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RESEARCH ARTICLE

Changes in growth and photosynthetic characteristics of *Ocimum sanctum* under growth regulator treatments

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Abstract A field experiment was conducted to investigate the effect of growth regulators on growth characteristics such as root length, shoot length, total leaf area, number of inflorescence per plant, number of flower per inflorescence, whole plant fresh weight and whole plant dry weight. Photosynthetic characteristics were also analyzed based on the same experiment. For this, various photosynthetic pigment contents such as chlorophyll, carotenoid, anthocyanin and xanthophyll content were calculated. The conventional growth regulator abscisic acid (ABA) and non-conventional growth regulator triazole compound paclobutrazol (PBZ) were used. Root length increased due to growth regulator treatment, but shoot length decreased. Leaf area was decreased due to growth regulator treatment. The number of inflorescence increased in ABA treated plants, but it was decreased in PBZ treated plants. In ABA treated plants, the number of flowers per inflorescence was increased. In PBZ treated plants the number of inflorescence was reduced. The whole plant fresh weight (FW) and dry weight (DW) were increased in ABA and PBZ treated plants. There was an increase in chlorophyll content in growth regulator treated plants compared to control, and it was more in PBZ treated plants. The carotenoid content was also increased in ABA and PBZ treated plants.

Keywords abscisic acid (ABA), growth characteristics, *Ocimum Sanctum*, paclobutrazol (PBZ), photosynthetic characteristics

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1 Introduction

With increasing realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, more people are interested in the use of plants, and plant-based drug throughout the world. So exploitation of medicinal plant has become popular (Tan et al., 2006). Over the recent years, several scales of physiology have been applied to the study of the response to plant growth regulators in field crops (Al-khasawneh et al., 2006). However, little understanding is gained about the physiological basis in terms of growth and photosynthetic characteristics under plant growth regulator treatment.

The term, plant growth regulator is not only restricted to synthetic compounds, but also includes the naturally occurring hormones. Hence, plant growth regulator can be defined as natural or synthetic compounds that modify plant growth and development patterns, exerting profound influence on many physiological processes (Makoto, 2003). Abscisic aid (ABA) is a plant growth regulator that has been identified as a messenger in stress–perception response pathways (Jia and Lu, 2003) such as drought, high temperature, low temperature and salinity stress (Zhang et al., 2001).

ABA is also involved in many physiological processes such as photosynthesis. It has been demonstrated that ABA plays an important role in stomatal movement, regulation of photosynthetic activities, and the stability of the photosynthetic apparatus, and gives expression involved in chloroplasts (Jia and Lu, 2003). The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones, including gibberellin (GA), ABA and cytokinins (Fletcher et al., 2000). They induce a variety of morphological and biochemical responses in plants including inhibiting shoot elongation, stimulating root growth, increasing cytokinin synthesis and transient rise in ABA, as well as conferring protection from various environmental stresses (Gopi et al., 2005; Jaleel et al., 2006, 2007).

In the present study, *Ocimum sanctum* L. (family lamiaceae), commonly known as sacred Tulsi, is a fragrant bushy plant found in semitropical and tropical parts of India. Different parts of the plant are used traditionally in the Ayurveda and Siddha system of medicine for treating infections, skin diseases, hepatic disorders, cold, cough, malarial fever and even as an antidote for snake bites (Satyavati and Gupta, 1987).

Ocimum sanctum has shown growth promoting, hypotensive, cardiac depressant, smooth muscle cell relaxant and anti-stress activities (Malaviya and Gupta, 1971; Singh et al., 1970; Bhargava and Singh, 1981). *Ocimum* leaf extract shows strong protective effect against radiation injury (Uma Devi and Ganasundari, 1995). The present investigation was undertaken to study the effect of paclobutrazol and ABA on the growth and photosynthetic pigments like chlorophyll a, chlorophyll b, total chlorophyll, xanthophylls and carotenoid.

