__Original Research Article__

Effect of Water Stress and Paclobutrazole onProline and Total Antioxidant Activity in Groundnut (*Arachis hypogaea* L.)

ABSTRACT

The present investigation entitled "Effect of Water Stress and Paclobutrazole on Proline and Total Antioxidant Activity in Groundnut (*Arachis hypogaea* L.)" was carried out during during the summer seasons of the year 2021 and 2022.Twelve treatments comprising of all possible combinations of three irrigation levels for water stress and four paclobutrazole levels were tested in a split plot design with three replications. Total anti-oxidant activity and proline content showed significantly higher with irrigation at 20 days interval. Whereas, minimum was recorded with irrigation at 10 days interval in pooled results.The significantly higher total anti-oxidant activity and proline content was recorded with application of PBZ @ 300 mg/l during both the years and in pooled analysis, respectively, whereas a minimum was noted with control (No spray of PBZ).

Keywords: Total Anti-oxidant activity, Proline, Paaclobutrazol, water stress, Groundnut

UNDER PEER REVIEW

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.), belongs to the legume family (Fabaceae). It's an annual herbaceous plant with prostrate growth, producing yellow, pea-like flowers that self-pollinate.Groundnut is a vital crop for its protein-rich seeds and oil content, cultivated worldwide. The groundnut seed is called kernel which is used in confectionary nut flour production, protein and peanut milk (Woodroof, 1966).Groundnut is the major oilseed crop in India and it plays a significant role in bridging the vegetable oil deficit within the country.

Abiotic stress such as drought, soil salinity and extreme temperatures adversely affect the productivity and quality of groundnut.Drought stress is one of the major limitations to crop productivity.Gujarat possesses an arid to semi-arid climate, and frequently suffers from drought due to failure of monsoon and occurrence of heat waves. The trend for the last 35 years shows that drought occurs almost every year, and leads to severe water-scarcity in many parts of Gujarat. (Bandyopadhyay *et al.*, 2020).

Plants are subjected to various abiotic stresses resulted in production of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and singlet oxygen ($^1O_2$) that can disturb plant homeostasis. To avoid this, plant adapts self defense system through accumulation of osmolytes and activation of antioxidative cascade (Shinde *et al.*, 2018). The ability of different plants to tolerate environmental stress may be significantly influenced by the modification of the activity of these enzymes.To improve the tolerance ability of plants during stress conditions, application of paclobutrazole (PBZ) was widely reported. . The most prominent and likely hypothesis on increasing plant production and stress tolerance induced by PBZ has been attributed to it sustaining the endogenous cytokinin concentration, maintaining water status, improving nutrient uptake and carbohydrate synthesis, improving chlorophyll biosynthesis, and promoting antioxidant capacity. (Kamran *et al.*, 2020). Therefore, the purpose of the current research was to assess the defensive interplaying roles of the PBZ application on groundnut under water stress conditions.

UNDER PEER REVIEW

2. METHODOLOGY

3.1 Experimental Site

The present research work entitled “Amelioration of water stress and effect of paclobutrazole on morpho-physiology, growth and yield in groundnut (*Arachis hypogaea* L.)” was carried out at Department of Plant Physiology, BACA and an experiment conducted at Regional Research Station, AAU, Anand during summer 2021 and 2022.

3.2 Details of Treatments and Statistical Design

Twelve treatments comprising of all possible combinations of three irrigation levels for water stress and four paclobutrazole levels were tested in a split plot design with three replications. Irrigation levels for water stress treatments were assigned into the main plots and paclobutrazole levels were allotted in sub plot as sub plot treatments. The treatments consisted of three irrigation levels for water stress *viz.*, I_1: Irrigations at 10 days interval (10 irrigation); I_2: Irrigation at 15 days interval (8 irrigation) and I_3: Irrigation at 20 days interval (6 irrigation) and four levels of paclobutrazole*viz.*, T_1: Control (No application of PBZ); T_2: PBZ @ 100 mg/l; T_3: PBZ @ 200 mg/l and T_4: PBZ @ 300 mg/l at 35 and 55 DAS.There were twelve treatment combinations were evaluated in the present study *viz*.,I_1T_1: Irrigation at 10 days interval + Control (No PBZ), I_1T_2: Irrigation at 10 days interval + PBZ @ 100 mg/l, I_1T_3: Irrigation at 10 days interval + PBZ @ 300 mg/l, I_1T_4: Irrigation at 10 days interval + PBZ @ 300 mg/l , I_2T_1: Irrigation at 15 days interval + Control (No PBZ) , I_2T_2: Irrigation at 15 days interval + PBZ @ 100 mg/l, I_2T_3: Irrigation at 15 days interval + PBZ @ 200 mg/l, I_2T_4: Irrigation at 15 days interval + PBZ @ 300 mg/l , I_3T_1: Irrigation at 20 days interval + Control (No PBZ), I_3T_2: Irrigation at 20 days interval + PBZ @ 100 mg/l, I_3T_3: Irrigation at 20 days interval + PBZ @ 200 mg/l , I3T4: Irrigation at 20 days interval + PBZ @ 300 mg/l. Paclobutrazol application was done through foliar spraying at 35 and 55 days after sowing.

