABA levels increase positively with decreasing leaf water potential during drought condition. During stress condition synthesis of ABA inside the plant system cause to growth retardant and it reduce the respiration and evaporation. Ultimately it reduces the yield loss.

Abiotic stress is known to modulate the content of abscisic acid in leaves of higher plants and this growth hormone is known to regulate many developmental events in plant growth including seed maturation, dormancy stress tolerance and water relations [34].

Endoplasmic Reticulum -localized small heat shock protein (sHSP), designated WAP20 (20-kD). It used for the promotion of the renaturation of chemically denaturated citrate synthases and prevention of heat stress-induced aggregation of the enzyme. Transcript levels of WAP20 in the bark tissue were seasonally changed, showing high expression levels from mid-October to mid-December, and the transcript levels were additionally increased and decreased by cold treatment and warm treatment, respectively. WAP20 transcripts were detected abundantly in bark tissue rather than xylem and winter bud tissues during seasonal cold acclimation. The bark tissue specificity of WAP20 accumulation was also observed by exogenous application of phytohormone abscisic acid (ABA) in de-acclimated twigs, whereas WAP20 transcripts were increased in all of these tissues by heat shock treatment at 37°C in summer twigs. Thus, ABA involved in the expression of the WAP20 gene in bark tissue of the mulberry tree during seasonal cold acclimation [35].

### 2.5 Inhibitors GA Biosynthesis

Plant growth retardants are applied in agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant

productivity. Most growth retardants act by inhibiting gibberellin (GA) biosynthesis. To date, four different types of inhibitors are known such as Onium compounds, such as chlormequat chloride, mepiquat chloride, chlorphonium, and AMO-1618, which block the cyclases copalyl-diphosphate synthase and ent-kaurene synthase involved in GA metabolism [36]. Inhibitors of GA biosynthesis may be capable of up-regulating expression of several GA biosynthesis genes in roots, possibly to maintain normal root growth by a feedback regulation [37].

CCC and B-Nine are two synthetic ABA type growth inhibitors commonly used. CCC which inhibits the GA biosynthesis, plant height and internodal length has increased the leaf yield [38]. CCC spray induced femaleness in Kosen variety [15]. Counteracts the effects of auxins and gibberellins. ABA have inhibitory effect on growth and development of roots at the concentrations of 1.0 – 1.0 ppm and also promotes the rooting of cuttings [39]. Continuous application of ABA at 10 ppm in solution form found to depress the shoot elongation [16]. 500 ppm of ABA, abscission was favoured.

### 2.6 Ethylene

Important role of ethylene is signaling the plants during onset of leaf senescence and abscission in deciduous plants and ripening the fruits. Ethrel at 400 ppm also induce femaleness in mulberry [40]. Ethrel at 0.1 ppm, induced the rooting in mulberry cuttings but not further root growth [39]. Ethylene spray to axillary bud stimulates the onset of new shoot elongation at low concentration [8]. Continuous application of ethylene in solution form found to depress the shoot elongation [16].

Dennis [41], showed that 2-chloroethylphosphonic acid (ethrel) at 2000  $\mu\text{g l}^{-1}$  in MS medium supplemented with 5  $\mu\text{M}$  BAP were maximize the female inflorescence (13.6%). Silver nitrate at 2500  $\mu\text{g l}^{-1}$  in MS medium supplemented with 5  $\mu\text{M}$  BAP were maximize the male inflorescence (22.4%). Bisexual flowers were also observed along with male and female flowers in silver nitrate treated plants.

## 3. COMBINATIONS

The ratio of auxins and cytokinins influences the outgrowth of plants. High auxin:cytokinin ratio promotes activation of shoot branching. Low auxin:cytokinin ratio promotes activation of lateral

roots. Auxin and cytokinin compound, a biologically derived plant growth regulator yielded 6904 kg/ha/yr over the control resulted in a net profit of Rs.5425. Increase in growth and yield of mulberry by 17% with the use of auxin and cytokinin formulation [3]. Both auxin and cytokinin precursors increased the chlorophyll a and a/b ratio, higher photosynthesis and thus enhances the Leaf area index and leaf yield [42]. Auxin and cytokinin compound also increased the protein content over the control. Combinations of cytokinin and auxin found to be effective with other *Morus* species were less effective for *M. laevigata* [31].

The combination of zeatin and 2,4-D induced highest percentage of cell divisions (29%) followed with zeatin and NAA (10%). Specific role of the auxin dicamba is inducing cell divisions in mulberry which is different from other auxins like NAA and 2,4-D [43].

The ratio of IAA: GA was almost equal portion in the vegetative phase of the plant growth. During reproductive phase there was decline in the ratio of IAA:GA in the androecious member and in case of gynoecious member the ratio is increased [44]. Petkov [45] showed GA at 100-500 ppm with IBA at 100 ppm + urea 1000 ppm gave best germination percentage to an extent of 96% on 21<sup>st</sup> day after treatment compared to the untreated and water soaked controls.

