



ENHANCED PHENOLS ACCRETION, PHOTOSYNTHETIC PIGMENTS AND PROTEIN CONTENTS IN
CASSIA ANGUSTIFOLIA BY USING PLANT GROWTH REGULATORS

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ABSTRACT

The present investigation was carried out to study the effect of Plant Growth Regulators (PGRs) on biochemical constituents of *Cassia angustifolia*. The foliar application of PGRs like Gibberellic acid (GA₃), Indole acetic acid (IAA) and the growth retardant Abscisic acid (ABA) were applied at the concentrations of 25, 50 and 100 mg/L as foliar spray at 45 DAS up to the flowering stage at the interval of fifteen days. The physiological analyses were carried out on pre-flowering stage (75 DAS), flowering stage (90 DAS) and post-flowering stage (120 DAS). Results revealed that there was positive and highly significant increase in photosynthetic pigments, proteins and phenols due to all the concentrations of PGRs used. The improvement in basic metabolites through PGRs application may probably lead to enhancement in secondary metabolite contents. From the results of the present investigation, we can conclude that all the treatments of PGRs enhance the contents of photosynthetic pigments, proteins and phenols. The most important antioxidant like phenols having pivotal role in abiotic stress tolerance, were significantly increased at pre flowering, flowering and even post flowering stage, due to the application of ABA. This increase of phenols by PGRs treatments may be further enhancing the antioxidant capacity of Senna. The increase in phenolics in the leaves of Senna might be playing important role for stimulating the secondary metabolites in it.

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INTRODUCTION

The *Cassia angustifolia* belongs to family Caesalpinaceae, which is commonly known as Senna. The medicinal active compound of Senna is sennoside A and B, are the two anthroquinone glycosides that are responsible for purgative action of it (Aktar *et al.*, 2008). However, leaves of this plant are in demand internationally and preferred as ingredient of herbal tea in Europe. India is also the largest producer and exporter of Senna leaves, pods and total sennosides to the world market. There is pressing global demand for Ayurvedic drugs, hence standardization of cultivation practices and improvement in productivity of therapeutically active secondary metabolites is the urgent need. For this physiological investigations were carried out on traditional medicinal plant *Cassia angustifolia* Vahl. by using foliar treatments of different PGRs. Different strategies like, use of dormancy breaking agrochemicals, proper irrigation and fertilizers, hybrid seeds, plant growth regulators (PGRs) etc. are generally used to achieve vigorous growth and to enhance the flowering, fruiting, yield and production of commercially important secondary metabolites. Amongst these, use of PGRs is proven and widely used technique for different crops including medicinal plant to achieve these objectives.

The term plant growth regulating substances include both naturally occurring and synthetic growth substances, and include both growth promoters and growth retardants. They control and regulate the growth, metabolic processes and developments in plants by physiological manipulation. The use of PGRs have emerged as an important tool in improving agricultural production and to help in removing many of the barriers imposed by heredity and/or environmental stress. The major types of plant bio-regulators are auxins, gibberellins, cytokinins, abscisic acid and ethylene.

Auxins is the first hormone to be discovered in plants, which regulate polar translocation, apical bud dominance, root initiation, delay in abscission, vascular differentiation, floral bud formation and fruit development. Gibberellins are commonly used as growth enhancers because they stimulate cell division, cell growth or increase wall plasticity and the transcription of genes for α -amylase synthesis. Growth retardants are also used in crop improvement programs. These induce variety of morphological and biochemical responses in plants, including retarded shoot elongation, stimulated rooting and protection from various environmental stresses. Hence the study was conducted to revise the effect of foliar spray of PGRs on the biochemical constituents of medicinal plant *Cassia angustifolia* under natural condition.

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MATERIALS AND METHODS

Authentic seeds of Senna were obtained from National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat. Field culture experiments were conducted in the research field of Department of Botany, University of Pune, from October 2007 to May 2010 to determine the effect of foliar application of PGRs on the biochemical constituents of Senna. All experiments were conducted in a completely Randomized Block Design. The pre-soaked seeds of Senna were sown in ridges and furrows cover with thin layer of soil. The distance between two plants was 30 cm and the distance between two rows was 40 cm. Each treatment had 3 replications and each replication had 10 seedlings. Light irrigation was immediately given after seed sowing. Standard inter cultivation practices were used throughout the experiment. Temperatures during the experiment were in the range of 28-30 °C during day and 19-21 °C at night. The foliar spraying of PGRs was applied at 45 DAS till flowering at the interval of 15 days. The control plants were sprayed with DW. Freshly prepared solutions of Gibberellic acid (GA₃), Indole acetic acid (IAA) and the growth retardant Abscisic acid (ABA) in the concentration of 25, 50 and 100mg/L using 1.5, 2.5 and 3.5 liter of solution respectively for each experimental plot. PGRs were dissolved in 95% ethanol and then brought to final concentration using distilled water (DW).

