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Photosynthetic pigment content alterations in Arachis hypogaea L. in relation to varied irrigation levels with growth hormone and triazoles

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ABSTRACT

Field experiments were conducted to identify the variation of photosynthetic pigment contents in peanut under drought stress, paclobutrazol (PBZ), and abscisic acid (ABA) and their combination. The pigment contents of the groundnut leave increased with age in control and treatments. Drought stress decreased the chlorophyll, carotenoid, xanthophyll, and anthocyanin contents. PBZ and ABA to the drought stressed plants increased the chlorophyll, carotenoid, xanthophyll, and anthocyanin contents when compared to stressed and unstressed plants.

KEY WORDS: Triazole, peanut, abscisic acid, pigments, drought

INTRODUCTION

Agricultural scientists employ various techniques, including developing high yielding cultivar, improved agronomical methods, and agrochemicals like plant growth regulators to improve the yield of crop plants. Plant growth regulators modify the source and sink relationship and enhance the yield in many crop plants. Manipulating the crop morphology by using plant growth regulators also increases the utilization of solar radiation and alter photo assimilate distribution in favor of yield increment (Prakash, 1997).

Plants are subjected to several environmental stresses that adversely affect growth, metabolism, and yield (Lawlor, 2002). The environmental stresses such as drought, temperature, salinity, air pollution, heavy metals, pesticides, and soil pH are major factors limiting crop production because, they affects almost all plant. The production of plants with tolerance to environmental stresses is one of the priorities in plant science research, and certain chemicals have been demonstrated to regulate the expression of tolerance (Fletcher *et al.,* 2000).

Drought is a major abiotic factor that limits agricultural crop production. Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high, and these two conditions often coincide under arid and semiarid climates. Water stress tolerance is seen in almost all plant species but its extent varies from species to species. Although the general effects of drought on plant growth are fairly well-known, the primary effects of water deficit at the biochemical and molecular levels are not well-understood (Hong-Bo *et al.,* 2006).

Triazole compounds are systemic fungicides having plant growth regulating properties. Protection of plants from apparently unrelated stress by triazole and also mediated by a reduction in free radical damage and increases in the antioxidant potential and has an efficient free-radical scavenging system that enables them to detoxify active oxygen (Manivannan *et al.,* 2007; Sankar *et al.,* 2007).

Paclobutrazol (PBZ) is a triazolic group of fungicide which has plant growth regulating properties. The growth regulating properties of PBZ are mediated by changes in the balance of important plant hormones including the gibberellins, abscisic acid (ABA), and cytokinins (Bora *et al.,* 2007). ABA is a plant growth regulator that has been identified as a messenger in stress-perception-response pathways such as drought, high temperature, low temperature, and salinity stress (Zhang *et al.,* 2001). The identification of the methods responsible for the manifestation of growth and yield under drought stress has now become one of the most important tasks to provide food security for the future generation.

Groundnut (*Arachis hypogaea* L.), is one of the most important oilseed crops grown as a major source of vegetable oil and protein, both for human consumption and as a fodder crop. Because groundnut is usually grown in rain fed condition, it has been hypothesized that improving growth under water limited conditions will serve better cultivation in water scarce areas also. The objectives of the present study were to understand the effect of drought, PBZ, ABA and in combination on the photosynthetic pigments constituents of *A. hypogaea* under field conditions.

MATERIALS AND METHODS

A. hypogaea L. seeds were obtained from the Krishi vigyan Kendra Form Science Center, Tamil Nadu Agricultural University, Thindivanam, Kerala, Tamil Nadu, India. PBZ is obtained as CULTAR 25% w/v from Zeneca ICI Agrochemical Ltd., Mumbai, Maharashtra, India and ABA from Sigma Chemicals, Bangalore. The experiments were conducted at the Botanical Garden and Stress Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India.

The peanut seeds were grown in a field, and the experiments were conducted in a randomized block design. The seeds were sown in plots measuring 3 × 3 M in three replications with spacing of 30 cm between rows and 15 cm between plants. There were 200 plants in each plot. Farmyard manure was given at the time of sowing. Control plants were treated with bore well water and irrigated every 10 days interval. Drought stressed plants were irrigated every 20 days interval. PBZ 10 mg/L and ABA 10 μg/L was used for treatments to stress and unstressed (control) plants. PBZ and ABA treatments were given by soil drenching and foliar spraying methods, respectively.

