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Original Article

Variation in growth of peanut plants under drought stress condition and in combination with paclobutrazol and abscisic acid

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ABSTRACT

Economically important oilseed crop peanut (*Arachis hypogaea* L. TVM-2) belonging to the family Fabaceae was selected for the present investigation. Field experiments were conducted to identify the variation under drought stress, paclobutrazol, and abscisic acid, and their combination on the growth of peanut plants. Drought stress decreased the morphological parameters like root length, stem length, total leaf area, fresh and dry weight on 40, 60 and 80 days after sowing (DAS) when compared to control. The paclobutrazol (PBZ) and abscisic acid (ABA) treatment to the drought stressed plants increased all the parameters when compared to drought stressed plants. PBZ treatment increased the root length when compared to control, while decreased the stem length and leaf area. ABA treated plants decreased root length, while increasing the stem length and leaf area when compared to control. Fresh and dry weight decreased in drought stressed plants when compared to control. PBZ and ABA to the drought stressed plants increased the fresh and dry weight. PBZ and ABA treatments decreased the fresh and dry weight when compared in the unstressed plants.

KEY WORDS: Abscisic acid, drought, growth, paclobutrazol, peanut

INTRODUCTION

Drought is a major abiotic factor that limits agricultural crop production. Drought is a meteorological term and is commonly defined as a period without significant rainfall (Turner, 1979). Drought stress occurs when the available water in the soil is reduced, and atmospheric conditions cause continuous loss of water by transpiration or evaporation. The FAO (Food and Agricultural Organization of the United Nations) pointed out that one way to ensure future food needs of the increasing world populations should involve a better use of water by the development of crop varieties which needs less amount of water and more tolerant to drought (Andersen *et al.,* 1999).

Triazole compounds are systemic fungicides having plant growth regulating properties. The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including gibberellic acid, abscisic acid (ABA), and cytokinins (Hajihashemi *et al.,* 2007). Triazoles induce a variety of morphological and biochemical responses in plants, inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis, and a transient rise in ABA, as well as conferring protection from various environmental stresses (Fletcher *et al.,* 2000). 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 (Kopyra and Gwozdz, 2003; Manivannan *et al.,* 2007; and Sankar *et al.,* 2007).

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 important roles in stomatal movements, the regulation of photosynthetic enzyme activities, stability of photosynthetic apparatus, and expressions involved in chloroplasts (Jia and Lu, 2003).

Groundnut (*Arachis hypogaea* L.), also known as peanut, 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. Groundnut is extensively cultivated in 107 countries of the world on 25.2 mha with an annual production of 36.5 m (Mace *et al.,* 2006). Groundnut seeds contain 44-56% oil and 22-30% protein on a dry seed basis. In addition, they are a good source of minerals (phosphorus, calcium magnesium, and potassium) and vitamins (E, K, and B group) (Dwivedi *et al.,* 1996). India ranks first in world's groundnut production, accounting for 40% of the world area (7.5 m ha) and 31.7% (5.7 m) of the total production in the world. At present, India accounts for 9.6% of the world's total output of oilseeds, with more than 25 million hectares of land under oilseeds. Despite considerable area and production of groundnut in the country, the average yield of groundnut is too poor, only about 900 kg/ha as against 1416 kg/ha Asian average and 1275 kg/ha the world average (Hegde, 2005).

The objectives of the present study were to understand the effect of drought, paclobutrazol (PBZ), ABA and in combination on the growth characters of *A. hypogaea* under field conditions.

MATERIALS AND METHODS

Economically important oilseed crop peanut (*A. hypogaea* L.) belongs to the family Fabaceae was selected for the present investigation. Seeds of peanut (*A. hypogaea* L. TMV-2) were obtained from the Krishivigyan Kendra Form Science Center, Tamil Nadu Agricultural University, Thindivanam, Tamil Nadu, India. PBZ is a triazolic group of fungicide having plant growth regulating properties, obtained as CULTAR 25% w/v from Zeneca ICI Agrochemical Ltd., Mumbai, India and ABA from Sigma Chemicals, Bangalore were used in the present study. The experiments were conducted at the Botanical Garden and Stress Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India.