2 Materials and methods

The seeds of the two varieties were collected from the Horticulture Department, Faculty of Agriculture, Annamalai University, Tamil Nadu. Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-trizol-1-yl)-pentan-3-ol], a triazolic group of fungicide having plant growth regulator (PGR) properties, was obtained as cultar 25% W/V paclobutrazol from Zeneca ICI Agrochemical Ltd., Mumbai, India, and used for the study. ABA was obtained from Sigma Chemicals, Bangalore.

The experimental part of this work was carried out in the Botanical Garden and stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. During the study, the average temperature was 26°C–32°C and the relative humidity (RH) varied between 60%–75%.

2.1 Cultivation methods

The plants were raised in the Botanical Garden during the months of January–May 2007. The seeds were sown separately in raised seed beds by broadcasting method and covered with paddy straw to ensure proper germination. The nursery beds were watered twice a day and weeded regularly in order to ensure the healthy growth of the seedlings. The seedlings were transplanted in 12 plants in each vial to 9 cement vials having 1 m diameters, in the vials filled with red soil, sand and farmyard manure (FYM) at a 1 : 1 : 1 ratio. The vials were irrigated immediately after transplantation and subsequent irrigation was done twice in a week to keep the optimum moisture level required in the soil.

2.2 PBZ and ABA treatments

In the preliminary experiments, 2, 5, 10, 15 and 20 $mg \cdot L^{-1}$ paclobutrazol and 2.5, 5, 7.5 and 10 $\mu mol \cdot L^{-1}$ ABA were used for treatments to determine the optimum concentration of paclobutrazol and ABA. Among the treatments, 15 $mg \cdot L^{-1}$ paclobutrazol and 7.5 $\mu mol \cdot L^{-1}$ ABA increased dry weight and higher concentrations slightly decreased the growth and dry weight. At the lower concentrations, there was no change in dry weight and growth of the plant. Hence, 15 $mg \cdot L^{-1}$ paclobutrazol and 7.5 $\mu mol \cdot L^{-1}$ ABA concentrations were used to study their effects on *Ocimum santum* plants.

Three vials each were taken for treatments with PBZ and ABA, respectively, and 3 vials were kept untreated and served as control. 15 $mg \cdot L^{-1}$ paclobutrazol and 7.5 $\mu mol \cdot L^{-1}$ ABA were given to each plant by soil drenching. The treatment was given on 50, 70 and 90 days after pollination (DAP). The plants were uprooted randomly on 60, 80 and 100 DAP and separated into root, stem, leaves and flowers used for determining growth, biochemical, antioxidant potential and phenol contents.

2.3 Growth parameters

The plant height was measured from the soil level to the tip of the shoot and expressed in cm. The plant root length was measured from the point of first cotyledonary node to the tip of the longest root and expressed in cm. The total number of leaves which were fully developed was counted and expressed as the number of leaves per plant. The total leaf area of the plants was measured using the LICOR Photo Electric Area Meter (model LI-3100, Lincoln, USA) and expressed in cm^2 per plant. The number of inflorescence per plant was counted and expressed as the number of inflorescence per plant. Numbers of flowers per inflorescence were counted and expressed as the number of flowers per inflorescence per plant.

After washing the plants in tap water, the fresh weight was determined by using an electronic balance 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 48 hours. After drying, the weight was measured and the values were expressed in grams.

2.4 Photosynthetic characteristics

2.4.1 Estimation of chlorophyll and carotenoid contents

Chlorophyll and carotenoid were extracted from the leaves, estimated by the method of Arnon (1949), and expressed in milligram per gram fresh weight. The carotenoid content was estimated using the formula of Kirk and Allen (1965), and was expressed in milligrams per gram fresh weight.

2.4.2 Anthocyanin

Anthocyanin was extracted and estimated from the fresh plant materials by the method of Beggs and Wellmann (1985). The results were expressed in milligrams per gram fresh weight.

2.4.3 Xanthophyll

Xanthophyll was extracted and estimated from the fresh plant materials by the method of Neogy et al (1990). The results were expressed in milligrams per gram fresh weight.

3 Results

3.1 The basical plant indices

3.1.1 Root length

The root length increased with PBZ and ABA treatments (Table 1; Fig. 1; Plate 1). It increased to a larger extent with PBZ treatment when compared to control and ABA treatments. The percentage over control increase was 121.07 in PBZ treated and 113.03 in ABA treated on 100 DAP, respectively.