Plants were harvested randomly on 40^{th}, 60^{th}, and 80^{th} days after showing (DAS) and washed with tap water and then with deionized water. The leaves were separated and used for determining photosynthetic pigment contents.

PIGMENT COMPOSITION

Chlorophyll and carotenoid

Chlorophyll and carotenoid contents were extracted from the leaves and estimated according to the method of Arnon (1949).

Extraction

About 400 mg of fresh leaf material was ground with 10 ml of 80% acetone at 4°C in a pestle and mortar and centrifuged at 2500 g for 10 min at 4°C. The residue was re-extracted with 80% acetone until the green color was disappear in the residue and the extracts were pooled and transferred to a graduated tube and made up to 20 ml with 80% acetone and assayed immediately.

Estimation

About 3 ml aliquots of the extract were transferred to a cuvette, and the absorbance was read at 645, 663 and 480 nm in a spectrophotometer (U-2001–Hitachi) against 80% acetone as a blank. Chlorophyll content was calculated using the formula of Arnon (1949).

Total chlorophyll (mg/ml) = 0.0202) × (A.645) + (0.00802) × (A.663)

Chlorophyll "a" (mg/ml) = (0.0127) × (A.663) – 0.00269) × (A. 645)

Chlorophyll "b" (mg/ml) = (0.0229) × (A.645) – (0.00468) × (A. 663)

Carotenoid content was calculated using the formula of Kirk and Allen (1965) and expressed in milligrams a gram fresh weight.

Carotenoid = A.480 + (0.114 × A.663 – 0.638 × A.645)

Xanthophyll

Xanthophyll contents were estimated by the method of Neogy *et al.* (2001).

400 mg of fresh leaf tissues (3^{rd} leaf discs) were ground with 10 ml of 80% acetone at 4°C in a pestle and mortar and centrifuged at 1000 g for 15 min. The residue was re-extracted with 80% acetone until the color was completely disappears in the residue. An aqueous acetone extracts were shaken thrice with an equal volume of hexane in separating funnel, and the combined hexane fractions were washed with equal volumes of water. To separate xanthophylls from carotenes, the hexane fraction containing carotenoid was extracted repeatedly with

90% methanol. The hexane fraction containing carotenes and the methanol fraction containing xanthophylls were measured by taking the values of absorbance at 425 and 450 nm, respectively. The results are expressed in per gram fresh weight.

Anthocyanin

Anthocyanin content was extracted and estimated by the method of Zhang and Quantick (1997).

In a pestle and mortar, 500 mg of the third leaf discs tissues were ground with 10 ml of 1% methanol and repeated 3 times. The homogenate was centrifuged at 19,000 g for 15 min. The resultant supernatant was diluted with 1% HCl-methanol to 50 ml. The absorption of diluents was measured at 530 nm. The anthocyanin contents are expressed mg gram fresh weight.

RESULTS

Chlorophyll Content

Chlorophyll "a" (Figure 1)

The chlorophyll "a" content was very much affected by drought stress treatment and it was 55.10% over control on 80 DAS. PBZ and ABA to the drought stressed plants increased the chlorophyll "a" content when compared to the drought stressed plants and it was 120.22% and 114.64% over control, respectively on 80 DAS. PBZ and ABA treatment to the unstressed plants increased the chlorophyll "a" content when compared with control and it was 146.16% and 129.85% over control on 80 DAS, respectively.

Chlorophyll "b" (Figure 1)

The chlorophyll "b" content increased with age in the control and treated plants. The chlorophyll 'b' content was decreased by drought stress and it was 52.92% over control on 80 DAS. Drought stress with PBZ and ABA treatments increased the chlorophyll "b" content and it was 112.66% and 111.66% over control respectively on 80 DAS. PBZ and ABA treatments increased the chlorophyll "b" content when compared with control and other treatments, and the increase was 127.88% and 124.18% over control on 80 DAS.