In the preliminary experiments, 2, 5, 10, 15, and 20 mg/L (active principle) concentrations were prepared from commercial preparations such as PBZ, and ABA 2, 5, 10, 15, and 20 µg/L were used for treatment to determine the optimum concentration of these compounds at which the dry weight increased significantly. Among these concentrations, 10 mg/L PBZ and 10 µg/L ABA were found to increase the dry weight significantly, and the higher concentration slightly decreased the growth and dry weight. Hence, 10 mg/L PBZ and 10 µg/L ABA were used to determine the effect of these plant growth regulators compound on the growth and metabolism of *A. hypogaea* L.

The peanut seeds were surface sterilized with 0.2% mercuric chloride solution for 2 min and rinsed thoroughly with distilled water. The peanut seeds were grown in a field, and the experiments were conducted during the months of February-May 2006 and 2007 in a randomized block design. The seeds were sown in plots measuring 3 m × 3 m in three replications with a 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 were used for treatments to stress and unstressed (control) plants. PBZ and ABA treatments were given by soil drenching and foliar spraying methods, respectively.

The treatment schedule was as shown below.

Serial number	Treatments
1	Control
2	Drought
3	Drought + PBZ
4	Drought + ABA
5	PBZ
6	ABA

ABA: Abscisic acid, PBZ: Paclobutrazol

Plants were harvested randomly on 40th, 60th, and 80th days after sowing (DAS) and washed with tap water and then with deionized water. The plants were separated into leaf, stem, and root and used for determining growth, anatomical, physiological, biochemical parameters, and the potential of enzymic and non-enzymic antioxidant parameters.

METHODS

Growth Parameters

Root length and stem length were recorded on 40th, 60th, and 80th DAS. Below the point of root-stem transition to the tap root and the length of lateral roots was taken as total root length. The length between shoot tip and point of root-stem transition region was taken as stem length. The root length and the stem length are expressed in centimeters per plant.

Total leaf area

Total leaf area was measured with LI-COR photoelectric leaf area meter (Model LI-3100, Lincoln, USA) and expressed in cm^2 per plant.

Fresh and dry weight

Plants were harvested and separated into leaves, stems, and roots, and fresh weight was recorded. The samples were dried in an oven at 60°C until the constant dry weight was obtained and dry weight was recorded. The fresh and dry weights are expressed in grams per plant.

RESULTS

Root Length (Table 1)

The root length decreased in drought-stressed *A. hypogaea* when compared to control, and it was 94.44% over control on 80 DAS. The root length increased in drought stress with PBZ and ABA treatments, when compared to control. The extent of increase was 146.31% and 122.13% over control on 80 DAS. PBZ treatment also increased the root length and it was 125.22% over control. In ABA-treated plants, the root length decreased when compared to control and it was 94.85% over control on 80 DAS.

Stem Length (Table 1)

Drought stress inhibited the stem length in peanut to a larger extent, and it was 75.35% over control on 80 DAS. Drought stress with PBZ and ABA treatments caused an enhancement in stem length when compared to drought-stressed plants in all sampling days. PBZ treatment decreased the stem length, and it was 68.53% over control on 80 DAS. ABA treatment significantly increased the stem length, and it was 118.06% over control on 80 DAS.

Total Leaf Area (Table 1)

Total leaf area reduced under drought stress when compared to control in peanut and it was 77.61% over control on 80 DAS. Drought stress with PBZ and ABA caused an increase in leaf area when compared to drought-stressed plants at all the sampling days, and it was 94.46% and 92.49% over control on 80 DAS. PBZ treatment decreased the total leaf area, and it was 97.54% over control. In ABA-treated plants, the total leaf area increased when compared to control and it was 102.95 on 80 DAS.

In drought-stressed plants, the root length, stem length, and total leaf area decreased to a larger extent. PBZ and ABA treatments to drought-stressed plants increased the root length, stem length, and total leaf area. PBZ-treated plants showed an increased root length when compared to control while it lowered the stem length and total leaf area. ABA-treated plants showed decreased root length while increasing the stem length and leaf area when compared to control.

Whole Plant Fresh Weight (Table 2)

Drought stress decreased the whole plant fresh weight to a larger extent, and it was 67.26% over control in *A. hypogaea*. The fresh weight increased in PBZ and ABA treatments under drought stress. The extent of increase was more in drought stress with PBZ-treated plants, and it was 78.12% over control and ABA-treated plants 75.58% over control on 80 DAS. PBZ and ABA treatments decreased the fresh weight significantly when compared to control, and it was 96.91% and 85.73% over control on 80 DAS.