Table 1 Effect of paclobutrazol and ABA on root length, shoot length, and leaf area of *Ocimum sanctum*

	growth stages (DAS)	control	paclobutrazol	ABA
root length /cm	60	21.00 ± 0.080	26.00 ± 0.845	23.00 ± 0.758
	80	22.00 ± 0.088	28.00 ± 0.951	25.00 ± 0.861
	100	26.10 ± 0.090	31.80 ± 1.083	29.50 ± 0.980
shoot length /cm	60	34.00 ± 0.120	18.50 ± 0.062	27.00 ± 0.111
	80	47.00 ± 0.150	21.00 ± 0.700	43.50 ± 0.150
	100	49.00 ± 0.160	24.10 ± 0.080	46.20 ± 0.160
total leaf area/cm^2	60	9.00 ± 0.360	5.40 ± 0.220	6.00 ± 0.240
	80	12.00 ± 0.411	6.30 ± 0.240	7.00 ± 0.264
	100	12.50 ± 0.421	8.00 ± 0.267	9.00 ± 0.320

Values are mean ± SD of 7 samples. ABA: abscisic acid; DAS: days after sowing.

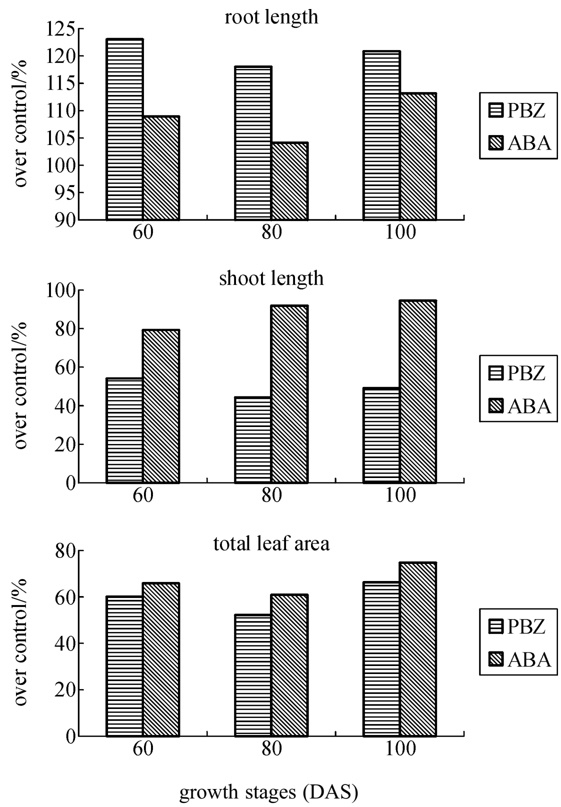

Fig. 1 Effect of paclobutrazol (PBZ) and abscisic acid (ABA) on morphological parameters of *Ocimum sanctum*. DAS: days after sowing.

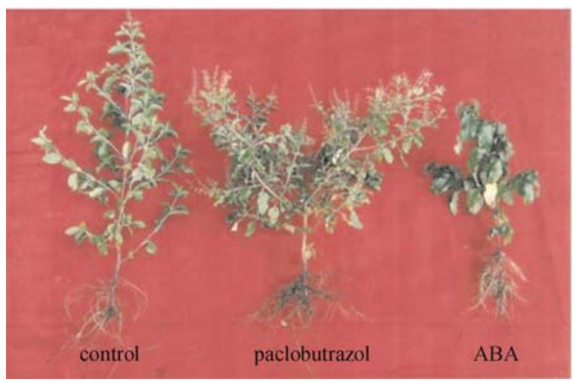

Plate 1 Effect of paclobutrazol and ABA on morphology of *Ocimum sanctum*

3.1.2 Shoot length

Growth regulator treated *Ocimum sanctum* plants shows moderate decrease in shoot length when compared to control. The reduction was 94.29% (ABA) and 49.18% (PBZ) over control on 100 DAP.

