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RESEARCH ARTICLE

# ABA and $GA_3$ affect the growth and pigment composition in *Andrographis paniculata* Wall.ex Nees., an important folk herb

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**Abstract** In this study, 5 μmol·$L^{-1}$ abscisic acid (ABA) and gibberellic acid ($GA_3$) were used to study the effect of both growth regulators on the morphological parameters and pigment composition of *Andrographis paniculata*. The growth regulators were applied by means of foliar spray during morning hours. ABA treatment inhibited the growth of the stem and internodal length when compared with control, whereas $GA_3$ treatment increased the plant height and internodal length. The total number of leaves per plant decreased in the ABA-treated plants, but $GA_3$ treatment increased the total number of leaves when compared with the control. Both growth regulators (ABA and $GA_3$) showed increased leaf area. ABA and $GA_3$ treatments slightly decreased the total root growth at all the stages of growth. The growth regulator treatments increased the whole plant fresh and dry weight at all stages of growth. ABA enhanced the fresh and dry weight to a larger extent when compared with $GA_3$. An increase in the total chlorophyll content was recorded in ABA and $GA_3$ treatments. The chlorophyll-a, chlorophyll-b, and carotenoids were increased by ABA and $GA_3$ treatments when compared with the control plants. The xanthophylls and anthocyanin content were increased with ABA and $GA_3$ treatments in *A. paniculata* plants.

**Keywords** Andrographis paniculata, chlorophyll, growth, xanthophylls, anthocyanin, carotenoids

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## 1 Introduction

Abscisic acid (ABA) is a plant growth regulator that has been identified as a messenger in stress-perception-response pathways (Zhang et al., 2006) such as drought, high temperature, low temperature, and salinity stress (Leul and Zhou, 1998). Many studies have shown that ABA can enhance the tolerance of plants to environmental stresses. ABA is involved in many physiological processes. It has been demonstrated that ABA plays an important role in stomatal movements, regulation of photosynthetic enzyme activities, stability of photosynthetic apparatus, and gene expressions involved in chloroplasts. A major physiological effect induced by ABA on plants is the closure of stomata, which inevitably leads to a decrease in photosynthetic $CO_2$ assimilation. Because $CO_2$ assimilation is the main sink for leaf-absorbed light energy, a decrease in $CO_2$ assimilation can potentially expose plants to excess excitation energy, which, if not safely dissipated, may result in photo damage to PSII because of an overreduction of reaction centers. Indeed, it has been reported that ABA-induced stomatal closure results in increased susceptibility to photo damage, which is associated with the destacking of thylakoid membranes and rupturing of the chloroplast envelopes (Jia and Lu, 2003).

Gibberellic acid ($GA_3$) is a member of a type of plant hormone called Gibberellins, which regulate the growth rate of plants. There are more than 100 gibberellins now known in plants, and many of them are intermediates or by-products of the biosynthetic pathway. Gibberellins are involved in several plant development processes, and they

promote a number of desirable effects including stem elongation, uniform flowering, reduced time to flowering, and increased flower number and size (Jaleel et al., 2007). However, certain concentrations of $GA_3$ may cause excessive stem elongation and result in poor-quality plants (Srivastava and Srivastava, 2007). It was apparent that treating plants with $GA_3$ increased the height of the plants compared with the control, which may be attributed to the growth-promoting effect of $GA_3$, which stimulated and accelerated cell division, thus increasing cell elongation and enlargement or both (Paroussi et al., 2002). Several studies have revealed the effectiveness of $GA_3$ on increasing plant height and flower stalk length (Makoto, 2003).