Ten randomly selected third leaf from top of each plants from control and treatment was selected at 75 DAS (pre-flowering stage), 90 DAS (flowering stage) and 120 DAS (post-flowering stage) for biochemical analysis. The veins of the compound leaves were removed and the composite sample of leaflet was used for biochemical analysis.

Biochemical analysis

Determination of chlorophyll

Extraction of chlorophyll pigments was carried out by the method of Shoaf and Lium (1976) by using dimethyl sulfoxide (DMSO). The fresh leaf samples (1 g) were incubated in 7.0 mL DMSO at 65 °C for 30min. At the end of the incubation period, supernatant was cleaned, discarding the leaf tissues. The volume was made up to 10 mL with DMSO and its absorbance was measured at 645 and 663 nm in spectrophotometer. (UV-VIS spectrophotometer) using DMSO as blank. Chlorophyll a, chlorophyll b and total chlorophylls were calculated by using different formulae.

Determination of protein

Proteins were estimated by using Lowry *et al.* (1951) method. The fresh samples (100 mg) were homogenized in 2.5 mL of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged and 0.2 mL extract was used to prepare the reaction mixture. Volume was adjusted to 1 mL with distilled water. 5.0 mL reagent 'C' (alkaline copper solution) was added. Then 0.5 mL reagent 'D' (Folin-Cicalteau reagent) was mixed and incubated at room temperature for 30 min. The blue colour developed was read at 660nm on UV-visible spectrophotometer (Shimadzu- 1601).

Determination of phenol

Fresh samples of seedlings and leaf (1 g) were homogenized in 80 % ethanol repetitively and final volume was made up to 10 mL. The mixture was sonicated for 15 min to complete the extraction and it was centrifuged at 9000 rpm for 10 min. The supernatant was utilized for analysis of total phenolic contents by Folin-Cicalteau reagent (Farkas and Kiraly 1962), using tannic acid as the standard. The total polyphenolic content was calculated from a calibration curve.

Statistical analysis

The data were presented as the pooled means of three replicates. The data of field analyses was recorded for three consecutive years (2007-08, 2008-09 and 2009-10) presented pooled means. The significance of the mean differences was explored through one-way-ANOVA statistics followed by DMRT (Duncan's multiple range test) at p=0.05 as a post hoc test. SPSS for Windows ver. 11.5 and Microsoft Excel 2007 were used to carry out statistical analyses and graphical data presentations, respectively.

RESULTS

Photosynthetic pigments

The results on photosynthetic pigments (Chl a, Chl b and total chlorophylls) at pre flowering, flowering and post flowering stages presented in Figs.1 a,b,c revealed that in general at all the stages with all the PGRs the photosynthetic pigments were enhanced over control. During pre flowering stage the treatment of GA₃ (100 mg/L) was found effective to enhance the total chlorophyll pigments by 9.34 % over control. However during flowering and post flowering stage it was less effective as compared to the other PGRs. During flowering and post flowering stage ABA (100 mg/L) caused increase in total chlorophyll contents by 18.23 % and 25.16 % as compared to other PGRs and control. Next to ABA (100 mg/L), the treatment of IAA (100 mg/L) was found very effective to enhance total chlorophylls by 14.27 % and 23.72 % over control respectively.

Proteins

In the present investigation the results on changes in protein contents due to the application of different concentrations of PGRs showed in Fig. 2 Indicated that with all the treatments of PGRs the contents of protein were increased over control at all the three stages. At pre flowering stage the protein contents were enhanced by GA₃, IAA and ABA (100 mg/L) by 21.53 %, 23.5 % and 21.53 % respectively over control. At flowering stage GA₃ was less effective as compared to IAA and ABA for enhancing protein contents. At flowering and post flowering stage proteins were increased to maximum due to the treatments of IAA and ABA (100 mg/L), which caused 28.13 % and 13.4 8% increase over control.