3.1.3 Total leaf area

The leaf area was increased with the age of the control and the treated *Ocimum* plants. The PGR treatments inhibited the leaf growth of *Ocimum sanctum* plant when compared to control. Among the treatments, PBZ inhibited the leaf area at 66.67% when compared to ABA treated plants on 100 DAP. In ABA treated plants, the leaf area also decreased and it was 75% over control on 100 DAP.

3.1.4 Number of inflorescence per plant

The number of inflorescence per plant increased with the age in the control and treated plants (Table 2; Fig. 2). PGR treatments caused an increase in the number of

Table 2 Effect of paclobutrazol and ABA on number of inflorescences per plant, length of inflorescence, and number of flowers per inflorescence of *Ocimum sanctum*

	growth stages (DAS)	control	paclobutrazol	ABA
number of inflorescences per plant	60	02.00 ± 0.091	01.00 ± 0.042	03.00 ± 0.104
	80	04.00 ± 0.140	03.00 ± 0.103	09.00 ± 0.350
	100	05.00 ± 0.170	04.00 ± 0.130	11.00 ± 0.380
length of inflorescence /cm	60	06.00 ± 0.240	04.00 ± 0.140	11.00 ± 0.035
	80	07.50 ± 0.310	04.00 ± 0.160	12.00 ± 0.041
	100	09.00 ± 0.350	06.10 ± 0.210	13.00 ± 0.045
number of flowers per inflorescence	60	39.00 ± 1.300	32.00 ± 1.100	21.00 ± 0.750
	80	148.0 ± 5.770	49.00 ± 1.880	162.0 ± 5.220
	100	154.0 ± 4.999	61.00 ± 2.110	169.00 ± 5.800

Values are mean ± SD of 7 samples. ABA: abscisic acid; DAS: days after sowing.

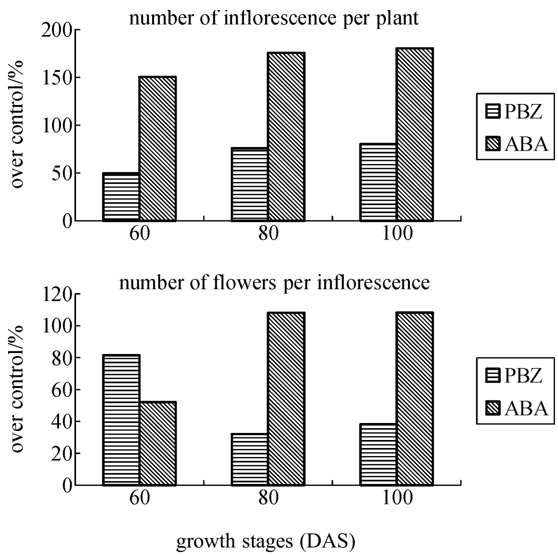

Fig. 2 Effect of paclobutrazol (PBZ) and abscisic acid (ABA) on morphological parameters of *Ocimum sanctum*. DAS: days after sowing.

inflorescence per plant to a larger extent when compared to control. Among the treatments ABA increased the number of inflorescence per plant more than that of PBZ treated plants, and it was 120% over control on 100 DAP. PBZ reduced the inflorescence per plant, and it was 80% over control on 100 DAP.

3.1.5 Number of flowers per inflorescence

The number of flowers per inflorescence increased with the age in the control and treated plants. PGR treatments increased the number of flowers per inflorescence when compared to control. Among the treatments, ABA increased the number of flowers per inflorescence more than that of PBZ treated plants, and it was 109.74% over control on 100 DAP. PBZ reduced the number of flowers per inflorescence, and it was 39.61% over control on 100 DAP.

3.1.6 Whole plant fresh weight

The whole plant fresh weight increased with PBZ and ABA treatments (Table 3; Fig. 3).

The whole plant fresh weight increased to a larger extent with ABA treatment when compared to the control and PBZ treatments. The percentage over control increase was 193.11 in ABA treated and 159.67 in PBZ treated on 100 DAP, respectively.