Total chlorophyll (Figure 1)

The total chlorophyll content increased with the age of the plant. Drought stress decreased the total chlorophyll content to a larger extent and it was 54.02%over control on 80 DAS. PBZ and ABA treatments to the drought-stressed plants increased the total chlorophyll content to a level higher than that of control and it were 116.48% and 113.17% over control. PBZ and ABA treatments increased the total chlorophyll content to a large extent and it was 137.11% and 127.04% over control on 80 DAS.

The chlorophyll "a," chlorophyll "b" and total chlorophyll contents decreased under drought stress condition. PBZ and ABA treatments to the drought stressed plants increased the chlorophyll "a," chlorophyll "b" and total chlorophyll contents when compared with stressed and unstressed plants. PBZ and ABA treatment increased the chlorophyll "a," chlorophyll "b" and total chlorophyll contents to a level above that of control.

Carotenoid Content (Figure 2)

The carotenoid content increased with age in control and stressed groundnut plants. Drought stress decreased the carotenoid content when compared to control and it was 76.59% over control on 80 DAS. Drought stress with PBZ and ABA increased the carotenoid content and it was 111.26% and 108.63% over control respectively on 80 DAS. PBZ and ABA treatments also showed an increased the carotenoid content when compared with stressed and unstressed plants.

Xanthophyll Content (Figure 2)

Drought stress decreased the xanthophyll content to a larger extent when compared to control, and the decrease was 81.60% over control on 80 DAS. Drought stressed plants treated with PBZ and ABA showed an increased xanthophyll content when compared with unstressed plants and it was 114.44%, 104.77% over control on 80 DAS. PBZ and ABA treatments also increased the xanthophyll content to a higher extent, and the increase was 130.89% and 124.95% over control on 80 DAS.

Anthocyanin Content (Figure 2)

Drought stress treatment decreased the anthocyanin content to a larger extent when compared to control, and the decrease was 87.89% over control on 80 DAS. PBZ and ABA treatments to the drought stressed plants increased the anthocyanin content when compared to control and drought stressed plants and it was 112.24% and 109.49% over control respectively on 80 DAS. PBZ and ABA treatments also increased the anthocyanin content to a higher extent, and the increase was 119.81% and 113.62% over control on 80 DAS, respectively.

DISCUSSION

Chlorophyll "a"

Drought stress decreased the chlorophyll content when compared to control. A reduction in chlorophyll content