Phenols

The results recorded in Fig 3 revealed that the phenols were increased with ABA (100 mg/L) at pre flowering, flowering

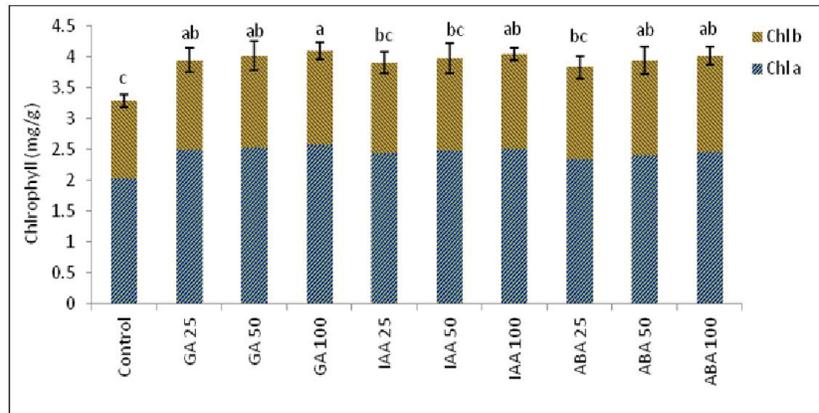


Figure 1a. Effect of PGRs on chlorophylls in field grown Senna at pre flowering stage

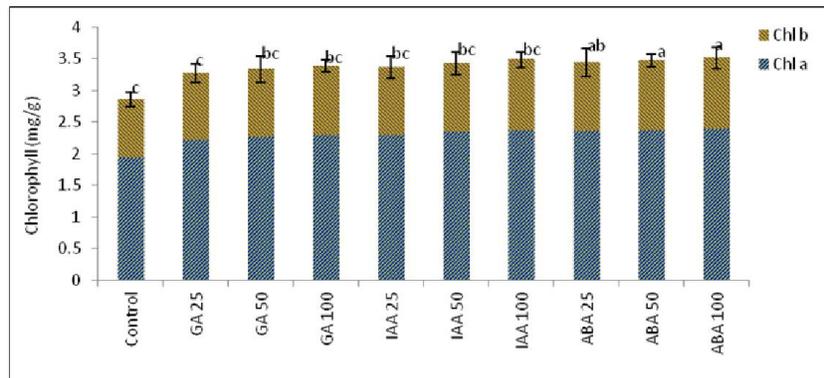


Figure 1b. Effect of PGRs on chlorophylls in field grown Senna at flowering stage

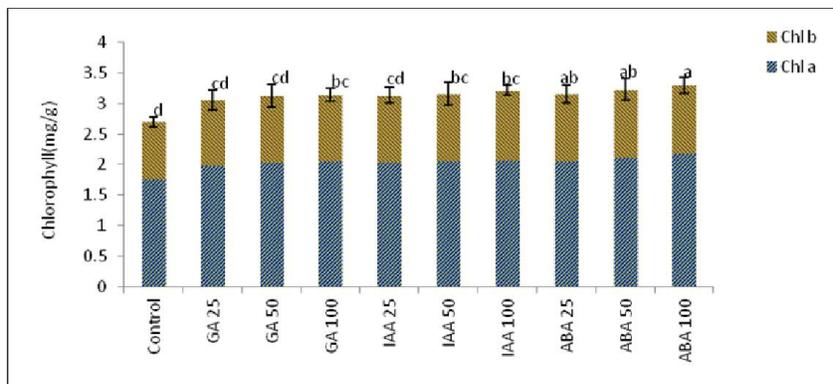


Figure 1c. Effect of PGRs on chlorophylls in field grown Senna at post flowering stage

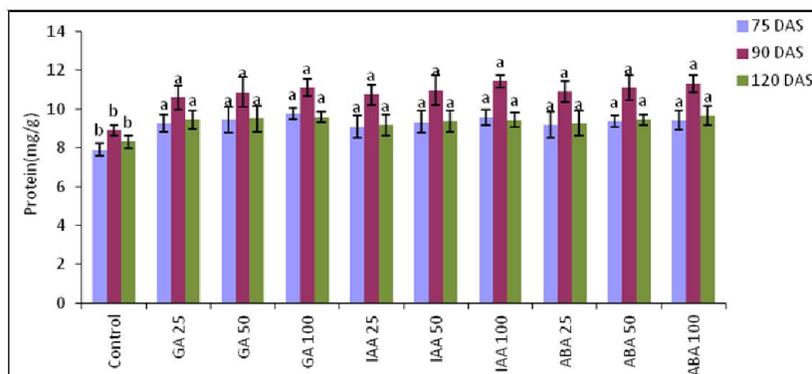


Figure 2. Effect of PGRs on protein in field grown Senna at different phenological stages