Table 1: Effect of PBZ, ABA and drought and their combination induced changes on root, stem length, and total leaf area of *Arachis hypogaea*

DAS	Control	Treatments					*F* value	CD (*P*=0.05)
		Drought	Drought+PBZ 10 mg/L	Drought+ABA 10 μg/L	PBZ 10 mg/L	ABA 10 μg/L		
Root length (cm/plant)								
40	11.25	12.79	15.42	13.26	13.32	10.15	24.334**	0.895
60	15.43	12.53	21.81	18.4	18.93	14.39	57.034**	1.258
80	19.24	18.17	28.15	23.51	24.09	18.25	48.462**	1.583
Stem length (cm/plant)								
40	19.54	14.05	14.99	18.6	12.17	21.89	67.412**	1.345
60	37.47	28.71	30.22	36.43	24.72	42.91	79.578**	1.979
80	45.95	35.51	37.81	42.57	31.49	54.25	87.946**	2.266
Total leaf area (cm^2/plant)								
40	103.56	75.17	85.72	82.61	93.58	109.37	89.428**	1.606
60	112.84	91.25	109.41	105.38	111.65	115.26	76.416**	2.080
80	124.19	96.38	117.31	114.86	121.14	127.85	36.319**	1.734

**Significantly different at 0.01 level; Values are the mean of seven replicates. DAS: Days after sowing, CD: Critical difference, ABA: Abscisic acid, PBZ: Paclobutrazol

Table 2: Effect of PBZ, ABA and drought and their combination induced changes on whole plant fresh and dry weight of *Arachis hypogaea*

DAS	Control	Treatments					*F* value	CD (*P*=0.05)
		Drought	Drought+PBZ 10 mg/L	Drought+ABA 10 μg/L	PBZ 10 mg/L	ABA 10 μg/L		
Whole plant fresh weight								
40	18.52	13.626	16.124	15.61	17.943	16.925	20.216**	1.243
60	27.642	19.435	23.243	21.938	25.857	23.786	42.281**	4.869
80	50.132	33.721	39.163	37.89	48.585	42.976	83.704**	1.844
Whole plant dry weight								
40	2.101	1.191	1.824	1.799	1.949	1.903	28.788**	0.150
60	3.308	2.271	2.711	2.621	3.197	2.984	17.826**	0.400
80	5.131	3.486	4.119	3.986	4.671	4.38	17.333**	0.389

**Significantly different at 0.01 level. Values are the mean of seven replicates and expressed in grams/plant. CD: Critical difference, DAS: Days after sowing, ABA: Abscisic acid, PBZ: Paclobutrazol

Whole Plant Dry Weight (Table 2)

Drought stress caused decreased dry weight accumulation in peanut plants, and it was 67.94% over control on 80 DAS. Drought stress with PBZ and ABA treatments increased the dry weight when compared with drought-stressed plants, and it was 80.28% and 77.68% over control on 80 DAS. PBZ and ABA treatments significantly decreased the dry weight, and it was 91.03% and 85.37% over control.

DISCUSSION

Root Length

The root length increased in drought-stressed *A. hypogaea* when compared to control on 40 DAS. Drought stress increased the root length in sunflower (Manivannan *et al.,* 2007); olive (Bacelar *et al.,* 2007); *Cannabis sativa* (Amaducci *et al.,* 2008), and oak species (Rodriguez-Calcerrada *et al.,* 2008). The development of root system may increase the water uptake under drought stress. On 60 and 80 DAS, the root growth was decreased when compared to control. The root growth increased initially, but in a later stage, it was lowered because of severe drought stress. The reduction in plant height might be associated with declined cell enlargement and cell growth due to the low turgor pressure under drought stress (Liang *et al.,* 2006) and parley (Petropoulos *et al.,* 2008). Drought stress decreased the root length in *Albizzia* seedlings (Sundaravalli *et al*., 2005). Similar results were observed in *Eucalyptus microtheca* seedlings (Li *et al*., 2000) and populus species (Yin *et al*., 2005). Water stress reduces the biomass of fibrous roots in pearl millet and soybean (Kusaka *et al*., 2005 and Zhang *et al.,* 2007).

The root length increased in PBZ treatment under drought stress when compared to all other treatments. PBZ treatment increased the root growth in rose plants (Jenks *et al.,* 2001) and olive (Thakur *et al.,* 1998) under drought stress. Similar results were observed in triadimefon treatment to the NaCl-stressed radish (Panneerselvam *et al.,* 1997).

