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Biochemical content variation in *Arachis hypogaea* under drought stress with or without paclobutrazol and abscisic acid

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ABSTRACT

An experiment was conducted to determine the changes in biochemical parameters (amino acid, protein, and proline) under drought stress, paclobutrazol (PBZ), and abscisic acid (ABA) and their combination. Drought stress caused a significant increase in the biochemical constituents such as amino acid and proline contents when compared with control in groundnut plants. PBZ and ABA treatment to the drought stressed plants also increased amino acid and proline contents. However, these contents were lower than that of drought stressed plants. PBZ and ABA treatments to the drought stressed plants increased the protein content when compared to drought stressed plants. Increase drought tolerance induced by PBZ and ABA in groundnut will be helpful for the farmers to cultivate peanut under drought condition.

KEY WORDS: Peanut, drought, biochemical, abscisic acid

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an ancient crop of the new world. The peanut is cultivated around the world in tropical, subtropical and warm temperate climates. About 13.5 million ha are grown in Asia, 5.3 million ha in Africa, 1.2 million ha in the Americas, and 0.1 million ha in other parts of the world. India and China are the largest producers of the (Fasina, 2008). However, when diseases are controlled and good management practices are used, yields of 3 or more mt/ha can be achieved (Lamb and Blankenship, 1996). The peanut seed has 36-54% oil, and more than half of the global crop is grown as an oilseed.

Environmental stresses, such as drought, high salinity and extreme temperatures, have adverse effects on plant productivity (Levitt, 1980). Tolerant plants respond and adapt to these stresses through various morphological, physiological, and molecular processes (Dramé *et al.*, 2007). Water is essential for crop production, and best use of available water must be made for efficient crop production and higher yields. Water deficit affects crop growth, depending on the stage of growth and the degree or intensity of water stress.

Triazoles induce a variety of morphological, physiological and biochemical responses in plants, including a reduction in shoot elongation, stimulation of rooting and increased chlorophyll content. The gibberellin biosynthesis and carbohydrate status altered with triazole treatments in plants and increased stress tolerance, delayed senescence, increased cytokinin synthesis and a transient rise in abscisic acid (ABA) synthesis (Leul and Zhou, 1999). Diniconazole treated *Nicotiana tabacum* plants showed higher ABA content and higher transcription levels of ABA response genes during rehydration than the untreated plants and were a more drought stress tolerant (Kitahata *et al.,* 2005).

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

MATERIALS AND METHODS

Peanut (*A. hypogaea* L. TMV-2) seeds 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, Bengaluru 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 the 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 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 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 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 biochemical parameters.

Estimation of Total Free Amino Acid Content

Total free amino acids were extracted and estimated by the following method of Moore and Stein (1948).

Extraction

About 500 mg of fresh plant material was homogenized in a mortar and pestle with 10 ml of 80% boiled ethanol. The extract was centrifuged at 800 g for 15 min, and the supernatant was made up to 10 ml with 80% ethanol and used for the estimation.

Estimation

In 25 ml test tube, 1 ml of ethanol extract was taken and neutralized with 0.1 N NaOH using methyl red indicators. To which, 1 ml of ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 min, and then 5 ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm in a Spectrophotometer (U-2001-Hitachi) against an appropriate blank. The standard graph was prepared by using leucine as standard, and the amino acid content was calculated using the standard graph, and the results are expressed in milligram a gram dry weight.

Determination of Proline Content

Proline was extracted and estimated following the method of Bates *et al*. (1973).

Extraction

500 mg of fresh plant material was homogenized in a mortar and pestle with 10 ml of 3% aqueous sulfosalicyclic acid. Then the homogenate was filtered through Whatmann No. 1 filter paper. The residue was re-extracted and pooled, and the filtrates were made up to 20 ml with aqueous sulfosalicylic acid and this extract was used for the estimation of proline.

Estimation

To 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added. The mixture was incubated for an hour at 100°C in a boiling water bath. Then, the test tubes were transferred to an ice bath to terminate the reaction. Then, 4 ml of toluene was added and mixed vigorously using a test tube stirrer for 20 s and the toluene containing the chromophore was separated from the aqueous phase with the help of a separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using a reagent blank. The proline content was determined from a standard curve with proline, and the results are expressed in milligrams a gram dry weight.

RESULTS AND DISCUSSIONS

Free Amino Acid (Table 1)

Root

The amino acid content increased in drought stress in all the stages of growth, and it was 139.51%, 147.21% and 151.36% over control on 40, 60 and 80 DAS respectively. PBZ and ABA treatments to the drought stressed plants

Table 1: Effect of PBZ, ABA and drought and their combination induced changes on amino acid content of *A. 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								
40	6.386	8.909	8.724	8.547	8.476	8.278	47.865**	0.335
60	8.811	12.971	12.843	12.164	12.094	11.978	30.891**	0.949
80	13.091	19.815	19.491	19.142	18.466	18.257	49.646**	0.705
Stem								
40	8.201	9.884	9.626	9.383	9.452	9.294	15.772**	0.503
60	9.273	11.816	11.368	11.344	11.102	10.948	12.718**	0.784
80	11.303	15.724	15.203	14.831	14.171	13.979	81.442**	0.529
Leaf								
40	7.557	9.863	9.206	9.012	8.634	8.414	12.119**	0.390
60	9.322	13.239	12.623	11.896	11.151	10.775	56.522**	0.597
80	10.032	15.06	14.197	13.299	12.524	12.193	42.587**	0.554