Table 3 Effect of paclobutrazol and ABA on fresh and dry weights of *Ocimum sanctum*

	growth stages (DAS)	control	paclobutrazol	ABA
whole plant fresh weight/g	60	6.088 ± 0.196	6.206 ± 0.222	6.097 ± 0.211
	80	11.65 ± 0.039	19.73 ± 0.071	23.70 ± 0.082
	100	12.92 ± 0.045	20.63 ± 0.076	24.95 ± 0.096
whole plant dry weight/g	60	1.987 ± 0.060	2.431 ± 0.080	2.210 ± 0.076
	80	3.927 ± 0.130	5.265 ± 0.169	6.291 ± 0.203
	100	3.863 ± 0.150	6.012 ± 0.200	6.892 ± 0.220

Values are mean ± SD of 7 samples. ABA: abscisic acid; DAS: days after sowing.

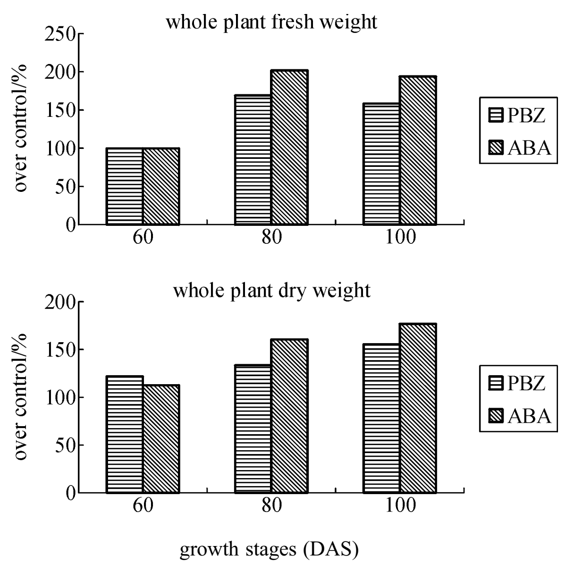

Fig. 3 Effect of paclobutrazol (PBZ) and abscisic acid (ABA) on fresh and dry weights of *Ocimum sanctum*. DAS: days after sowing.

3.1.7 Whole plant dry weight

The whole plant dry weight increased with PBZ and ABA treatments (Table 3; Fig. 3).

The whole plant dry weight increased to a larger extent with ABA treatment when compared to control and PBZ treatments. The percentage over control increase was 177.63 in ABA treated and 155.63 PBZ treated on 100 DAP respectively.

3.2 Photosynthetic pigments

3.2.1 Chlorophyll ‘a’

The chlorophyll ‘a’ content was also considerably increased by the PBZ and ABA treatments at all the stages of *Ocimum* plant (Table 4; Fig. 4). The PBZ increased the chlorophyll ‘a’ content. The maximum recording was 160% over control on 100 DAP. When compared to PBZ, the ABA treated plants increased the chlorophyll ‘a’ content, and it was 133.33% over control on 100 DAP.

Table 4 Effect of paclobutrazol and ABA on chlorophyll contents of *Ocimum sanctum* mg·g^{-1} fr. wt.

	growth stages (DAS)	control	paclobutrazol	ABA
chlorophyll ‘a’	60	0.024 ± 0.005	0.031 ± 0.001	0.030 ± 0.013
	80	0.086 ± 0.007	0.013 ± 0.001	0.112 ± 0.004
	100	0.030 ± 0.004	0.048 ± 0.002	0.040 ± 0.002
chlorophyll ‘b’	60	0.019 ± 0.001	0.026 ± 0.001	0.024 ± 0.001
	80	0.058 ± 0.0013	0.088 ± 0.003	0.081 ± 0.002
	100	0.024 ± 0.0019	0.038 ± 0.001	0.036 ± 0.001
total chlorophyll	60	0.043 ± 0.004	0.054 ± 0.003	0.056 ± 0.002
	80	0.144 ± 0.002	0.185 ± 0.006	0.193 ± 0.006
	100	0.054 ± 0.0024	0.086 ± 0.004	0.076 ± 0.003

Values are mean ± SD of 7 samples. ABA: abscisic acid; DAS: days after sowing.