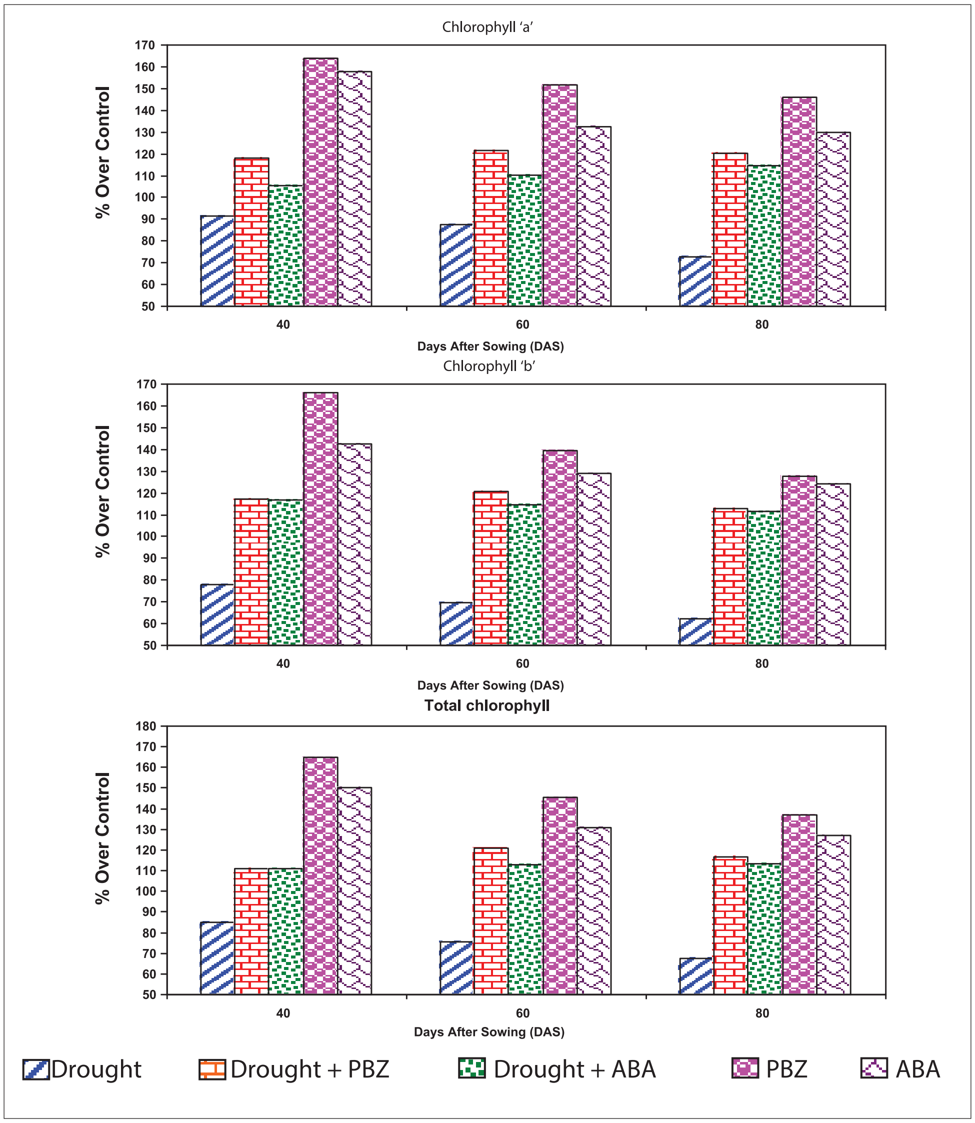

Figure 1: Effect of paclobutrazol, abscisic acid and drought and their combination induced changes in chlorophyll "a," chlorophyll "b" and total chlorophyll content of *Arachis hypogaea*. (Values are expressed in per cent over control)

was reported in drought stressed *Helianthus annuus* (Manivannan *et al.,* 2007). Similar results were observed in Wheat (Loggini *et al.,* 1999); *Pinus halepensis* (Alonso *et al.,* 2001); Cherry (Sawhney and Singh, 2002).

PBZ treatment to the drought stressed peanut plants increased the chlorophyll content when compared to control. PBZ and drought stress treatment increased the pigments in olive (Thakur *et al.,* 1998) and rose plants (Jenks *et al.,* 2001). Similar results were observed in triazole treatment to the NaCl stressed peanut (Muthukumarasamy and Panneerselvam, 1997).

ABA treatment with drought stressed groundnut plants increased the chlorophyll content when compared to control. Similar results were observed in citrus (Norman *et al.,* 1990); tobacco (Imai *et al.,* 1995); *Betula pendula* (Li *et al.,* 2003); *Kentucky bluegrass* (Wang *et al.,* 2003).

PBZ increased the chlorophyll content when compared to unstressed plants in all the sampling days. Sebastian *et al.* (2002) reported the enhanced chlorophyll synthesis in *Dianthus caryophyllus* treated with PBZ. PBZ treated leaves were dark green due to high chlorophyll a and b in *Chrysanthemum*; *Zea mays*; cowpea and potato. Sunitha *et al.* (2004) reported that the barley seedlings treated with PBZ appeared greener and thicker due to increased chlorophyll contents.

ABA treatment increased the chlorophyll content when compared to unstressed plants. ABA plays a direct role in mediating the photosynthesis to respiration in leaves and