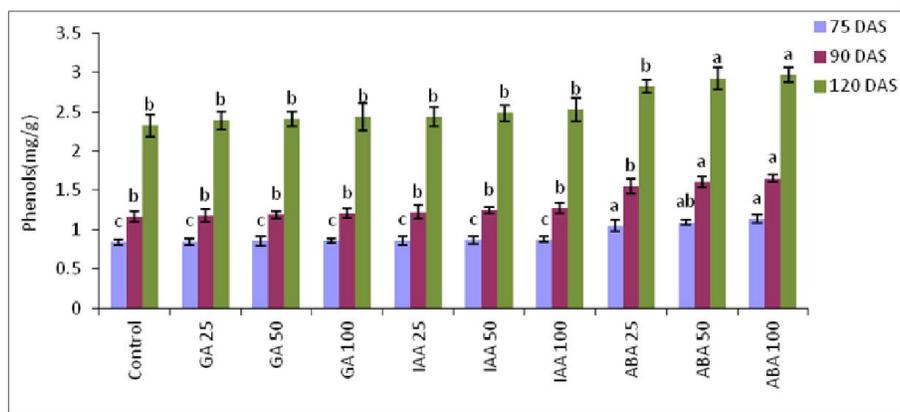


Figure 3. Effect of PGRs on phenols in field grown Senna at different phenological stages

and post flowering stage by 35.48 %, 42.36 % and 27.86 % respectively over control. Next effective treatment was IAA 100 mg/L at the all stages of growth of Senna, which caused the increase by 3.8 %, 9.26 % and 8.91 % respectively over control. While GA₃ treatments were not so effective to enhance phenol contents. The results of present investigation on phenol contents, revealed significant increase due to the treatment of ABA (100 mg/L) at pre flowering, flowering and post flowering stages over control in Senna. Next effective treatment was IAA (100 mg/L), while all the GA₃ treatments were not so effective to enhance phenol contents.

DISCUSSION

Photosynthetic pigments

The treatments of PGRs are generally influence to cause increase/decrease in photosynthetic pigments, and *Cassia angustifolia* has followed the same trend. The results regarding photosynthetic pigments when treated with different concentrations of GA₃, IAA and ABA caused increase in total chlorophyll contents in many medicinal plants is well documented for *Mentha spicata* (Singh and Mishra, 2001) and black cumin (Shah *et al.*, 2007; Shah, 2008). The results recorded in the present investigation with GA₃ (100 mg/L) in *Cassia angustifolia* are in agreement with above findings. Similarly Afroz *et al.* (2005) had also reported the increase in chlorophyll contents with the application of GA₃ 50 ppm in mustard. Yadav *et al.* (2008) also reported that GA₃, IAA and CCC have caused increase in the chlorophyll contents of pea. Kar *et al.* (1989) in safflower showed that growth retardants like Cycocel and Aminozide maintained which level of chlorophylls.

Tomas *et al.* (2005) have reported that the GA₃ and IAA were responsible for increasing chlorophyll contents in flax (*Linum usitatissimum*). Increase in photosynthetic pigments was reported by Sriharan *et al.* (2005) in black gram with the application of different PGRs. Effect of IAA for increasing the chlorophylls was reported in *Cymbopogon flexuosus* (Misra and Srivastava, 1991) and *Solanum khasianum* (Borse *et al.*, 2000). Increase in photosynthetic pigments was reported by Sriharan *et al.* (2005) in black gram with the application of

different PGRs. Enhancement in chlorophylls due to the treatments of different growth retardants was also reported in *Solanum tuberosum* (Sharma *et al.*, 1998). The treatments of different growth retardants responsible to increase the chlorophylls in tomato (Muller and Schuphan, 1976). Mepiquat chloride, chloromequat chloride, and daminozide also have been associated with increased photosynthesis (Wu *et al.*, 1985, Gardner, 1988, Nepomuceno *et al.*, 1997) through increased total chlorophylls. Zakaria *et al.* (2001) noted that increased photosynthesis greatly increased flowering, boll retention, and yield of *Gossypium barbadense*.

The increase in chlorophyll contents in PGRs treated plants may be attributed to increased content of Fe, Mg and N in these plants, as these elements have significant role in chlorophyll structure, biosynthesis and functioning. Plant growth regulators are known to regulate primary and secondary metabolisms through regulation of enzymatic activities (Normanly *et al.*, 1995, Heldt, 1997). The changes in metabolic activities might be indirectly or directly contributing to the synthesis of photosynthetic pigments. The increase in chlorophyll contents of *Cassia* with different PGRs such as GA₃, IAA and ABA can be attributed to above mention reasons. There was significant increase in nitrogen, iron and manganese content of *Cassia* with the treatments of PGRs and micronutrients. As recorded by Gadallah (1999) the PGRs like ABA might be giving stability to chlorophylls and which might be resulting into their enhancement and improved photosynthetic. In resulting in to improvement in growth, yield and other attributes.