3.3 Determination of the Total Antioxidant Activity:

Antioxidant activity was measured using ferric reducing antioxidant power (FRAP) method as described by Arnao *et al.* (2001).

UNDER PEER REVIEW

3.3.1 Sample Extraction

For the preparation of sample 0.1 g of sample was taken in 10 ml centrifuge tubes containing 10 ml of 60% methanol at room temperature followed by centrifugation at 10,000 rpm and 10°C for 15 min. The supernatant was used directly for FRAP assay. (The principle of this method is based on the reduction of a ferric tripyridyl triazine complex to its ferrous, colored form in the presence of antioxidants).

3.3.2 Methodology

The FRAP reagent contained 2.5 ml of a 10 mM TPTZ (2,4,6- tripyridyls triazine, Sigma) solution in 40 mM HCL plus 2.5 ml of 20 mM FeCl, 6H2O and 25 ml of 0.3 M acetate buffer, pH 3.6 and was prepared freshly and warmed at 37 °C. Aliquots of 1 ml sample were mixed with 3.0 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured using spectrophotometer after incubation at 37 °C for 10 minutes. Ferrous sulphate was used as the standard in the range of 0.2, 0.4, 0.6, 0.8 and 1.0 ml.

3.4 Determination of the Proline content of leaf (65 DAS)

Sample Extraction

Fresh leaf samples (0.5 g) were collected, ground well in a mortar using pestle and extracted in 10 ml of three per cent sulphosalicylic acid. The extract was filtered through Whatman No. 1 filter paper and the filtrate was used for proline estimation.

Methodology

An aliquot of 2.0 ml from each sample was taken in separate test tubes and to each test tube, 2.0 ml of acid ninhydrin reagent and 2.0 ml of glacial acetic acid were added and boiled on hot water bath for an hour. Then the test tubes were transferred to ice water bath for cooling and the contents of each test tube were transferred to a separating funnel. To this, 4.0 ml of toluene was added, shaken thoroughly and allowed to form two separate layers.The upper toluene layer containing the colour complex due to proline-ninhydrin interaction was taken into a separate test tube and the colour was read in ultra-spec double beam spectrophotometer at 520 nm (Bates *et al.*, 1973).

4. RESULT AND DISCUSSION

The observations recorded were analyzed statistically and presented and discussed under the following heads:

4.1 Effect of Water Stress on Total Anti-Oxidant Activity and Proline Content of Leaf

4.1.1 Effect of Water Stress on Total Anti-Oxidant Activity

The results regarding total anti-oxidant activity at 65 DAS (2.30, 2.32 and 2.31%) showed that significantly higher with irrigation at 20 days interval (I_3) during 2021, 2022 and in pooled results (Table 1), respectively. Whereas, minimum total anti-oxidantactivity at 65 DAS (0.94, 0.97 and 0.95%) was recorded with irrigation at 10 days interval (I_1) in pooled results, respectively

UNDER PEER REVIEW

Data clearly indicated that the significantly higher total anti-oxidant activity at harvest (2.16, 2.07 and 2.12%) was recorded with irrigation at 20 days interval (I_3) during the year 2021, 2022 and in pooled data, respectively which was found statistically at par with I_2 in both the years. While, minimum total anti-oxidant activity at harvest (0.76, 0.87 and 0.81%) was observed with irrigation at 10 days interval (I_1) during both the years and in pooled mean, respectively.