3.2.2 Chlorophyll ‘b’

The chlorophyll ‘b’ content was also considerably increased by the PBZ and ABA treatments at all the stages of *Ocimum* (Table 4; Fig. 4).

The PBZ treatment increased the chlorophyll ‘b’ content. The maximum recording was 158.33% over control on 100 DAP when compared to ABA treated plants. Also in ABA treated plants, the chlorophyll ‘b’ content was increased, and it was 150% over control on 100 DAP.

3.2.3 Total chlorophyll

The total chlorophyll content of leaves was increased with the age of the plant. The PBZ and ABA treatments increased the total chlorophyll content to a higher level than the control plants. The increase percentage was 159.26% in PBZ treated plants and 140% in ABA treated plants on 100 DAP.

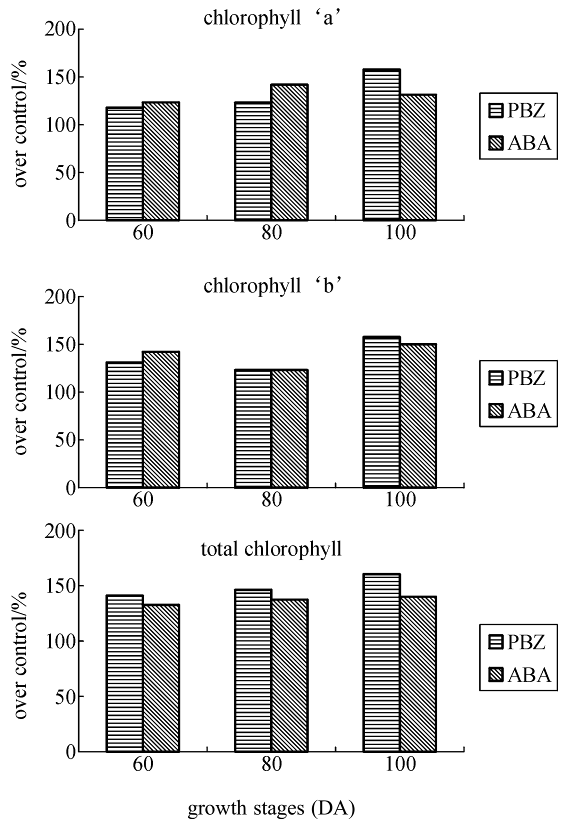

Fig. 4 Effect of paclobutrazol (PBZ) and abscisic acid (ABA) on chlorophyll contents of *Ocimum sanctum*. DAP: days after sowing.

3.2.4 Carotenoid

The carotenoid content of the leaves also increased in the PBZ and ABA treated plants and control with the age of the plant (Table 5; Fig. 5). Among the treatments, PBZ increased 140.67% and ABA 140.54% over control treated plants on 100 DAP.

Table 5 Effect of paclobutrazol and ABA on photosynthetic pigment contents of *Ocimum sanctum* mg·g^{-1} fr. wt.

	growth stages (DAS)	control	paclobutrazol	ABA
carotenoid	60	1.078 ± 0.037	0.811 ± 0.031	0.765 ± 0.029
	80	1.216 ± 0.044	1.631 ± 0.058	1.521 ± 0.054
	100	1.091 ± 0.038	1.535 ± 0.061	1.533 ± 0.054
anthocyanin	60	56.95 ± 1.964	74.1 ± 2.390	61.75 ± 1.990
	80	3.150 ± 0.109	4.25 ± 1.370	3.96 ± 0.132
	100	2.800 ± 0.096	3.91 ± 0.126	3.690 ± 0.123
xanthophylls	60	120.2 ± 4.001	168.3 ± 6.000	117.8 ± 4.210
	80	118.5 ± 5.210	148.9 ± 5.320	140.7 ± 4.810
	100	136.7 ± 5.230	165.3 ± 5.521	162.6 ± 5.245

Values are mean ± SD of 7 samples. ABA: abscisic acid; DAS: days after sowing.