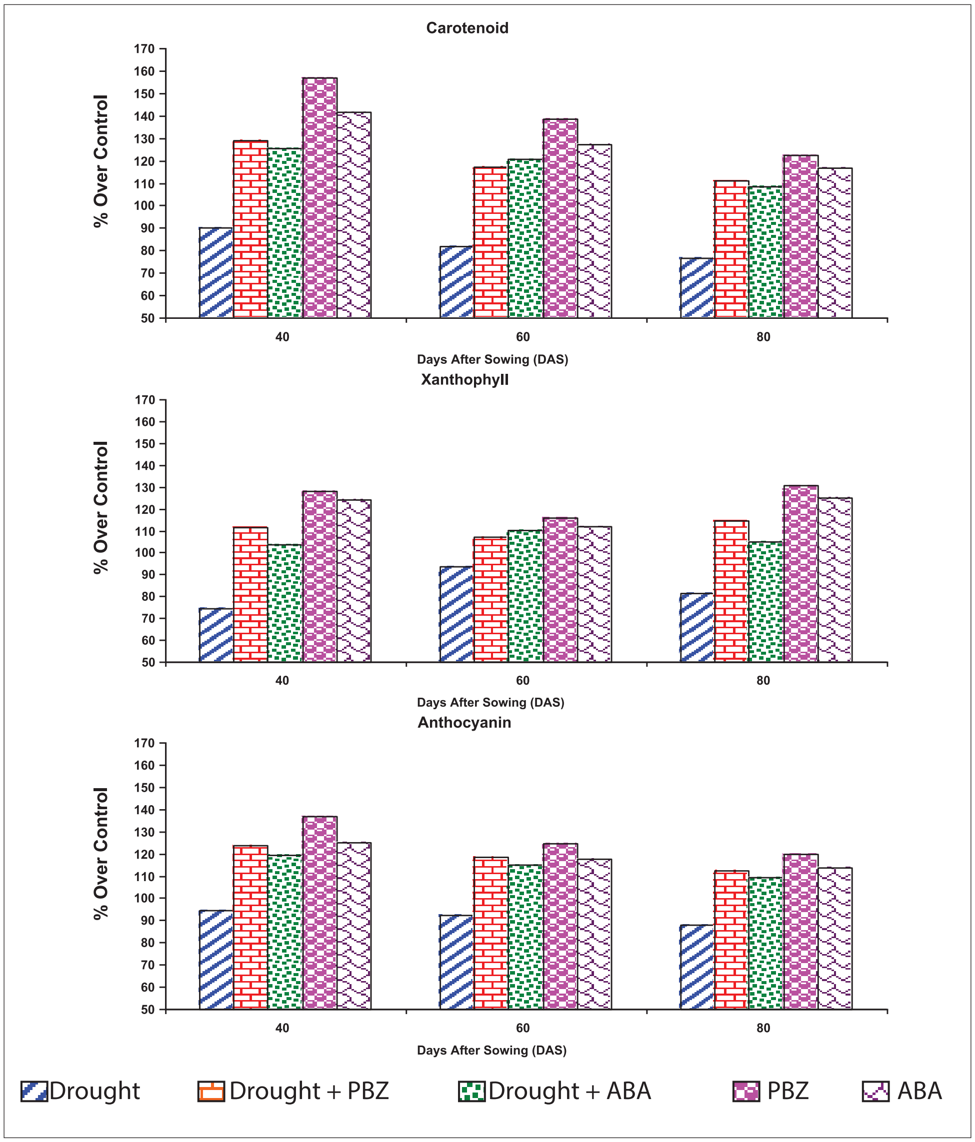

Figure 2: Effect of paclobutrazol, abscisic acid and drought and their combination induced changes in carotenoid, xanthophyll and anthocyanin content of *Arachis hypogaea*. (Values are expressed in per cent over control)

also the inhibition of lateral root development as reported in *Arabidopsis* (Zhou *et al.,* 1998).

Chlorophyll "b"

The chlorophyll "b" content of groundnut leaves also increased with age in the control and treated plants. Drought induced a reduction in chlorophyll "b" content in the leaves of groundnut when compared to control plants.

PBZ treatment to the drought stressed groundnut plants increased the chlorophyll 'b' content when compared to control. PBZ with drought stress treatment increased the pigments in olive and rose plants. Similar results were observed in apple seedling; pea plant (Wang *et al.,* 1992). PBZ and ABA treatments increased the chlorophyll 'b' content. The higher chlorophyll content in triazole treated radish 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). ABA treated plants increased the chlorophyll content. Similar results were observed in tomato (Thompson *et al.,* 2000); *K. bluegrass* (Wang *et al.,* 2003).