**Significantly different at 0.01 level, values are the mean of three replicates and expressed in mg/g dry weight. PBZ: Paclobutrazol, ABA: Abscisic acid, *A. hypogaea: Arachis hypogaea*, CD: Critical difference, DAS: Days after sowing

lowered the amino acid content when compared to drought stressed plants and it was 148.89% and 146.22% over control on 80 DAS. The amino acid content of the roots increased under individual PBZ and ABA treatments and it was 141.05% and 139.46% over control on 80 DAS.

Stem

Amino acid content was high in drought stressed stem of *A. hypogaea* when compared to control and it was 139.11% over control on 80 DAS. PBZ and ABA to the drought stressed plants lowered the amino acid content when compared to the drought stressed plants and it was 134.51% and 131.21% over control on 80 DAS. PBZ and ABA-treated plants showed an increase in the amino acid content when compared to control and the increase was 125.37% and 123.68% over control on 80 DAS.

Leaf

Drought stress increased the amino acid content in the leaves of peanut plants, and it was 150.12% over control on 80 DAS. PBZ and ABA treatments to the drought stressed plants showed a lower amino acid content when compared with drought stressed plants and it was 141.52% and 132.57% over control on 80 DAS. PBZ and ABA treatments increased the amino acid content when compared with control, and it was 124.84% and 121.54% over control on 80 DAS respectively. In the drought stressed plants the leaves and roots showed higher amino acid content when compared to the stem.

Drought stress increased the amino acid content when compared to control in *A. hypogaea*. The amino acid content increased under drought condition in sunflower (Manivannan *et al*., 2007). The amino acid content increased under drought condition in *A. hypogaea*; *Sorghum*; *Phragmites australis*; pepper; *Radix astragali*; *Molus domestica* (Sircelj *et al*., 2005) and marsh grasses. The accumulated amino acid may be occurring in response to the change in the osmotic adjustment of their cellular contents. PBZ treatment to the drought stressed peanut plants lowered the amino acid content when compared to drought stress, but it was higher than that of control. Similar results were observed in PBZ and triacontanol in olive varieties under water stress (Thakur *et al*., 1998) and PBZ treated wheat seedlings under low-temperature stress (Berova *et al*., 2002). ABA treatment to the drought stressed groundnut plants increased the amino acid content in all the sampling days when compared to control, but it was lower than that of drought stressed plants. Similar observations were made in *Populus koreana* (Cochard *et al*., 1996); Kentucky bluegrass and maize (Ren *et al*., 2007). Similar results were observed in triadimefon treatment increased the amino acid content in radish (Muthukumarasamy *et al*., 2000) and soybean.

Protein (Table 2)

Root

In the roots, the protein content decreased in the drought stress when compared with control and it was 73.98%, 70.16% and 63.40% over control on 40, 60 and 80 DAS. Drought stressed plants with PBZ and ABA treatments increased the protein content when compared to drought stressed plants, and it was 69.89% and 81.48% over control on 80 DAS. PBZ and ABA treatments caused a decrease in the protein content when compared to the control of the root, and it was 75.83% and 85.03% over control on 80 DAS.

Stem

Drought stress decreased the protein content in the stem of *A. hypogaea* and it was 59.11% over control on 80 DAS. PBZ and ABA treatments to the drought stressed plants increased the protein content of the stem when compared with drought stressed plants, and it was 63.82% and

71.09% over control on 80 DAS. PBZ and ABA-treated plants showed a decreased protein content when compared to control, and it was 86.13% and 78.26% over control on 80 DAS.

Leaf

The protein content of the leaf significantly reduced by the drought stress treatment when compared to control and it was 68.45%, 64.29% and 60.52% over control on 40, 60 and 80 DAS respectively. PBZ and ABA treatments to the drought stressed plants increased the protein content when compared to drought stressed plants, and it was 76.44% and 75.41% over control. The protein content of the leaf decreased under PBZ and ABA treatments, and it was 79.48% and 84.53% over control on 80 DAS. Among the organs, the roots showed a higher protein content followed by leaf and stem in all the treatments.

In *A. hypogaea*, drought stress caused a decrease in protein content of the groundnut plants at all stages of growth. Drought stress caused a decrease in the protein content in all parts of the plants to a larger extent in groundnut. Similar results were observed in maize; groundnut and wheat (Gong *et al*., 2005). The drought stressed plants reduced the quantity of soluble proteins observed in the present experiment can be related to reduced rate of protein biosynthesis and increased breakdown of proteins under water limited environment. There was a significant reduction in protein content under stress due to the increase in proline contents. The reduction in protein content in the chilling stressed plants was correlated with increased proline accumulation. Thus, may be due to the hydrolysis of protein or the inhibition of protein synthesis by oxidative stress leading to the accumulation of proline. Application of PBZ with drought stress treatment resulted in increased protein content in *A. hypogaea* when compared to drought stress, but it was lower than that of control. PBZ treated wheat seedlings had more soluble protein (Kraus *et al*., 1995) and *Brassica carinata*. Triadimefon treatment increased the protein content in *Raphanus sativus* (Muthukumarasamy *et al*., 2000), and cucumber seedlings (Panneerselvam *et al*., 1998).