*Andrographis paniculata*, commonly known as the "king of bitters," belongs to Acanthaceae family. It is an ancient medicinal herb with extensive ethnobotanical uses. It is a perennial herb found throughout India especially in dense forests. It is under cultivation in many states of India. In Ayurveda, the whole plant is extensively used for various ailments. The extract of the whole plant is used as coolant, laxative, vulnerary, antipyretic, antiperiodic, anti-inflammatory, and expectorant. In Ayurveda, the extract of the whole plant along with some other plant extracts is used to treat fever. *Andrographis* is also used in homeopathic drug as an active ingredient along with other plant extracts. It is a principal herb in the domestic medicine called "Alui", which is given to infants for treating fever, dysentery, and skin diseases.

The aim of the present study was to study the effect of plant growth regulators such as ABA and $GA_3$ on growth, dry matter production, and pigment composition of *A. paniculata*.

## 2 Materials and methods

### 2.1 Seeds and growth regulators

The seeds of *A. paniculata* were obtained from the Herbal Folklore Research Center, Andhra Pradesh, India. The plant growth regulators, ABA and $GA_3$, were purchased from Himedia India Ltd., Mumbai. During the study, the average temperature was 26°C to 32°C (maximum/minimum), and relative humidity (RH) varied between 60% and 75%.

### 2.2 Cultivation methods

The seeds of *A. paniculata* were surface sterilized with 0.2% mercuric chloride solution for 5 min with frequent shaking and thoroughly washed with tap water. The seeds were sown, and plants were raised in nursery beds. The seedlings of 30 days old were transplanted to plastic pots. The pots were filled with 3 kg uniform soil mixture containing red soil, sand, and farm yard manure at 1:1:1 ratio. No inorganic fertilizer or systemic pesticide was used during the experiment. Ground water was used for irrigation. The experiment was laid out in a completely randomized block design.

### 2.3 ABA and $GA_3$ treatments

In the preliminary experiments, 2, 3, 4, 5, and 6 $\mu mol \cdot L^{-1}$ ABA and $GA_3$ were used for treatment to determine the optimum concentration. Among the treatments, 5 $\mu mol \cdot L^{-1}$ ABA and $GA_3$ increased the dry weight significantly, and higher concentration slightly decreased the growth and dry weight. At lower concentrations, there was no significant change in dry weight and growth. Hence, 5 $\mu mol \cdot L^{-1}$ ABA and $GA_3$ concentrations were used to study the effect of ABA and $GA_3$ on the morphological parameters and photosynthetic pigments of *A. paniculata*.

The growth regulators were applied by means of foliar spray during morning hours. Before spraying, each pot was covered with foil paper to prevent any runoff the foliage to enter the media. A control treatment was included in which the plants were not treated with any growth regulators. The growth regulators were given through foliar sprays on $10^{th}$, $30^{th}$, and $50^{th}$ days after transplantation (DAT). The plants were taken randomly on 20, 40, and 60 DAT and separated into roots, stems, and leaves used for determining morphological parameters and pigment contents.

### 2.4 Growth parameters

The plant height was measured from the soil level to the tip of the shoot and expressed in centimeters. The plant root length was measured from the point of first cotyledonary node to the tip of the root and expressed in centimeters. The total number of leaves that were fully developed was counted and expressed as number of leaves per plant. The total leaf area of the plants was measured using LICOR Photo Electric Area Meter (Model LI-3100, Lincoln, USA) and expressed in square centimeters per plant.

After washing the plants in tap water, fresh weight was determined using an electronic balance (Model-XK3190-A7M), and the values were expressed in grams. After the fresh weight was measured, the plants were dried at 60°C in a hot air oven for 24 hours. After drying, the weight was measured, and the values were expressed in grams.

### 2.5 Pigment analysis

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949) and expressed in milligrams per gram fresh weight. Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight. Xanthophyll contents were estimated by the method of Neogy et al. (2001). The results were expressed in absorbance per gram fresh weight. Anthocyanin was extracted and estimated by the method of Kim et al. (2002).