Total Chlorophyll

The total chlorophyll content of the leaves of *A. hypogaea* increased with age of plant in control. However, drought stress caused a reduction in total chlorophyll content in

A. hypogaea plants. The chlorophyll content in the wheat leaf decreased due to chemical desiccation treatments.

PBZ treatment to the drought stressed *A. hypogaea* plants increased the total chlorophyll content when compared to control. PBZ treatment to the drought stressed plants treatment increased the pigments in olive and jack pine, white spruce and black spruce (Marshall *et al.,* 1991). Similar results were observed in triazole treatment to the NaCl stressed peanut (Muthukumarasamy and Panneerselvam, 1997).

ABA treatment with drought stressed groundnut plants increased the total chlorophyll content when compared to control. Similar results were observed in citrus; *E. camaldulensis*; tobacco (Imai *et al.,* 1995).

PBZ treatment to the unstressed plants increased the total chlorophyll content. Sunitha *et al.* (2004) reported that the barley seedlings treated with PBZ appeared greener and thicker due to increased chlorophyll contents.

ABA treatment increased the total chlorophyll content in the unstressed plants. Similar observations were made in *Arabidopsis* (Zhou *et al.,* 1998).

Carotenoid

The carotenoid content of the *A. hypogaea* leaves increased with age in control and treated plants. Drought stress induced a reduction in carotenoid content in the leaves of peanut when compared to control. Similar results were observed in tomato (Berova *et al.,* 2000, wheat and barley seedling (Sunitha *et al.,* 2004).

PBZ to the drought stressed *A. hypogaea* plants increased the carotenoid content when compared to control and drought stressed plants. PBZ with drought stress treatment increased the pigments in olive. Similar results were observed in triazole treatment to the NaCl stressed peanut (Muthukumarasamy and Panneerselvam, 1997).

ABA treatment to the drought stressed groundnut plants increased the carotenoid content when compared to control. Similar results were observed in citrus (Norman *et al.,* 1990); tobacco (Imai *et al.,* 1995); *B. pendula*; *Populus koveana*; *K. bluegrass* (Wang *et al.,* 2003).

The treatment with PBZ increased the carotenoid content in the unstressed plants. Similar results were observed in PBZ treated barley seedlings. Sunitha *et al.* (2004) reported that the barley seedlings treated with PBZ appeared greener and thicker due to increased pigment contents. Similar results were observed in growth regulators treated *Catharanthus* plants (Jaleel *et al.*, 2006a). Triadimefon treatment increased the carotenoid content to a higher level in cucumber. PBZ treatment increased the carotenoid content in *Raphanus sativus* plants (Sankari *et al.*, 2006).

ABA treatment increases to the unstressed plants carotenoid content when compared to control. ABA plays a direct role in mediating the photosynthesis to respiration in leaves and also the inhibition of lateral root development as reported in *Arabidopsis* (Zhou *et al.,* 1998).

Xanthophyll

Drought stress decreased the xanthophyll content when compared to control. Similar results were observed in wheat (Berova *et al.,* 2000) and barley seedling (Sunitha *et al.,* 2004).

PBZ and ABA treatment with drought stress increased the xanthophyll content in groundnut plants. The unsaturated hydrocarbons not only give color to fruits and flowers but also have multiple functions in photosynthesis. They participate in light-harvesting in photosynthetic membranes and protect the photosynthetic apparatus from excessive light energy by quenching triplet chlorophylls and singlet oxygen.

Anthocyanin

Drought stressed peanut plants showed a decreased anthocyanin content when compared to control. Similar results were observed in tomato (Berova *et al.,* 2000; barley seedling (Sunitha *et al.,* 2004).

PBZ treatment increased the anthocyanin content in *A. hypogaea* plants, but drought stress has no significant effect upon this. Triadimefon increased the chlorophyll and anthocyanin content in radish cotyledons. Triazoles greatly increase anthocyanin accumulation in carrot tissue cultures. Tetraconazole increased the anthocyanin content in maize (Angela *et al.,* 1997). In *A. hypogaea* the anthocyanin content increased under drought with ABA treatments. Treatment with ABA increased the anthocyanin accumulation in strawberry fruits. Triazoles induced a transient raise in abscissic acid content in the bean. This increased ABA content induced by triazole might be the cause for the increased anthocyanin content.

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