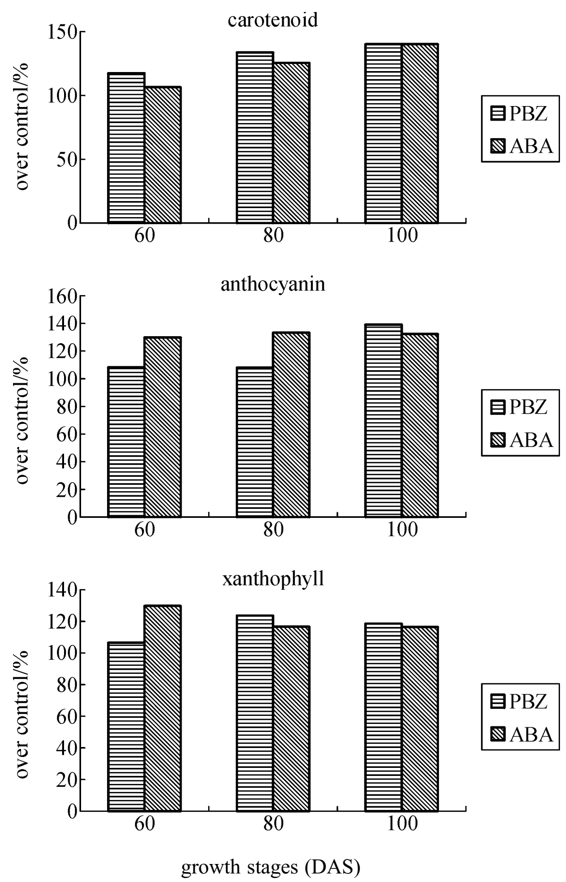

Fig. 5 Effect of paclobutrazol (PBZ) and abscisic acid (ABA) on photosynthetic pigment contents of *Ocimum sanctum*. DAS: days after sowing.

3.2.5 Anthocyanin

In the leaves, the anthocyanin content increased in both PBZ and ABA treated plants (Table 5; Fig. 5). PBZ treatment increased the anthocyanin content 139.64% in the leaves when compared to ABA treatment (132.14% over control) on 100 DAP.

3.2.6 Xanthophyll

The xanthophyll content of the *Ocimum* leaves increased in the control and treated plants with age (Table 5; Fig. 5). The PBZ treated plants had higher xanthophyll content when compared to ABA treated and control plants. The highest xanthophyll content was observed in the PBZ treated plants followed by ABA treated plants, and it was 120.92% and 118.95% over control on 100 DAP, respectively.

4 Discussion

The present investigation was conducted to determine the effect of PBZ and ABA on the growth and antioxidant status of *Ocimum sanctum.* The changes in parameters such as growth, photosynthetic pigments, non-enzymatic antioxidants, antioxidant enzymes and phenolic compound accumulation are discussed.

The root length was increased by PBZ and ABA treatments in *Ocimum sanctum* when compared to the control on 100 DAP. Paclobutrazol increased the length of roots and enhanced the lateral root formation in tomato plants (Berova et al., 2000). An increase in root length was reported in radish due to triazole treatment in peanut (Panneerselvam et al., 1997; Muthukumarasamy and Panneerselvam, 1997). This stimulation of root growth may be related to the increased partitioning of assimilates towards the roots due to a decreased demand on the shoot (Symons et al., 1990). The root to shoot ratio increases under stress conditions to facilitate water absorption (Jaleel et al., 2007) and is related to ABA content of roots and shoots (Sharp et al., 2000). The morphological responses to exogenous ABA application shows that ABA could play an important role in control of stress tolerance in two *Poplar* sp. (Yin et al., 2004).

PBZ and ABA treatments in *Ocimum sanctum* decreased the plant height to a larger extent when compared to control on all sampling days. PBZ treatment alone decreased the plant height when compared to ABA treatments. Triazole treatments reduced stem elongation and plant height in *Echinochloa frumentacea* (Sankhla et al., 1992). Barley seedlings show reduced growth under treatment with paclobutrazol (Sunitha et al., 2004). The growth retarding effect of triazole is caused by the inhibition of GA as observed in *Cucurbita maxima* (Izumi et al., 1985). The ABA induced growth inhibition resulted from signal transduction at the single-cell level, and thereby induced closure of stomata (Trejo et al., 1995).

