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Leaf anatomical changes in peanut plants in relation to drought stress with or without paclobutrazol and abscisic acid

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ABSTRACT

Peanut (*Arachis hypogaea* L. TVM-2) was grown in field under drought stress in combination with paclobutrazol (PBZ) and abscisic acid (ABA) to study their individual and combined effects on leaf anatomical characteristics. The thickness of the leaf, upper and lower epidermis and the number of cells per unit area in the palisade and spongy regions were very much reduced under drought stress. The palisade and spongy layers of mesophylls were well-differentiated, and the cells are wider and longer as compared to shorter palisade and spongy parenchyma of control. The number of the palisade and spongy cells increased per unit area with all treatments as compared with drought stressed and unstressed plants. The vascular bundles of the PBZ-treated plants were narrow and dense when compared to control. The xylem vessels of PBZ and ABA-treated leaves were much narrow when compared to control plants. Among the treatments, the present findings revealed that the growth regulator treatments to the drought stressed plants have great impact on the anatomy of *A. hypogaea* plants.

KEY WORDS: Absisic acid, leaf anatomy, peanut, paclabutrazol

INTRODUCTION

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 (Zhu, 2002; Chaitanya *et al.,* 2003; Chaves *et al.,* 2003; Shao *et al.,* 2006).

Paclobutrazol (PBZ) is a triazolic group of fungicide which have plant growth regulating properties (Milandri *et al.,* 2008). Abscisic acid (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).

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 oil seeds, with more than 25 million hectares of land under oil seeds. 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).

Groundnut (*A. hypogaea* L.), is one of the most important oilseed crops and green manure as it roots have nodules harboring nitrogen fixing bacteria helps to fix the nitrogen in the soil and increase the soil fertility. Because groundnut is usually grown in rain fed condition, it has been hypothesized that improving water use efficiency would be the best strategy to cope with episodes of intermittent drought (Krishnamurthy *et al.,* 2007). The objectives of the present study were to understand the effect of drought, PBZ, ABA and in combination on the leaf anatomical characters of *A. hypogaea* under field conditions.

MATERIALS AND METHODS

Seeds of *A. hypogaea* L. belongs to the family Fabaceae were obtained from the Krishivigyan Kendra Form Science Center, Tamil Nadu Agricultural University, Thindivanam, Tamil Nadu, India. PBZ is obtained from Zeneca ICI Agrochemical Ltd., Mumbai, Maharashtra, India and ABA from Sigma Chemicals, Bangalore, Karnataka, India 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.

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 in a Randomized block design. 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 40th, 60th, and 80th days after shows (DAS) and washed with tap water and then with deionized water. The plants were separated into leaf, stem and root and used for determining anatomical parameters.

Leaf Anatomical Studies

The leaves were washed thoroughly with water and fixed in formalin acetic acid (FAA). For studying the internal structure of a leaf, thin transverse sections were taken by using rotary microtome, stained, and observed under calibrated light microscope and the thickness of leaf was measured by a pre-calibrated ocular micrometer. For stomatal studies, the epidermal peels were taken out from the basal, middle and apical regions by adopting direct peel method. The epidermal peels were stained with 1% Delafield's hematoxylin and mounted in 50% glycerin. The observations were taken in peels of both controls and in each treatment. The leaf, upper and lower epidermis thicknesses are expressed in micrometers and number stomata in upper, and lower epidermal cells per unit area (mm^2) were calculated by using the generally followed formula of Metcalfe and Chalk (1979). The number of spongy and palisade cells per unit area cells (mm^2) were also calculated separately to find out the relative effect of growth regulator compounds.

RESULTS AND DISCUSSION

Leaf Anatomy (Table 1, Figure 1)

Thickness of leaf (Table 1)

Drought stress decreased the leaf thickness to a larger extent when compared with control, and it was 89.49% over control on 80 DAS. Drought stress with PBZ and ABA-treated plants showed an increased leaf thickness when compared with control and drought stressed plants and it was 108.86% and 103.69% over control