Table 2: Effect of PBZ, ABA and drought and their combination induced changes on protein content of *A. 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								
40	13.868	10.259	10.976	12.831	12.427	13.133	16.021**	0.151
60	10.566	7.413	7.956	9.255	8.489	9.469	18.861**	0.496
80	12.932	8.199	9.038	10.537	9.806	10.996	34.282**	0.844
Stem								
40	3.583	2.495	2.68	3.2110	3.386	3.517	15.758**	0.151
60	3.645	2.308	2.748	2.954	3.295	3.136	13.361**	0.773
80	4.397	2.599	2.806	3.126	3.787	3.441	11.400**	0.276
Leaf								
40	4.409	3.018	3.219	3.695	3.866	4.058	18.340**	0.224
60	5.341	3.434	4.258	4.453	4.447	4.845	12.456**	0.591
80	6.354	3.845	4.857	4.792	5.05	5.371	49.292**	0.379

**Significantly different at 0.01 level, values are the mean of three replicates and expressed in mg/g dry weight. PBZ: Paclobutrazol, ABA: Abscisic acid, *A. hypogaea: Arachis hypogaea*, CD: Critical difference, DAS: Days after sowing

Table 3: Effect of PBZ, ABA and drought and their combination induced changes on proline content of *A. 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								
40	0.329	0.513	0.411	0.399	0.439	0.426	14.575**	0.061
60	0.528	0.894	0.769	0.664	0.746	0.724	23.164**	0.072
80	0.863	1.438	1.323	1.115	1.239	1.219	15.588**	0.131
Stem								
40	0.307	0.546	0.423	0.42	0.389	0.369	10.288**	0.077
60	0.493	0.793	0.691	0.697	0.676	0.648	48.406**	0.033
80	0.868	1.438	1.172	1.153	1.219	1.201	14.263**	0.158
Leaf								
40	0.356	0.581	0.481	0.476	0.432	0.421	21.648**	0.050
60	0.543	0.794	0.753	0.739	0.721	0.687	19.038**	0.022
80	0.935	1.486	1.251	1.237	1.327	1.308	14.406**	0.299

**Significantly different at 0.01 level, values are the mean of three replicates and expressed in mg/g dry weight. PBZ: Paclobutrazol, ABA: Abscisic acid, *A. hypogaea: Arachis hypogaea,* CD: Critical difference, DAS: Days after sowing

Proline (Table 3)

Root

In the roots, the proline accumulation was increased by the drought stress at all stages of growth. Drought stressed plants increased the proline content when compared with control, and it was 155.93%, 169.32% and 166.63% over control on 40, 60 and 80 DAS. Drought stressed plants with PBZ and ABA treatments showed a decreased proline content when compared to drought stressed plants but, it was higher than that of control, and it was 134.30% and 129.20% over control on 80 DAS. PBZ and ABA treatment caused an increase in the proline content of the roots, and it was 143.57% and 141.25% over control on 80 DAS.

Stem

The proline content of the stem increased with drought stress even above the level of control and the increase was 165.67% over control on 80 DAS. Drought stressed plants treated with PBZ and ABA treatments increased the proline content in the stem when compared to control and it was 135.07% and 132.81% over control on 80 DAS. The proline content was increased under individual PBZ and ABA treatments, and it was 140.44% and 138.36% over control on 80 DAS.

Leaf

Drought stress caused an increase in the proline content of the leaf and it was 158.93% over control on 80 DAS. PBZ and ABA to the drought stressed plants decreased the proline content when compared to drought stressed plants, and it was 133.77% and 132.32% over control on 80 DAS. PBZ and ABA-treated plants showed an increase in the proline content and the increase was 141.93% and 139.89% over control on 80 DAS.

In *A. hypogaea*, drought stress caused increased accumulation of proline content at all stages of growth. Water stress resulted in an increase in proline accumulation in *Sorghum* (Yadav *et al*., 2005). The similar results were observed in *Sorghum* wheat (Nayyar and Gupta, 2003); soybean (Heerden and Kruger, 2002).

PBZ and ABA treatment caused an enhancement in proline content when compared to control, but it was lower than that of drought stressed groundnut plants. PBZ and ABA also resulted in increased proline content in *A. hypogaea*. PBZ increased the proline content in *Eruca sativa* seedlings (Mathur and Bohra, 1992). ABA increased the proline content in *Phaseolus vulgaris* (Mackay *et al*., 1990) and *Arabidopsis thaliana* (Verslues and Elizabeth, 2006). Proline accumulation in plants might be a scavenger and acting as an osmolyte. Increased proline in the stressed plants may be an adaptation to overcome the stress conditions.

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