PBZ and ABA treatments in *Ocimum sanctum* reduced the leaf area when compared to control. Moreover, individual PBZ and ABA treatments resulted in decreases in leaf area when compared to control. Paclobutrazol reduced the leaf area in tomatoes (Berova et al., 2000) and barley (Sunitha et al., 2004).

The leaf production was reduced in *Catharanthus* plants under PBZ treatment (Jaleel et al., 2006). Uniconazole reduced the leaf number in *pyrecantha* species (Norcini and Knox, 1989). Application of ABA at room temperature results in reduced leaf production in many plants. The leaf production was reduced in strawberry and soybean by triazole treatment (Davis, 1987). Uniconazole reduced the leaf number in *pyrecantha* species (Norcini and Knox, 1989).

PBZ and ABA treatments in *Ocimum sanctum* increased the whole plant fresh weight to a large extent. Similar results were reported in PBZ treated *Catharanthus* plants under salt stress (Jaleel et al., 2007). Triazole compounds inhibited gibberellin biosynthesis. Cytokinin and abscisic acid stimulated tuberization and reduced stolen length in potato by counteracting the gibberellin action (Xu et al., 1998). Cytokinin and abscisic acid content induced by these triazoles might be the cause for increased root growth.

PBZ and ABA treatments increased the dry weight considerably in *Ocimum sanctum* when compared to control plants. ABA plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance (Zhu, 2002), which might be the reason for the increased dry weight. Triadimefon and paclobutrazol altered the shoot dry weight in peanuts (Muthukumarasamy and Panneerselvam, 1997) and tomatoes (Still and Pill, 2004).

Treatment with PBZ and ABA increased the chlorophyll content in the leaf of *Ocimum sanctum*. Similar results were observed in paclobutrazol-treated barley seedlings (Sunitha et al., 2004) and tomatoes (Still and Pill, 2004). Paclobutrazol-treated leaves were dark green due to high chlorophyll 'a' and 'b' in potatoes (Tekalign et al., 2005).

The chlorophyll 'b' content of *Ocimum sanctum* leaves also increased in PBZ and ABA treated plants. The higher chlorophyll content in triazole-treated *Ocimum sanctum* may be related to the influence of triazole on endogenous cytokinin levels. It has been proposed that triazoles stimulate cytokinin synthesis that enhances chloroplast differentiation, chlorophyll biosynthesis and prevents chlorophyll degradation (Fletcher et al., 2000).

The total chlorophyll content of the *Ocimum sanctum* leaves increased with age of the plants in control, PBZ and ABA treatments. Paclobutrazol treatment increased the chlorophyll a, b and carotenoid pigments in the leaves of tomatoes (Berova et al., 2000; Still and Pill, 2004), wheat (Berova et al., 2002) and barley seedlings (Sunitha et al., 2004).

The carotenoid content of the *Ocimum sanctum* leaves increased with age in control and treated plants. Treatment with PBZ and ABA increased the carotenoid content. Paclobutrazol treatment increased the carotenoid content in *Catharanthus* plants (Jaleel et al., 2006). Triadimefon treatment increased the carotenoid content to a high level in cucumbers (Feng et al., 2003).

Increased anthocyanin and xanthophyll contents were found in PBZ- and ABA-treated *O. sanctum* plants. Similar results were reported in *Catharanthus* (Jaleel et al., 2006). Triazoles greatly increased anthocyanin accumulation in carrot tissue cultures (Ilan and Dougall, 1992). Tetraconazole increased the anthocyanin content in maize (Angela et al., 1997). Similar results were obtained in the leaves of Kentucky blue grass (Kane and Smiley, 1983). Paclobutrazol treatment increased the xanthophyll content in flowers of *Catharanthus* plants (Jaleel et al., 2006). Treatment with ABA increased anthocyanin accumulation in strawberry fruits (Jiang and Joyce, 2003).

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