ABA treatment to the drought-stressed groundnut plants increased the root length when compared to control and drought-stressed plants. The morphological and physiological responses to exogenous ABA application showed that ABA could play an important role to control drought tolerance in wheat (Quarrie and Jones, 1977); tomato (Sharp and Lenoble, 2002), and two populus species (Yin *et al.,* 2005).

PBZ treatment increased root growth when compared to unstressed plants in all the sampling days. PBZ increased the diameter and length of fibrous roots and enhanced the lateral root formation in tomato plants (Berova *et al.,* 2000). Similar results were observed in soybean treated with PBZ (Sankhla *et al.,* 1985); *Lycopersicon esculentum* (Baruah *et al.,* 1995); *Dianthus caryophyllus* (Banon *et al.,* 2002), and wheat (Berova *et al.,* 2002).

ABA treatment decreases the root growth of peanut in all the sampling days. ABA was found to decrease primary root growth moderately, whereas it had no significant effect on the number of lateral roots initiated. Similar results were observed in peas (Zhang and Davies, 1987); wheat (Cammue *et al.,* 1989); soybean (Creelman *et al.,* 1990); maize (Saab *et al.,* 1990), and poplar species (Yin *et al.,* 2005 and Li *et al.,* 2004).

Stem Length

Stem length decreased in *Eucalyptus* seedlings under drought stress (Li *et al.,* 2000). The plant height was reduced under drought stress in *Populus* species (Yin *et al.,* 2005). Reduced plant height was reported in *Albizzia* seedlings due to reduced stem length under drought stress (Sundaravalli *et al.,* 2005). Similar results were observed in avocado (Chartzoulakis *et al.,* 2002); soybean; *Abelmoschus esculentus* (Sankar *et al.,* 2007), and in olive (Bacelar *et al.,* 2007).

PBZ treatment to the drought-stressed plants increased the stem length when compared to drought-stressed plants.

PBZ with drought stress treatment increased the stem growth 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 to the drought-stressed plants increased the stem length of the peanut plants when compared to drought stress, but it was lower than that of control. Similar results were observed in drought-stressed tomato plants treated with ABA (Sharp *et al.,* 2000; Sharp and LeNoble, 2002) and two populus species (Yin *et al.,* 2005).

PBZ treatment to the unstressed plants decreased the stem length of groundnut plants when compared with control. Barley seedlings showed reduced growth under treatment with PBZ (Sunitha *et al.,* 2004). PBZ has been found to reduce stem elongation in a number of plants such as citrus (Aron *et al.,* 1985) and mango (Singh, 2000; Shinde *et al.,* 2000). Triazole treatments reduced the stem elongation in grape vines (Hunter and Procter, 1992), citrus (Mehouachi *et al.,* 1996), and maize seedlings (Fletcher *et al.,* 2000). Triazoles acts as antigibberellins, controls shoot growth by inhibiting the production of gibberellins, which are responsible for the cell elongation of shoots and leaves (Barrett, 2001).

ABA treatment increases the stem length when compared to control and other treatments. Similar results were observed in two contrasting poplar species to drought stress and exogenous ABA application (Yin *et al.,* 2005). ABA-induced growth was resulted from signal transduction at the single-cell level and thereby induces closure of stomata in peas (Zhang and Davies, 1987). Similar results were observed in wheat (Cammue *et al.,* 1989), soybean (Creelman *et al.,* 1990), and maize (Saab *et al.,* 1990).

Total Leaf Area

Drought stress reduced the leaf area when compared to control in *A. hypogaea*. The leaf growth was more sensitive to water stress in maize (Nayyar and Gupta, 2006). Similar results were observed under drought stress in *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005); *Eucalyptus globulus* (Pita and Pardos, 2001); wheat (Gong *et al.,* 2003); cowpea (Anyia and Herzog, 2004); Olive (Chartzoulakis *et al.*, 1999; Bosabalidis and Kofidis, 2002; Bacelar *et al.,* 2004; and Bacelar *et al.,* 2007) (Figure 1).

Figure 1: Effect of paclobutrazol, abscisic acid and drought and their combination induced changes on morphology of *Arachis hypogaea* on 40 days after sowing

PBZ treatment to the drought-stressed plants resulted in increased leaf area in groundnut plants when compared to drought-stressed plants. PBZ treatment increased the leaf area in olive (Thakur *et al.,* 1998) and rose plants (Jenks *et al.,* 2001) under drought stress. Similar results were observed in triazole treatment to the NaCl-stressed peanut (Muthukumarasamy and Panneerselvam, 1997).

