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Bhendi growth and biochemical characteristics in response to foliar application of plant growth regulators

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Abstract

The present study was aimed to find the stimulatory effect of certain plant growth regulators such as IAA, BAP, GA, Spic Cytozyme, and Aminos on the growth and biochemical characteristics of bhendi (*Abelmoschus esculentus* (L.) Moench). The results revealed that the plants growth regulators had a positive impact on bhendi plant growth as well as in the some biochemical aspects when they were sprayed as foliar. Further, the stimulatory effect with reference to shoot and root development was higher in plants sprayed with Spic Cytozyme. In the case of biochemical parameters, the stimulatory effect was varied among the plant growth regulators. There was a significant increase in all biochemical parameters except NR activity. There was not much variation in the NR activity with all plant growth regulators but better than the control plants.

Key words : Plant growth regulators, bhendi, growth, biochemical

INTRODUCTION

Plant hormones are signal molecules produced within the plant and occur in extremely low concentrations. They are not nutrients, but promote and influence the growth, development and differentiation of cells and tissues. The biosynthesis of plant hormones within plant tissues is often diffuse and not always localized. Plant hormones regulate cellular processes in targeted cells leads to determination of formation of flowers, stems, leaves and development and ripening of fruits. Plant hormones are affecting the seed growth, time of flowering, sex of flowers, senescence of leaves and fruits. The synthetic chemicals used to regulate the growth of cultivated plants, weeds and *in vitro* grown plants and plant cells; these manmade compounds are called Plant Growth Regulators (PGRs) [1].

In general, there are five major classes of plant hormones such as Auxins, Cytokinins, Gibberellins, Abscisic acid and Ethylene. These chemicals are each grouped together into one of these classes based on their structural similarities and on their effects on plant physiology. Some other plant hormones and growth regulators are not easily grouped into these classes; they exist naturally or are synthesized by humans or other organisms, including chemicals that inhibit plant growth or interrupt the physiological processes within plants. Each class has positive as well as inhibitory functions, and most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation.

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. The most common auxin found in plants is indole-3-acetic acid or IAA. Synthetic auxins are Naphthalene acetic acid, 2, 4 Dichlorophenoxy acetic acid *etc.* Cytokinins are a group of chemicals that influence cell division and shoot formation. The important cytokinins are Kinetin, Zeatin *etc.* Gibberellins are hormones that include a large range of chemicals that are produced naturally within plants and by fungi. They are the most powerful growth promoters because they increase internodes spacing, promote flowering, modify the flower sex expression, stem elongation, lowering, and breaking dormancy of seeds, buds, corms, and bulbs. There are over ninety forms of gibberellins, but GA is the most commonly used form. They are named as GA1, GA2, GA3 *etc*. Like natural phytohormones, they are various types of synthetic hormones commonly using in agricultural practices especially in horticultural crops. Spic Cytozyme is a biochemically active stimulant constituted by cytokinins, auxins, micronutrients. It supports the nutritional needs of plants during foliar spray of it since it contains auxins and cytokinins. It is mainly used by foliar spray. It is most commonly used in economically important plant for their improve yield. Aminos is the one of another plant growth regulator used to increase the growth and yield of crop plants. It contains amino acid, metal chelators, hydrolyzed proteins and phytostimulants [2].

The growth regulators stimulate and promote growth phenomenon in plants. Application of PGRs through foliar spray enhances crop nutrition in both conventional and alternative production systems. Observed effects of foliar fertilization have included yield increases, resistance to diseases and insect pests, improved drought tolerance and enhance the crop quality. The response is dependent on species, nature of PGR, concentration and frequency of application, as well as the stage of plant growth of horticultural and agronomic crops. But it is highly used for horticultural crops because horticultural crops are of higher value and their nutrient status is more carefully examined [3]. The role of foliar application of plant growth regulators on various physiological processes in plants is well known, which enable rapid change in the phenotype of the plant to achieve desirable results. The PGR are required by the plants for normal growth and development and have an important role in the translocation of photosynthates to the reproductive sinks, besides improving quality. Now-a-days foliar application of PGRs have assumed a greater importance in view of lower cost and higher efficiency compared to the traditional method of soil application. A considerable improvement in biochemical attributes of spinach was recorded due to the foliar application of PGRs [4 5].