## 3 Results

### 3.1 Growth

The total height of the plant increased with the age in control and growth regulator-treated plants (Table 1; Fig. 1). ABA treatment inhibited the growth of the stem when compared with the control, and it was 93.70% over the control on 60 DAT. $GA_3$ treatment increased the plant height when compared with the control and ABA treatment, and it was 129.96% over the control on 60 DAT.

**Table 1** Growth regulator-induced changes in the growth parameters of *A. paniculata*

| growth stages (DAT) | control | ABA | $GA_3$ |
|---|---|---|---|
| plant height/cm | | | |
| 20 | 33.5±1.29 | 32.7±1.30 | 39.4±1.35 |
| 40 | 48.7±1.57 | 46.0±1.72 | 60.9±1.96 |
| 60 | 52.4±1.80 | 49.0±1.96 | 68.1±2.72 |
| internodal length/cm | | | |
| 20 | 2.7±0.10 | 2.6±0.10 | 3.2±0.13 |
| 40 | 3.2±0.11 | 3.0±0.12 | 3.9±0.15 |
| 60 | 3.6±0.12 | 3.3±0.13 | 4.5±0.18 |
| number of leaves | | | |
| 20 | 44±1.69 | 43±1.48 | 49±1.18 |
| 40 | 58±2.23 | 56±1.93 | 67±2.31 |
| 60 | 61±2.35 | 58±2.32 | 70±2.41 |
| root length | | | |
| 20 | 10.7±0.41 | 10.3±0.39 | 10.0±0.34 |
| 40 | 13.2±0.47 | 12.9±0.43 | 12.7±0.41 |
| 60 | 14.8±0.46 | 14.6±0.50 | 14.3±0.53 |
| whole plant fresh weight/g | | | |
| 20 | 6.285±0.24 | 7.188±0.25 | 6.649±0.22 |
| 40 | 19.941±0.71 | 23.352±0.80 | 22.467±0.81 |
| 60 | 28.810±1.06 | 34.443±1.15 | 31.869±1.18 |
| whole plant dry weight/g | | | |
| 20 | 1.992±0.08 | 2.361±0.09 | 2.214±0.07 |
| 40 | 5.176±0.18 | 6.272±0.23 | 5.869±0.22 |
| 60 | 8.331±0.28 | 10.301±0.40 | 9.601±0.34 |

Values are mean±SD of three replicates. DAT: days after transplantation; ABA: abscisic acid; $GA_3$: gibberellic acid.