ABA treatment to the drought-stressed peanut plants increased the leaf area when compared to drought-stressed plants, but it was lower than that of control. Similar results were observed in ABA treatment under drought stress treatment in tomato (Sharp *et al.,* 2000; Sharp and LeNoble, 2002) and two populus species (Yin *et al.,* 2005).

PBZ treatment to the unstressed plants decreased the leaf area when compared to control. PBZ reduced the leaf area in wheat (Tonkinson *et al.,* 1995), tomato (Berova *et al.,* 2000), and barley (Sunitha *et al.*, 2004). The leaf area is reduced in *Catharanthus* plants under PBZ treatment (Jaleel *et al.*, 2007). The reduced leaf area in triazole treatments may be due to the increased ABA content and reduced gibberellin biosynthesis induced by triazoles. PBZ reduces cell number and length, so PBZ-treated plants characteristically have smaller leaf (Fletcher *et al.,* 2000). Uniconazole reduced the leaf number in *pyrecantha* (Norcini and Knox, 1989).

ABA treatment increased the leaf area when compared to control. Similar results were observed in the application of ABA at room temperature results in reduced leaf production in many plants (Swamy and Smith, 2001). The exogenous application of ABA will initiate stomatal closure and protect the plants from stress (Saab *et al.,* 1990). The involvement of ABA in mediating drought stress has been extensively studied. 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). The increased leaf area under drought stress by ABA is a part of drought stress mitigation mechanisms (Luan, 2002 and Zhu, 2002).

Whole Plant Fresh Weight

Drought stress treatment decreased the whole plant fresh weight in peanut plants. Similar results were observed in higher plants such as *Pearl millet* (Kusaka *et al.,* 2005) and *A. esculentum* (Bhatt and Srinivasa Rao, 2005). The reduction in fresh weight under drought condition might be due to suppression of cell expansion and cell growth due to the low turgor pressure, and partial root drying caused a significant reduction in shoot biomass when compared to control as observed in wheat (Gong *et al.,* 2003 and Shao *et al.,* 2005).

PBZ treatment to drought-stressed plants increased the fresh weight, but it was lower than that of control. Similar results were reported in *Catharanthus* plants under salt stress (Jaleel *et al*., 2007a). The triazole-treated plants have exhibited thickened and fleshy roots with increased root diameter and weight in radish (Panneerselvam *et al.,* 1997); tomato (Berova *et al.,* 2000), and wheat (Berova *et al.,* 2002). The main reason for the increase in fresh weight may be due to the increased root growth under PBZ treatments.

ABA treatment to the drought-stressed groundnut plants increased the whole plant fresh weight when compared to drought stress, but it was lower than that of control. Similar results were observed in ABA with drought stress treatment in tomato (Sharp *et al.,* 2000; Sharp and LeNoble, 2002) and two populus species; two contrasting poplar species to drought stress and exogenous ABA application (Yin *et al.,* 2005).

PBZ treatment lowered the whole plant fresh weight when compared to control in groundnut plants. Similar results were observed in triazole-treated plants are a reduction in fresh weight (Thakur and Thakkur, 1993). Triadimefon and PBZ decreased the shoot fresh weight in peanut (Muthukumarasamy and Panneerselvam, 1997).

ABA treatment decreased the whole plant fresh weight when compared to control in peanut plants. Similar results were observed in two contrasting poplar species to drought stress and exogenous ABA application (Yin *et al.,* 2005).

Whole Plant Dry Weight

Drought stress decreased the dry weight in groundnut plants. A decrease in plant biomass was reported in drought-stressed wheat (Shao *et al.,* 2007) and in *Asteriscus maritimus*. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism, and finally dying (Liang *et al*., 2006). Similar results were observed in NaCl-treated tomato and chickpea (Muthukumarasamy and Panneerselvam, 1997).

PBZ treatment to the drought-stressed peanut plants increased the whole plant dry weight when compared to drought-stressed plants. PBZ and drought stress treatment increased the whole plant dry weight in olive (Thakur *et al.,* 1998) and rose plants (Jenks *et al.,* 2001). The alteration of hormonal status induced by PBZ might be the cause for the increased dry weight under drought stress.

ABA treatment to the drought-stressed *A. hypogaea* plants increased the whole plant dry weight when compared to drought-stressed plants, but it was lower than that of control. 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 (Luan, 2002) which might be the reason for increased dry weight under drought stress (Yin *et al.,* 2005).

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