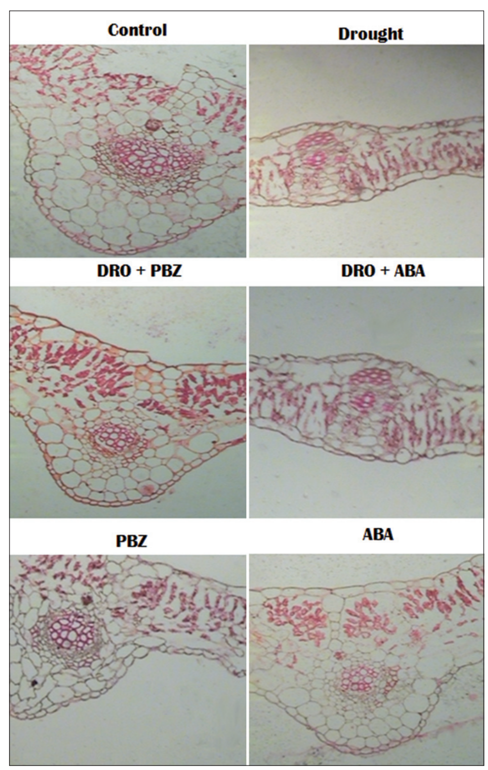

Figure 1: Effect of paclobutrazol, abscisic acid and drought and their combination induced changes on leaf anatomy of *Arachis hypogaea* on 80 days after showing

Table 1: Effect of PBZ, ABA and drought and their combination induced changes in the leaf anatomy of *Arachis hypogaea* on 80 DAS

Parameters	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		
Leaf thickness	215.21	192.6	234.27	223.15	243.4	228.41	160.694**	3.664
Epidermal thickness	10	6	9	8	13	11	125.415**	1.400
Number of palisade	39.54	25.47	42.13	41.18	49.39	45.24	138.050**	2.016
Palisade mesophyll	98	84	137	117	143	126	228.776**	4.014
Number of spongy cell	35.47	23.62	38.62	36.43	43.96	41.33	154.004**	9.630
Spongy mesophyll	124	97	158	146	165	152	411.515**	3.789
Xylem vessel	19	23	21	15	17	16	112.600**	2.430

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

on 80 DAS. PBZ and ABA-treated plants also increased the leaf thickness when compared with control and the increase was 113.09% and 106.13% over control on 80 DAS.

Epidermal thickness (Table 1)

The thickness of the epidermis was decreased by the drought stressed peanut plants and it was 60.00% over control on 80 DAS. PBZ and ABA treatments to the drought stressed plants increased the thickness of the epidermis and it was 90.00% and 80.00% over control on 80 DAS, but it was lower than that of control. PBZ and ABA treatments increased the thickness of the epidermis to a level even above that of control and other treatments, and it was 130.00% and 110.00% over control on 80 DAS.

Number of Palisade Cells per Unit Area (Table 1)

Drought stressed peanut plants decreased the number of palisade cells, and the decrease was 64.42% over control on 80 DAS. PBZ and ABA treatments to the drought stressed plants increased the number of palisade cells and it was 106.55% and 104.15% over control and drought stressed plants on 80 DAS. PBZ and ABA treatments also increased the palisade cell number when compared with control to a larger extent and it was 124.91% and 114.42% over control on 80 DAS, respectively.

Palisade Mesophyll (Table 1)

Drought stress decreased the palisade mesophyll length to a larger extent when compared with control and it was 85.71% over control on 80 DAS. Drought stressed PBZ and ABA-treated plants showed an increased palisade mesophyll length when compared with control and drought stressed plants and it was 139.79% and 119.39% over control on 80 DAS. PBZ and ABA-treated plants increased the palisade mesophyll length when compared with control and other treatments and the increase was 145.92% and 128.57% over control on 80 DAS.

Number of Spongy Cells per Unit Area (Table 1)

Distribution of spongy cells per unit area was reduced to 66.59% by the drought stress when compared with control. PBZ and ABA treatments to the drought stressed plants increased the number of spongy cells when compared to control and drought stressed plants and it was 108.88% and 102.71% over control on 80 DAS. PBZ and ABA treatments increased the number of spongy cells to a larger extent when compared with control and the increase was 123.94% and 116.52% over control on 80 DAS.

Drought stress treatment reduced the number of the palisade and spongy cells to a larger extent and PBZ and ABA treatments to the drought stressed plants increased the number of the palisade and spongy cells to the level of control. PBZ and ABA treatments also increased the number of the palisade and spongy cells to a level higher than that of control.