MATERIAL AND METHODS

The seeds of bhendi *Abelmoschus esculentus* (L). Moench. and growth regulators/hormones were purchased from Agricultural Research Station, Kovilpatti, Tamil Nadu.

Experimental Details

Healthy and uniform bhendi seeds were chosen and sown in the pots contained red, black and sand soils (1:1:1). The experimental details were T 1 - Indole Acetic Acid (IAA); T 2 - BAP (Benzyl Amino Purine); T 3 - GA (Gibberellin); T 4 - Spic Cytozyme (Commercial formulation); T 5 - Aminos (Commercial formulation) and T 6 - Control.

Growth Analysis

Plant growth regulators were sprayed at 7 days interval. The growth parameters such as shoot length, root length, fresh and dry weight were analyzed in the treated and control plants. Plants were uprooted without causing any damage to the seedlings and it was thoroughly washed with tap water in order to remove soil and debris particle. Then the shoot length was measured with the help of meter scale. In uprooted plants, the root length was measured with the help of meter scale and expressed in centimeter. The fresh weight of whole plant parts (shoot, leaves and root) was weighed using the electronic balance. The fresh undamaged whole plant systems of seedlings were kept in the oven at 80°C 4-6 hours and the dried seedlings were weighed using the electronic balance.

Biochemical Analysis

The biochemical parameters such as chlorophyll [6], protein [7], glucose [8], free amino acid [8] and nitrate reductase activity [9] were analyzed. The chlorophyll content was calculated using the following formulae.

$$\text{Chlorophyll a (mg/L)} = (11.75 \times A_{662}) - (2.35 \times A_{645})$$

$$\text{Chlorophyll b (mg/L)} = (18.61 \times A_{645}) - (3.96 \times A_{662})$$

$$\text{Total Chlorophyll (mg/L)} = (7.79 \times A_{662}) + (16.26 \times A_{645})$$

Protein was estimated in the fresh leaves (500 mg) ground with 10 ml of distilled water using a mortar and pestle. The homogenate was centrifuged at 3000 rpm for 5 minutes. To 0.5 ml of the test solution, 5ml of alkaline copper mixture and 0.5 ml of Folin phenol were added. It was mixed thoroughly and kept in an undisturbed condition for 10 minutes for the development of blue colour. 5 ml of alkaline copper reagent, 4 ml of distilled water, and 0.5 ml of folin phenol reagent was used as blank. The absorbance was read at 650 nm using spectrophotometer (ELICO SL 171). The Protein content was estimated from a standard graph of Bovine serum albumin. The total glucose content was estimated using anthrone method. Fresh leaf material 100 mg was ground in 10 ml of distilled water using a mortar and pestle. The homogenate was transferred to a beaker and filtered through a cheese cloth. To 0.1 ml of supernatant, 0.9 ml of distilled water and 4 ml of anthrone were added in the test tube. The tubes were incubated in boiling water bath for 10 minutes. The tubes were allowed to cool and absorbance was read at 620nm. The quantity of glucose was estimated using standard graph prepared with glucose.

Fresh leaf material (100 mg) was ground in 10ml of ethanol using a mortar and pestle. The homogenate was centrifuged at 5000 rpm for 5 minutes. To 0.5 ml of supernatant, 0.5 ml of ethanol was taken in a test tube and added 2 ml of ninhydrin reagent. The tubes were incubated in boiling water bath for few seconds. The absorbance was read at 570 nm using ethanol as blank. The quantity of free amino acid was estimated by standard graph prepared with glycine.