Water stress can lead to reduced photosynthetic activity in leaves, resulting in the accumulation of excess energy in the chloroplasts. This excess energy can lead to the generation of ROS through a process known as photooxidative stress. To counteract this, plants increase their antioxidant activity to scavenge the excess ROS and protect the photosynthetic machinery. The activation of both enzymatic and non-enzymatic antioxidants helps the plant cope with the oxidative stress and maintain cellular integrity and function.Chakraborty *et al.* (2015) observed that in 25-30 days water deficit stress conditions during peg as well as pod development stages antioxidant enzymes activity of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase activity was increased in peanut. Sunitha *et al.* (2015) also observed higher antioxidant activity as water stress increases.

UNDER PEER REVIEW

4.1.2 Effect of water stress on Proline Content of Leaf

The significantly (Table 2) higher proline content of leaf at 65 DAS (243.83, 242.45 and 243.14 µg/g fr.wt) was recorded with irrigation at 20 days interval (I_3) in the years 2021, 2022 and pooled data, respectively.The treatment irrigation at 10 days interval (I_1) resulted minimum proline content of leaf at 65 DAS (176.69, 172.18 and 174.44 µg/g fr.wt) during both the years 2021, 2022 and pooled data, respectively.Madhusudhan and Sudhakar (2023) reported similar results reveal a significant increase in the proline content the leaves groundnut under water-stressed conditions. Shinde *et al.* (2018**)**examined results of thirty days grown seedlings of groundnut which were subjected to water stress by withholding an irrigation for 15 days recorded similar treands of increase in proline concentration as stress increases.Nautiyal *et al.* (2017) observed increase in proline concentration in the treatments in which water cut was 50 percent compared to the fully irrigated treatments. Solanki and Sarangi (2014) investigated changes infree proline in the leaves of drought susceptible (JL-24) and drought tolerant (K-1375) varieties of peanut plants exposed to different durations of drought stress. They observed that the increase in the proline content under increasing drought stress (21 days) was found to be more than 2 folds in the tolerant variety when compared with susceptible variety.These results are in conformity with the results Davari *et al.* (2021), Solichatun*et al.* (2021), Yooyongwech*et al.*, (2017),Sunitha *et al.* (2015),Rady and Maybelle (2012), Mohamed *et al.* (2011) and Azevedo neto*et al.* (2010).

When a plant experiences water stress, the availability of water in the soil decreases, leading to reduced water uptake by the roots. As a result, the plant cells lose water, causing cellular dehydration and increased osmotic stress. To counteract this, plants accumulate compatible solutes like proline, which helps maintain cellular turgor pressure and prevents water loss from the cells. Proline is one of the most important compatible osmolytes in water deficit stressed plants. Proline may act as a non-toxic osmolyte in the cytoplasm andsustains the composition of macromolecules and organelles. Its accumulation supports to maintain turgor and stimulates continued growth under water stress. Proline acts as a scavenger of ROS, neutralizing them and protecting the cellular structures from oxidative damage. In water stress, as protein folding can be disrupted due to dehydration, the increased levels of proline help maintain protein stability and function.

4.2 Effect of Paclobutrazol on Total Anti-Oxidant Activity and Proline Content of Leaf

4.2.1 Effect of Paclobutrazolon Total Anti-Oxidant Activity

Total anti-oxidant activity at 65 DAS (Table 1) was recorded significantly higher (1.94, 1.93 and 1.93%) with application of PBZ @ 300 mg/l (T_4) during 2021, 2022 as well as in pooled results, respectively. It was at par with T_3 in the year 2022. While, minimum total anti-oxidant activity at 65 DAS (1.21, 1.34 and 1.28 %) were recorded with control (T_1) during 2021, 2022 and in pooled results, respectively.

Significantly higher total anti-oxidant activity at harvest (1.79, 1.73 and 1.76%) was recorded with application of PBZ @ 300 mg/l (T_4) during the year 2021, 2022 and in pooled mean, respectively. While, minimum total anti-oxidant activity at harvest (1.04, 1.12 and 1.08%) was noted in control (T_1) during both the years and in pooled mean, respectively.