Spongy Mesophyll (Table 1)

Drought stress decreased the spongy mesophyll length to a larger extent in *A. hypogaea* when compared with control and it was 78.23% over control on 80 DAS. Drought stressed with PBZ and ABA-treated plants increased the spongy mesophyll length when compared with control and drought stressed plants and it was 127.42% and 117.74% over control on 80 DAS. PBZ and ABA-treated plants also increased the palisade mesophyll length when compared with control and the increase was 133.06% and 122.58% over control on 80 DAS.

Xylem Vessel Diameter (Table 1)

The xylem vessel diameter was significantly decreased by the drought stressed plants when compared with control and it was 82.43% over control on 80 DAS. PBZ and ABA treatments to the drought stressed plants increased the xylem vessel diameter when compared with drought stressed plants. However, it was lower than that of control and it was 89.47% and 84.21% over control on 80 DAS. PBZ and ABA treatments to the unstressed plants increased the xylem vessel diameter when compared with control and it was 121.05% and 110.53% over control, respectively, on 80 DAS.

Leaf anatomy changed in water-stressed leaves, which could have accounted for the decreased stomatal conductance (Mori and Schroeder, 2004). The leaf thickness of *A. hypogaea* leaves increased with PBZ and ABA treatments. Among the treatments, PBZ increased the lamina thickness to a greater extent when compared to other treatments. PBZ treatment increased leaf thickness in potato (Tekalign *et al.,* 2005), *Chrysanthemum* (Burrows *et al.,* 1992), soybean and sugar beet (Dalziel and Lawrence 1984). The increased leaf thickness is attributed to an increase in epidermal cell diameter, palisade layer and spongy mesophyll depth (Mori and Schroeder, 2004).

PBZ and ABA treatments increased the total leaf thickness and epidermal thickness to a level higher than that of control in peanut plants. Similar results were observed in leaves of triadimefon treated wheat (Gao *et al.,* 1988) and PBZ treated pecon (Wood, 1984); *Aechmea faciata* (Ziv *et al.,* 1986) and *Chrysanthemum* (Burrows *et al.,* 1992).

Tekalign *et al.* (2005) reported that PBZ treated plants exhibited thicker epicuticular wax layers, larger epidermal cells compared to the control. PBZ increases the thickness of the epicuticular wax layer in rose cultivars (Jenks *et al.,* 2001) and maize (Sopher *et al.,* 1999). Our results are in accordance with the above observations.

PBZ and ABA treatments increased the number of the palisade and spongy layer in *A. hypogaea* leaves. Gao *et al.* (1988) found that growth regulators treated leaflets of wheat had more palisade cells per unit area. Induction of additional layers of palisade parenchyma was observed with the PBZ treatment in *Chrysanthemum* (Burrows *et al.,* 1992). PBZ increased the palisade cell length and width in potato (Tekalign *et al.,* 2005).

Increased cell division and mesophyll thickness, also chloroplast size was reported in triadimefon treated wheat leaves (Gao *et al.,* 1988). Similar is the case with PBZ treated sugar beet leaves (Dalziel and Lawrence, 1984), *Chrysanthemum* (Burrows *et al.,* 1992) and in soybean.

Triazoles increased the cytokinin levels in various plants like cucumber (Fletcher and Arnold, 1986) and rice. The increased cytokinin levels might have induced the cell division thereby increasing the number of cells in the palisade and spongy layers (Tekalign *et al.,* 2005).

Among the treatments PBZ treated leaves showed higher stomata per unit area both in upper and lower epidermis. Treatment with triazoles caused an increase in the number of stomata per unit area both in the upper and lower epidermis when compared to control. Among the epidermis, lower epidermis showed a higher level of number of stomata per unit area when compared to upper epidermis. Triadimefon treatment increased the stomatal number per unit area in wheat (Gao *et al.,* 1988). The increase in stomatal distribution could be due to the effect of triazoles on the hormonal balance as reported in wheat (Fletcher, 1985; Fletcher and Hofstra, 1985), and can be attributed to cell division induced through the increased cytokinin content as reported earlier by Fletcher and Arnold (1986).

Growth regulators could directly regulate stomatal opening and photosynthesis rate compared with plants under well-watered condition; plants under drought stress had higher ABA accumulation, lower stomatal conductance (Tardieu *et al.,* 1996; Aasamaa *et al.,* 2002); and lower photosynthesis rate (Meyer and Genty, 1999; Horvath *et al.,* 2000). Accordingly, PBZ and ABA may play an important role in the process for drought adaptation and enhance drought tolerance in the most groups of higher plants (Yin *et al.,* 2005).

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