NRA was estimated with leaf tissue (100mg) - fragmented into 2 mm segments and it was kept in incubation vials containing 5 ml of the medium for 1 hour at room temperature with periodical shaking. Aliquots of 0.5 ml from the vials of both control and experimental sets were analyzed for nitrate after 1 hour of incubation. To 0.5 ml of test solution, 1 ml of sulphanilamide and 1 ml of N-1-N were added and it was made up to 4 ml by adding distilled water. The mixture was allowed to colour development

Table 1: Response of plant growth regulators on the growth attributes of *Abelmoschus esculentus*

S. No.	Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
1.	Control	23.33 ± 0.788 (100)	8.3 ± 0.115 (100)	1.47 ±0.732 (100)	0.23 ± 0.680 (100)
2.	IAA	26.83 ± 0.272 (115)	13.0 ± 0.115 (116)	1.57 ± 0.010 (106)	0.30 ± 0.259 (130)
3.	BAP	30.7 ± 0.321 (131)	11.33 ± 0.219 (136)	2.25 ± 0.049 (152)	0.53 ± 0.017 (231)
4.	GA	29.8 ± 0.115 (127)	10.7 ± 0.058 (128)	1.70 ± 0.026 (115)	0.45 ± 0.016 (199)
5.	Spic Cytozyme	32.46 ± 1.010 (139)	13.8 ± 0.458 (159)	2.78 ± 0.032 (188)	0.60 ± 0.021 (264)
6.	Aminos	29.0 ± 0.265 (127)	10.3 ± 0.115 (124)	1.64 ± 0.381 (111)	0.35 ± 0.025 (153)

Table 2: Response of plant growth regulators on the biochemical attributes of *Abelmoschus esculentus*

S. No.	Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Free amino acid (mg/g LFW)	NRA (mg/g LFW)
1.	Control	0.91 ± 0.50 (100)	2.40 ± 0.41 (100)	0.56 ± 0.046 (100)	0.84 ± 0.014 (100)	0.47 ± 0.014 (100)
2.	IAA	0.97 ± 0.022 (105)	2.90 ± 0.23 (116)	0.88 ± 0.24 (157)	0.93 ± 0.739 (108)	0.52 ± 0.51 (113)
3.	BAP	1.26 ± 0.016 (137)	3.49 ± 0.03 (140)	1.60 ± 0.06 (285)	1.17 ± 0.061 (139)	0.58 ± 0.641 (123)
4.	GA	1.16 ± 0.96 (126)	3.36 ± 0.038 (135)	1.36 ± 0.16 (242)	1.11 ± 0.542 (131)	0.57 ± 0.381 (131)
5.	Spic Cytozyme	1.43 ± 0.016 (155)	3.25 ± 0.344 (130)	1.84 ± 0.16 (328)	1.44 ± 0.155 (170)	0.58 ± 0.848 (120)
6.	Aminos	1.02 ± 0.54 (110)	3.05 ± 0.027 (122)	1.12 ± 0.34 (200)	0.98 ± 0.027 (116)	0.55 ± 0.438 (117)

and absorbance was read at 540 nm.

Statistical Analysis

Morphological parameters were determined with five independent replicates. Biochemical characters and enzymatic assay were carried out at least three times. The data were reported as mean ± SE and in the figure parentheses represent the present activity.

RESULTS

The effect of foliar application of plant growth regulators such as IAA, BAP, GA, Spic Cytozyme and Aminos were studies on growth and biochemical characteristics of bhendi (*Abelmoschus esculentus*). Effect of growth regulators on the growth characters such as shoot length, root length, fresh weight and dry weight were analyzed in control as well as treated plants.