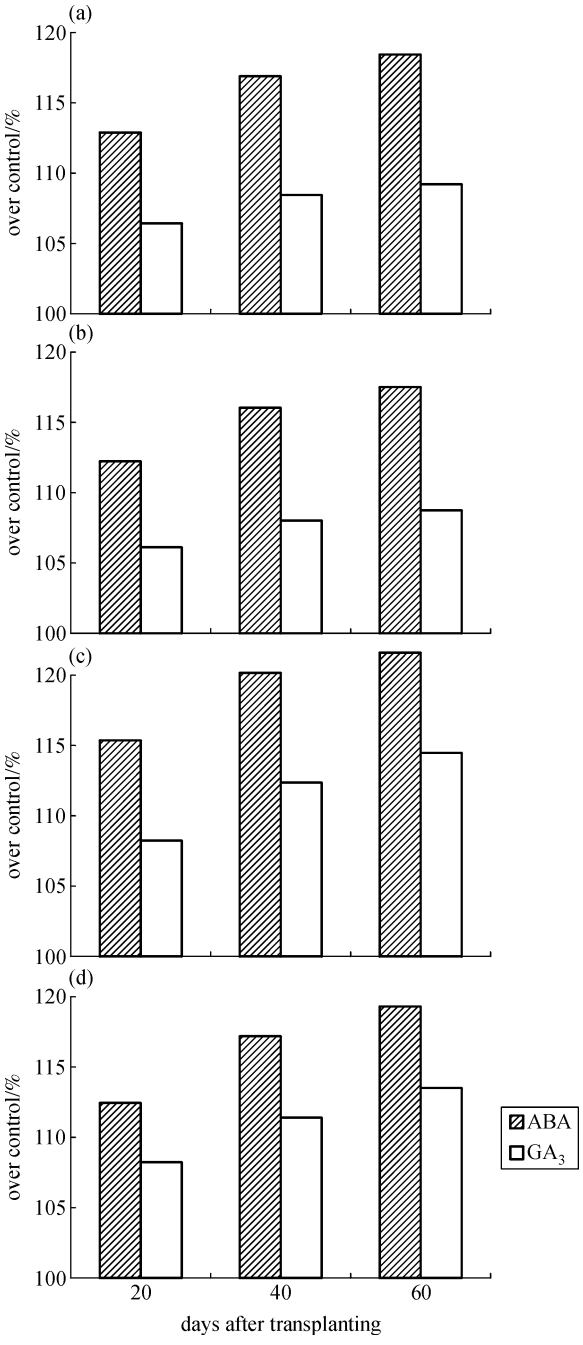


**Fig. 1** Growth regulator-induced changes in total chlorophyll, carotenoid, anthocyanin, and xanthophyll contents in the leaves of *A. paniculata*

The internodal growth was inhibited in ABA treatment. ABA inhibited the internodal length when compared with the control, and it was 8.34% lower than the control on 60 DAT, whereas $GA_3$ enhanced the internodal length significantly when compared with the control and ABA-treated plants, and it was 125% higher than the control on 60 DAT.

The total number of leaves per plant decreased in the ABA-treated plants, and it was lesser by 4.92% when compared with the control on 60 DAT. However, $GA_3$ treatment increased the total number of leaves when compared with the control, and it was 114.75% higher when compared with the control.

The total leaf area increased with the age in the control and treated plants. In the initial stages of growth period, the ABA-treated plants increased the leaf area, but at the maturation stage, the leaf area was not significantly increased. The $GA_3$-treated plants showed increased leaf

area when compared with the control at all the stages of growth, and it was 123.80% higher than the control on 60 DAT.

ABA and $GA_3$ treatments slightly decreased the total root growth at all the stages of growth, and it was 98.64% and 96.62%, respectively, over the control on 60 DAT.

The total fresh weight of *A. paniculata* increased with age of the plant. Both the treatments increased the whole plant fresh weight at all stages of growth. ABA enhanced the fresh weight to a larger extent when compared with $GA_3$ and the control, and it was 119.54% over the control on 60 DAT.

*A. paniculata* plants subjected to plant growth regulator treatments showed an increased whole plant dry weight when compared with the control. The total dry weight was increased with the age, and the rate of increase was very high in the ABA-treated plants when compared with $GA_3$-treated plants. ABA increased the total dry weight to 123.64% over the control, and in $GA_3$, it was 115.24% over the control on 60 DAT.

### 3.2 Pigments

The total chlorophyll content of the leaves increased with the age in the control and growth regulator–treated plants up to 40 DAT and then gradually decreased. ABA treatment increased the total chlorophyll content to a larger extent when compared with the control. ABA and $GA_3$ treatments increased the total chlorophyll content to 118.46% and 109.23%, respectively, over the control on 60 DAT.

Chlorophyll "a" and "b" content in the *A. paniculata* leaves increased with age in the control and treated plants. Plant growth regulator treatments increased the chlorophyll "a" and "b" content to a higher level. ABA-treated plants showed higher total chlorophyll and chlorophyll "a" and "b" content when compared with $GA_3$-treated plants.

Plant growth regulator treatments increased the carotenoid content of the leaves of *A. paniculata* when compared with the control. ABA and $GA_3$ increased the carotenoid content to 122.60% and 110.53%, respectively, over the control on 60 DAT.