Proteins

The results on changes in protein contents due to the application of different concentrations of PGRs showed that with all the treatments proteins have increased over control. Further it was noted that upto flowering stage the contents of proteins in leaves of Senna were increased. However during post flowering stage there was slight decrease. During pre flowering and flowering stage the proteins were enhanced by IAA (100 mg/L), while during post flowering stage it was less effective as compared to GA₃ (100 mg/L) and ABA (100 mg/L). Similar trend was recorded by Borse *et al.* (2000) in *Solanum khasianum*. They noted significant enhancement in

proteins due to IAA. The positive influence of NAA and IAA on protein contents was also reported by Sivakumar *et al.* (2001) in pearl millet. The results of present study are in agreement with the above findings. The results on protein content in tomato (Muller and Schupan, 1976) have supported the above trend. Increase in protein content due to GA₃ treatment was recorded in tobacco (Ho and Loo, 1979). In present investigation the contents of leaf proteins were enhanced in GA₃ (100 mg/L) treated *Cassia angustifolia* which is in agreement with the above findings. Shah *et al.* (2007) in black cumin and Vani *et al.* (2004) in baby corn had noted significant increase in proteins due to GA₃ treatments.

Similar trend indicating increase in proteins due to PGRs treatment in different medicinal and crop plants was recorded by Gadil *et al.* (2006), Sritharan *et al.* (2005), Muthukumar *et al.* (2005) and Sivakumar *et al.* (2001) in various plants including medicinal plants like ber, black gram, baby corn etc. Mahmoud *et al.* (1986) also reported increased protein contents in tomato and *Capsicum* due to the treatments of CCC. While Sawan *et al.* (1991) studied that application of growth retardant like mepiquat chloride, daminozide and Cycocel to cotton plants increased protein contents. Hedin *et al.* (1988) found that Cycocel increased protein content by 17–50% in leaves of cotton. Kar *et al.* (1989) in safflower showed that Cycocel and Aminoazide caused increased protein contents. Kler *et al.* (1991) found that foliar application of Cycocel (40, 60 and 80 ppm) caused the increase in proteins. The increase in protein contents due to the application of various PGRs like GA₃, NAA, ABA, CCC etc was attributed to increased uptake of nutrients particularly of nitrogen from the soil, due to PGRs, which further assimilated in protein. The general effect of the PGRs on enzymes involved in protein synthesis. The increased protein content may be ascribed to stimulation of protein synthesis process in PGRs treated plants. The increased protein contents in PGRs treated *Cassia* may be attributed to above cited reasons.

Phenols

Enhancement in phenolic contents due to application of ABA was reported in *Ocimum sanctum* by Gopi *et al.* (2009). The results of present investigation are in agreement with the above findings. Mahmood and Saxena (1986) recorded increase in phenols due to the application of growth regulators like IAA, IBA and IPA in tomato. Reda *et al.* (1972) also noted enhancement in phenols due to GA₃ in *Atropa belladonna*. Kadiogul and Atalay (2002) noted decrease in phenolic due to the treatments of various PGRs like GA₃, IAA etc in *Diospyros lotus*.

The enhancement in phenolics due to the treatments of various types of PGRs had different types of impact on normal functioning of the plants. Usually these compounds had greatly influence on auxins and their by cause positive influence on growth and developments of plants (Gantzer, 1960, Kefeli and Kutacek, 1977; Basak *et al.*, 1995). As suggested by Stenlid (1968) plants also interfere with GA₃ and Cytokinins and manipulate the growth as well as yield in treatments. They also influence the uptake of nitrates and phosphates. As suggested by Jaleel *et al.* (2007) phenols had great role in abiotic stress

tolerance as they are intermediately ROS acceptors. The phenolic compounds prevent auxin oxidation. There by increasing the auxin level in plants. This increased auxin level might be resulting in to vigorous growth of treated plants of Senna. The increased carbohydrates noted in present investigations might be associated with the enhanced level of auxin and polyphenols.

Abscisic acid commonly considered as a stress hormone has been implicated on plant acclimatization and protection against various biotic and abiotic stresses. It has been suggested that peroxidase could act as efficient H₂O₂ scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbate (Jaleel *et al.*, 2007). There proposed a hypothesize that a cycle where H₂O₂ is scavenged by phenolic compounds. Phenolics are oxidized to phenoxyl radicals. This phenoxyl radicals reduces the ascorbic acid into mono dehydro-ascorbate. This increase of phenols by triazoles may be further enhancing the antioxidant capacity of Senna. The increase in phenolics in the leaves of Senna might be playing similar role.

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