GROWTH CHARACTERS

The foliar application of Plant Growth Regulator (PGRs) were significantly increased the growth characters of bhendi. The response was varied with respect to types of PGRs. The application of plant growth regulators significantly increased the shoot length of seedlings which were sprayed with IAA, BAP, GA, Spic Cytozyme and Aminos. The increments over the control were found to be Spic Cytozyme sprayed plants (34.2 cm) followed by the BAP. Likewise, the root length was also found to be increased over the control as Spic Cytozyme and IAA produced taller root in bhendi followed by BAP, GA and Aminos. It was observed that the fresh weight was found to be increased in all growth regulators sprayed plants than control. The result revealed that the plant fresh weight was comparatively maximum (2.842 gm) in plant treated with Spic Cytozyme and least in the control plants. The plant dry weight was higher in plants sprayed with Spic Cytocyme and BAP than other treatments. Same trend was observed with respect to plant dry weight in plants sprayed with growth regulators (Table 1).

BIOCHEMICAL CHARACTERS

The foliar application of PGRs has a positive effect on the biochemical characters of bhendi (*Abelmoschus esculentus*) such as total chlorophyll, protein, amino acids, glucose content and NR activity. The results revealed that the biochemical characters of bhendi greatly affected by the application of various PGRs. The total chlorophyll content was higher in plants sprayed with Spic Cytozyme followed by BAP. The results also revealed that the protein content was found more in those seedlings sprayed with BAP and least in IAA and control. There was not much variation in protein content in plants sprayed with GA, Spic Cytozyme and Aminos. The glucose content was found to be increased in plants sprayed with Spic Cytozyme followed by BAP. Likewise, the free amino content was higher in plants with Spic Cytozyme followed by BAP. NRA was found to be increased in PGR sprayed plants over the control. There was not much variation between the treatments (Table 2).

DISCUSSION

GROWTH ATTRIBUTES

It has been observed that the plant growth regulators such as IAA, BAP, GA, Spic Cytozyme and Aminos in their individual had increased the growth characters such as shoot length, root length, plant fresh weight and dry weight. In the PGR sprayed plants, the growth characters of bhendi such as shoot length, root length, plant fresh weight, plant dry weight were significantly increased. The effect was varied based on the nature of action of different growth regulators. In general, all plant growth regulators are having their own morphological and biochemical/ physiological role in plants. Based on their role, the effect was differed in bhendi plant. The obtained results were supported by several researchers. The foliar application of NAA in

combination with $MgSO_4$ increased the number of monopodial and sympodial branches, number of good bolls per plant, average boll weight, seed weight per plant, number of seeds per boll compared to control. Further, the application of NAA @ 40ppm significantly increased the boll set percentage, boll weight, number of bolls and seed cotton yield per plant [10 11] .

The foliar application of growth hormones had a unique role in the up regulation of vegetative and reproductive growth of plants [12] and also documented the role of GA_3 as a regulator of plant growth [13]. The application of GA3, IAA and Kinetin in *Cicer arietinum* L. significantly increased the length of shoot. Further, the IAA and Kinetin more or less promoted expansion in diameter but decreased the extension growth. IAA and Kinetin promoted the initiation of cambium and maturity of metaxylem elements. In the mixed doses of GA3 + IAA and GA3 + Kinetin, increase in length as well as in the diameter was observed [14].

The plant growth regulators are having the ability to increase inter node and promote following. The similar result was obtained in the bhendi plant in their study [14].

Plant Growth Regulators (PGRs) such as cytokinin, auxins, naphthylacetic acid, and chlorophenoxyacetic acid were induced the tomato yields by increasing the truss mass of tomatoes (*Lycopersicum esculentum*). Synthetic auxins were increased the average fruit mass as well as the number of fruit with a diameter. Further, the application of PGR provided the most promising results by improving the yield of marketable fruit through increases in the number of fruit set, the number of fruit per truss and the overall truss mass [15].

The role of various commercial plant growth regulators such as synthetic cytokinin (CPPU), auxins, 4-chlorophenoxyacetic acid (4-CPA), gibberellins (GA3), 'ProGibb and SupaGibb were improved the fruiting characteristics tomatoes up to three times successively. Further, the promising results by improving the yield of marketable fruit through increases in the number of fruit set, the number of fruits truss and the overall truss mass [15].