Foliar spray of IAA and GA<sub>3</sub> were significantly improving leaf lobation and sex expression of Kajli and Mysore local cultivars of mulberry [33].

GA<sub>3</sub> and (S)-(+)-ABA, was significantly affect the generative growth of wild type 'Ichibei'. But vegetative growth was not affected by these treatments. The application of GA<sub>3</sub> had the strongest effect on the number of flowers in 'Ichibei'. In 'Ryoumenguwa', both vegetative and generative growth was significantly affected by GA<sub>3</sub> and (S)-(+)-ABA. It had the effect on the number of leaves and flowers. Adding GA<sub>3</sub> along with the BAP enhanced bud break frequency. MS medium supplemented with 4.4 μM BAP and 0.6 μM GA<sub>3</sub> accelerated the bud break from nodal explants and apical shoot buds and it enhanced frequency of *M. australis* in micro propagation techniques. Adventitious buds formed only on the MS medium supplemented with 4.40 μM BA and 0.54 μM NAA. Treatment with 8.8 μM BAP was most beneficial, producing the maximum number of cultures that showed shoot regeneration. These callus-derived shoots were then rooted on

2.69 μM NAA. BA (4.4 or 8.9 μM) and IBA (4.9 or 9.8 μM) added medium and its effect showed the maximum induction rate of 13.6% [11].

Ponchia and Gardiman [46] found that MS media supplemented with BA and NAA induced shoot proliferation in mulberry ('Florio' and 'Morettiana') at various times throughout the growing season.

The PGR combination zeatin (2.3 μM) and 2,4-D (2.3 μM) resulted in the highest number (29%) of cell divisions. Whole plants were obtained after culture of microcalli on MS medium containing TDZ at 4.5 μM and IAA at 17.1 μM. The regenerated shoots were rooted on MS medium supplemented with 4.9 μM IBA. With low revival rate during acclimation regenerated plants were established in the green house. Mesophyll tissue of mulberry is a good source material for protoplast isolation and culture [47].

Yew Lee [48] showed, Adding auxin such as IAA, 2,4-D and NAA enhanced the development of callus and adventitious roots and also increased the protein and rutin contents. Adding cytokinin such as BA and KN retarded callus and adventitious root development as well as the protein and rutin contents. The highest level of rutin was produced when adventitious roots were grown in a 34/66 ammonium/nitrate full-strength standard MS medium containing 5mg l<sup>-1</sup> IAA. The roots of the Sugye (*M. alba* L.) had the highest levels (242.2 μg/g fresh tissue) of rutin.

Incubation of the immobilized Subong cells in a full strength MS liquid medium containing 1 mg l<sup>-1</sup> of 2,4-D and 0.1 mg l<sup>-1</sup> of KN, produced the highest amount of rutin (8.2 μg/g callus cells, which is sum of 1.9 μg of endogenous rutin and 6.3 μg secreted rutin/g callus) level by 95% and GABA (305 μg/g callus cells- which is sum of 80 μg endogenous GABA and 225 μg secreted GABA/g callus) and secreted the largest amounts into the suspension media [13].

TDZ has shown both auxin and cytokinin like effects, although chemically, it is totally different from commonly used auxins and cytokinins. TDZ may modify endogenous plant growth regulators, either directly or indirectly and produce reactions in cell/tissue, necessary for its division/regeneration. Application of TDZ was used to induce the shoot formation and shoot formation with growth of large leaves, which the leaf and seeding were used as explant [49].

Primary cultures of *Morus alba* L. on MS medium supplemented with BAP (2 mg l<sup>-1</sup>) and NAA (0.2

mg l<sup>-1</sup>), proved best for multiple shoot formation. Multiplication was also achieved by MS medium fortified with BAP (2 mg l<sup>-1</sup>), NAA (0.2 mg l<sup>-1</sup>), aspergine (25 mg l<sup>-1</sup>) and glutamine (1.0 mg l<sup>-1</sup>). This medium facilitated the elongation of shoots and sprouting of axillary buds of in vitro grown shoots. About 80% rooting was obtained from shoot cultured on the MS medium supplemented with NAA (1.0 mg l<sup>-1</sup>). Plants with well-developed roots were transferred to soil with survival frequency of 70% [50].

Apical buds of mulberry (*Morus indica* L.) were cultured on LSBM fortified with 8.88 µM BAP in combination with 2 µM TIBA was the most suitable medium for initiation (94% response) and multiplication of shoots (10.6) in variety S54 [51].

#### 4. BRASSINOSTEROIDS

Brassinosteroids are a new type of polyhydroxy steroidal phytohormones with significant growth promoting influence [52,53]. Brassinosteroids (BRs) were discovered in 1970 by Mitchell and his co-workers [54] and were later extracted from the pollen of *Brassica napus* L. were discovered in 1979 by Grove and his co-workers [55]. BRs are considered ubiquitous in plant kingdom as they are found in almost all the phyla of the plant kingdom like alga, pteridophyte, gymnosperms, dicots and monocots [56]. BRs are a new group of phytohormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis, senescence etc. and also confer resistance to plants against various abiotic stresses [57].