Paclobutrazole can lead to the accumulation of reactive oxygen species in plant cells. ROS are highly reactive molecules generated as by-products of various metabolic processes,

UNDER PEER REVIEW

including those affected by paclobutrazole. The accumulation of ROS can trigger oxidative stress, leading to the activation of antioxidant defence mechanisms. In response to increased ROS levels caused by PBZ, plants often induce the synthesis and activation of antioxidant enzymes..Chen *et al.* (2019) reported that the application of paclobutrazole increased the antioxidant enzyme activity in groundnut plants. PBZ affects hormone levels in plants, including abscisic acid. ABA is involved in the regulation of stress-responsive genes, including those related to antioxidant synthesis. PBZ-induced changes in ABA levels may influence the expression of antioxidant genes and impact the total antioxidant activity of plants. Similar results were reported by Sofy *et al.* (2020).

4.2.2 Effect of paclobutrazole on Proline Content of Leaf

The results presented in Table 2 indicated that the significantly higher proline content of leaf at 65 DAS (191.65, 189.39 and 190.52 µg/g fr.wt) was recorded withapplication of PBZ @ 300 mg/l (T_4) during both the years and in pooled analysis, respectively. Whereas, minimum proline content of leaf at 65 DAS (211.35, 209.25 and 210.30 µg/g fr.wt) was noted with control (T_1) during the years 2021, 2022 and pooled results, respectively.

Paclobutrazole application has been found to increase the accumulation of proline. This effect is often associated with the plant's response to stress conditions, especially water stress. PBZ-induced water stress can trigger the synthesis of proline as a protective mechanism to counteract cellular dehydration and maintain osmotic balance. Similar results were reported by Davari *et al.* (2021), Solichatun*et al.* (2021), Rady and Maybelle (2012) and Mohamed *et al.* (2011).

4.3 Interaction Effect of Water Stress and Paclobutrazole on Total Anti-oxidant activity and Proline Content of Leaf

Interaction effect of water stress and paclobutrazole levels was found non-significant with respect to total anti-oxidant activity at 65 DAS as well as at harvest during individual year as well as pooled basis. However, Contrasting results were reported by many researchers regarding positive interaction of applications of PBZ during water stress conditions.Mohamed *et al.* (2011) observed that in 50 mg/l paclobutrazole-treated tomato plants grown under 60% field

UNDER PEER REVIEW

capacity, proline was recorded 1.52-fold compared to control. Similarly, Increase in anti-oxidant enzymes also recorded by them that helps to minimize the negative impacts of water stress.Davari *et al.* (2021)pointed out that application of PBZ under drought enhances the proline content as well as photosynthesis pigments. Chen *et al.* (2019) also reported similar results in groundnut and they concluded that PBZ helped the plants to cope with water stress.

CONCLUSION

In conclusion, our study unequivocally demonstrates a significant increase in both proline content and total antioxidant activity under water stress conditions, highlighting the adaptive responses of the studied system to mitigate the adverse effects of water stress conditions.

UNDER PEER REVIEW

Table 1: Effect of water stress and paclobutrazole on total anti-oxidant activity in groundnut during 2021, 2022 and pooled analysis

Treatments		**Total anti-oxidant activity**					
		65 DAS			**At harvest**		
		2021	**2022**	**Pooled**	**2021**	**2022**	**Pooled**
I_1	Irrigation at 10 days interval (10 irrigation)	0.94	0.97	0.95	0.76	0.87	0.81
I_2	Irrigation at 15 days interval (8 irrigation)	1.58	1.69	1.64	1.41	1.46	1.43
I_3	Irrigation at 20 days interval (6 irrigation)	2.30	2.32	2.31	2.16	2.07	2.12
S.Em. ±		0.03	0.04	0.02	0.03	0.02	0.02
C.D. at 5%		0.12	0.14	0.08	0.11	0.10	0.06
C.V.%		6.37	7.66	7.07	6.51	5.80	6.16
T_1	CONTROL(No PBZ)	1.21	1.34	1.28	1.04	1.12	1.08
T_2	PBZ @ 100 mg/l	1.55	1.54	1.55	1.39	1.42	1.41
T_3	PBZ @ 200 mg/l	1.72	1.82	1.77	1.55	1.59	1.57
T_4	PBZ @ 300 mg/l	1.94	1.93	1.93	1.79	1.73	1.76
S.Em. ±		0.02	0.04	0.02	0.03	0.02	0.02
C.D. at 5%		0.07	0.11	0.06	0.08	0.07	0.05
Interaction		NS	NS	NS	NS	NS	NS
C.V.%		4.46	6.65	5.70	5.88	5.01	5.46