BIOCHEMICAL ATTRIBUTES

Further, it was observed that the bhendi seedlings treated with the different growth regulators significantly increased the biochemical characters such as chlorophyll contents, protein content, free amino acid, glucose content over the control plants. The application of NAA had increased the rate of photosynthesis besides other promotional effects in cotton. Protein and glucose were the other two important biochemical constituents which recorded phenomenal enhancement in the treated plants over the control plants [16 17]. With the increase on the amount of protein there was a concomitant increase in the concentration of free amino acid also as observed by [18].

Similarly, the effect of plant growth regulators resulted in increase in chlorophyll content and fruiting nodes in cotton [19] and physiological effect of PGRs on groundnut at 120 ppm increased chlorophyll content and total chlorophyll and NR activity [20]. The enhancement of chlorophyll content in cycocel treated cotton leaves [21 22]. The foliar application of NAA, Triacontanal and Salicylic acid increased the content of chlorophyll, soluble protein, Nitrate reductase activity (NRA), grain yield, grain protein and total sugar content in pearl millet [23]. Foliar application of benzyl adenine (b-benzyl amino purine (BAP)) three or four times during the entire life period of the crop resulted delay in decline of the photosynthetic rate as observed by Srivastava and Sairam [24]. With the increase on the amount of protein, there is a concomitant increase in the concentration of free amino acid also as observed by Kutty and Phillips [18].

Nitrate reductase is the first and most important enzyme in the overall nitrogen metabolism of the plant. In the leaves, nitrate reduction is closely associated with photosynthetic process. Reduced pyridine nucleotides for reduction of nitrate are obtained from the oxidation of Calvin cycle intermediates [25 26]. It is believed that the reduction of nitrate to nitrite by nitrate reductase activity is the rate of limiting process to the utilization of nitrogen in the form of nitrate.

Response of field grown mature tea to the foliar application of Biozyme Crop Plus, a commercial plant growth regulator, applied at different concentrations ranging from 250 to 1000 ppm was evaluated. Tea shoots were analyzed to determine their N, P, K, Ca, and Mg contents. The treated tea plant shoots contained significantly higher contents of N and K followed by Ca and Mg than the untreated control plant shoots. The total chlorophyll content of Biozyme treated tea shoots was also increased significantly [27]. An increase in the amount of total chlorophyll content in apple leaves and proved to be a photosynthetic rate promoter [28 29] with the foliar application of plant growth regulators.

CONCLUSION

The application of plant growth regulators (PGRs) such as IAA, BAP, GA, Spic Cytozyme and Aminos were significantly increased the growth such as shoot length, root length, fresh weight and dry weight and biochemical characteristics of *Abelmoschus esculentus*. The growth response was varied with respect to PGRs. It was observed that the both shoot and root length was higher in plants treated with Spic Cytozyme. The plant fresh and dry weight was higher in plants sprayed with Spic Cytozyme followed by BAP. Like growth characters, the biochemical characters were also responding well to the PGRs. The total chlorophyll and glucose content was higher in plants sprayed with Spic Cytozyme but in the case of protein it was found more in seedlings treated with BAP. Nitrate reductase activity was higher in plants those sprayed with Spic Cytozyme and BAP. Among the PGRs, the Spic Cytozyme was respond better to bhendi (*Abelmoschus esculentus* (L).Moench) with respect to growth as well as biochemical characters.

The role of foliar application of plant growth regulators to enable rapid change in the phenotype of the plant to achieve desirable results. The PGR are required by the plants for normal growth and development and have an important role in the translocation of photosynthates to the reproductive sinks, besides improving quality. Now-a-days foliar application of PGRs have assumed a greater importance in view of lower cost and higher efficiency compared to the traditional method of soil application.

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