Brassinosteroids (BRs) are a family of about 70 structurally related polyhydroxy steroidal phytohormones that regulate a number of physiological processes in plants. Among these, brassinolide (BL), 28-homobrassinolide (28-homoBL) and 24-epibrassinolide (24-EpiBL) are more common. Exogenous application of 24-EpiBL, increase the total soluble protein content and protect the plasma membrane from oxidative damage in maize plant [58].

Brassinosteroids are considered as plant growth regulators with pleiotropic effects, as they influence varied developmental processes like growth, germination of seeds, rhizogenesis, flowering and senescence. Brassinosteroids also confer resistance to plants against various abiotic stresses [57]. The function of BR is similar to auxin (promote stem elongation). Exogenous application of Brassinosteroid at 5µg/ml

increased the leaf yields in translocation of N and P [59].

Arteca and Arteca [60] reported that BRs induce exaggerated growth in hydroponically grown *Arabidopsis thaliana* and also control the proliferation of its leaf cells. BRs are a new group of phytohormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis, senescence etc. and also confer resistance to plants against various abiotic stresses [61,57]. BRs promote the growth of apical meristems in potato tubers [62], accelerate the rate of cell division in isolated protoplasts of *Petunia hybrida* [63] and also induce callus growth and regeneration ability in *Spartina patens* of poaceae [64]. BRs also play a prominent role in nodulation and nitrogenase activity of groundnut [65] and soya bean [66].

#### 5. PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

Rhizobacteria inhibit plant roots and exert a positive effect ranging from direct influence mechanism to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to the plants are termed as PGPR. Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth.

PGPR helps in stimulating plant growth in general and roots in particular as they serve various growth promoting regulators like auxin, gibberellins etc., vitamins and also for better nutrient uptake [67].

There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism: suppression of plant disease (Bioprotectants), improved nutrient acquisition (Biofertilizers), or phytohormone production (Biostimulants). If the crop attacked by pathogenic organisms Siderophores produced by some PGPR scavenge heavy metal micronutrients in the rhizosphere (eg., Iron) starving pathogenic organisms of proper nutrition to mount an attack of the crop.

Plant growth promoting bacterium applied as foliar spray on *Lycopersicon esculentum* and *Cucumis sativus* increased biochemical contents and growth parameters of plants. VAM also well known for phosphorus solubilization, increased plant nutrients uptake and in control root disease.

The biofertilizer formulations are Seri-Azo, Seri-Phos and Potash Mobilizing Bacteria. Application of VAM and Azotobacter has shown a way of saving on expensive fertilizers like Nitrogen and Phosphorus thereby improving leaf yield and quality [33].

The application of *Azotobacter*, Phosphate Solubilizing bacteria and VA-Mycorrhiza, were increased in yield at 20 kg/ha/yr *Azotobacter*, 5 kg/ha/yr Phosphate Solubilizing bacteria and 10,025 kg/ha/yr VA-Mycorrhiza compared with the control followed by the treatment with 50% reduction of nitrogen and phosphorus fertilizers (10,427 kg leaf yield/ha/yr) [68].

PGPR increased the systemic resistance against leaf rust and leaf spot. The disease severity of leaf rust was reduced to 34.8 and 38.5% in mulberry plant precultivated with bacterial isolates, *P. fluorescens* and *B. subtilis*, respectively, as against 81.3% in control. The severity of leaf spot was reduced to 31.2 and 35.2%, with *P. fluorescens* and *B. subtilis*, respectively as against 84.4% in control [68].

The *Azotobacter chroococcum* (Chamarajanagar or mysore strain) at 20 kg recorded minimum leaf spot disease severity from 5.1-9.6% followed by 10 kg/ha/yr Chamarajanagar or mysore strain (10.1%) [68].

## 6. VERMIWASH

Vermiwash contains valuable vitamins, enzymes and regulators like auxins, gibberellins etc. and 50 bacterial isolates obtained from the rhizosphere. It contains 86% could produce auxins, 58% gibberellins and 90% kinetin-like substances.

Plant growth promoter 'Phalda' is used as foliar spray which increase the growth and leaf yield of mulberry [33].

## 7. CONCLUSION

Due to increasing demand of silk and the limited arable land in the country, stress has been laid on the higher production and improvement of foliage quality to meet the growing demand of sericulture industry of the country. Mulberry (*Morus alba* L.) leaf is the main basic food plant of the silkworm (*Bombyx mori* L.), which converts leaf and protein into silk. The production of quality foliage can be increased by the increasing assimilation rate of the plant and directing its

movements to the foliage through the application of the plant growth regulators.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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