The xanthophyll content in the *A. paniculata* increased with the plant growth regulator treatments. ABA treatment increased it to a higher level than $GA_3$, and it was 123.26% and 115.54%, respectively, over the control on 60 DAT.

Anthocyanin content in the *A. paniculata* leaves increased with plant growth regulator treatments. ABA and $GA_3$ treatments increased the anthocyanin content in the leaves to 119.54% and 113.67%, respectively, over the control on 60 DAT.

## 4 Discussion

The total height of the plant increased with the age in the control and growth regulator-treated plants. $GA_3$-treated plants exhibited more pronounced effects on increasing stem length than the control and ABA. ABA-treated plants showed reduced stem length than the control plants.

The plant hormones $GA_3$ and ABA exert profound effects on fundamental processes of plant growth and development (Kieber and Araki, 2006). GA is widely regarded as a growth-promoting compound that positively regulates processes such as seed germination, stem elongation, leaf expansion, pollen-tube growth, flower and fruit development, and floral transition (Swain and Singh, 2005).

Gibberellins are involved in several plant development processes and promote a number of desirable effects including stem elongation, uniform flowering, reduced time to flowering, and increased flower number and size (Hopkins, 1995).

It was apparent that treating plants with $GA_3$ increased the height of the plant over the control, which may be attributed to the growth promotion effect of $GA_3$ in stimulating and accelerating cell division, increasing cell elongation and enlargement, or both (Al-Khassawneh et al., 2006). $GA_3$ application was reported to increase the weight of aerial plant parts in Viola (Vlahos, 1991), and the flower diameter doubled in Persian violet (Neumaier et al., 1987). GA and ABA have mostly antagonistic effects, in which $GA_3$ promotes and ABA inhibits the processes (Mahouachi et al., 2005). In the present study, it was clearly observed that ABA treatment reduced the stem length when compared with $GA_3$ treatment.

ABA treatment reduced the stem elongation and internodal length. The internodal growth was inhibited in ABA treatment, whereas $GA_3$ enhanced the internodal length significantly when compared with the control. The growth-retarding effect of ABA is caused by the inhibition of $GA_3$ biosynthesis as observed in *Cucurbita maxima* (Izumi et al., 1985).

Treatment with growth regulators affected the leaf growth. Among the treatments, ABA lowered the leaf area when compared with the control, whereas $GA_3$ increased the leaf area when compared with the control. The reduced leaf area in ABA-treated plants may be due to the increased ABA content and reduced gibberellin biosynthesis induced by ABA.

ABA and $GA_3$ treatments slightly decreased the total root growth at all the stages of growth when compared with the control. In terrestrial plants, externally applied gibberellins have been shown to promote stem elongation, leaf enlargement and elongation, and the inhibition of root formation (Srivastava and Srivastava, 2007). ABA also has inhibitory action on lateral root development (Zhou et al., 1998). Both the growth regulator treatments increased the whole plant fresh and dry weight at all stages of growth. Among the plant growth regulators, ABA enhances the fresh and dry weight to a larger extent when compared with $GA_3$ and the control.

ABA and $GA_3$ treatments increased the total chlorophyll

and chlorophyll "a" and "b" content in the leaves of *A. paniculata*. $GA_3$ increased the vegetative growth and pigment concentration in Elsanta (Tehranifar and Battey, 1997). Plant growth regulator treatments increased the carotenoid content of the leaves of *A. paniculata* when compared with the control.

Plant growth regulator-treated *A. paniculata* leaves showed increased xanthophylls content at all stages of growth. ABA treatment increased it to a higher level than $GA_3$. ABA is basically involved in xanthophylls formation and acts as a precursor compound.

Plant growth regulators increased the anthocyanin content in the leaves of *A. paniculata*. Treatment with ABA increased anthocyanin accumulation in strawberry fruits (Jiang and Joyce, 2003). This increased ABA content induced a transient raise in ABA content in bean (Asare-Boamah et al., 1986), resulting in increased anthocyanin content.

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