UNDER PEER REVIEW

Table 2: Effect of water stress and paclobutrazole on proline content of leaf in groundnut during 2021, 2022 and pooled analysis

Treatments		**Proline content of leaf at 65 DAS**		
		2021	**2022**	**Pooled**
I_1	Irrigation at 10 days interval (10 irrigation)	176.69	172.18	174.44
I_2	Irrigation at 15 days interval (8 irrigation)	189.79	187.41	188.60
I_3	Irrigation at 20 days interval (6 irrigation)	243.83	242.45	243.14
S.Em. ±		1.75	1.41	1.12
C.D. at 5%		6.87	5.53	3.66
C.V.%		2.98	2.43	2.72
T_1	CONTROL (No application of PBZ)	191.65	189.39	190.52
T_2	PBZ @ 100 mg/l	203.17	200.06	201.62
T_3	PBZ @ 200 mg/l	207.58	204.02	205.80
T_4	PBZ @ 300 mg/l	211.35	209.25	210.30
S.Em. ±		1.22	0.89	0.75
C.D. at 5%		3.63	2.63	2.16
Interaction		NS	NS	NS
C.V.%		1.80	1.32	1.58

UNDER PEER REVIEW

.REFERENCES

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food chemistry*, 73, 239-244.

Azevedo Neto, A. D., Nogueira, R. J. M. C., Melo Filho, P. A., & Santos, R. C. (2010). Physiological and biochemical responses of peanut genotypes to water deficit, *Journal of Plant Interactions*, 5(1), 1-10.

Bandyopadhyay, N., Bhuiyan, C., & Saha, A. K. (2020). Drought mitigation: Critical analysis and proposal for a new drought policy with special reference to Gujarat (India). *Progress in Disaster Science,* 5,1-13.

Bates, R., Waldren, R. P., & Teare I. D. (1973). A rapid determination of free proline for water stress studies. *Plant and soil,* 39, 205-207.

Chakraborty, K., Singh, A. L., Kalariya, K. A., & Goswami, N. (2015). Physiological responses of peanut (*Arachis hypogaea* L.) cultivars to water deficit stress: status of oxidative stress and antioxidant enzyme activities. *Acta Botanica Croatica*, 74(1), 123-142.

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in plant science*, 9, 1945.

Davari, K., Rokhzadi, A., Mohammadi, K., &Pasari, B. (2021). Paclobutrazol and amino acid-based biostimulant as beneficial compounds in alleviating the drought stress effects on Safflower (*Carthamus tinctorius* L.). *Journal of Soil Science and Plant Nutrition*, 1-17.

Kamran, M., Ahmad, S., Ahmad, I., Hussain, I., Meng, X., Zhang, X., & Han, Q. (2020). Paclobutrazol application favors yield improvement of maize under semiarid regions by delaying leaf senescence and regulating photosynthetic capacity and antioxidant system during grain-filling stage. *Agronomy*, 10(2), 187.

UNDER PEER REVIEW

Madhusudhan, K. V., & Sudhakar, C. (2023). Differential responses of growth, antioxidant enzymes and osmolytes in the leaves of two groundnut (*Arachis hypogaea* L.) cultivars subjected to water stress. *Journal of Stress Physiology & Biochemistry*, 19(3), 110-124.

Mohamed, G. F., Agamy, R. A., & Rady, M. M. (2011). Ameliorative effects of some antioxidants on water-stressed tomato (*Lycopersicon esculentum* Mill.) plants. *Journal of Applied Sciences Research*, 7(12), 2470-2478.

Nautiyal, P. C., Kulkarni, G., Singh, A. L., & Basu, M. S. (2017). Evaluation of water-deficit stress tolerance in Bambara groundnut land races for cultivation in sub-tropical environments in India. *Indian Journal of Plant Physiology*, 22, 190-196.

Rady, M. M., & Maybelle, S. G. (2012). Improving barley yield grown under water stressconditions. *Research Journal of Recent Sciences*, 1(16), 2277-2250.

Shinde, S. S., Kachare, D. P., Satbhai, R. D., & Naik, R. M. (2018). Water stress induced proline accumulation and antioxidative enzymes in groundnut (*Arachis hypogaea*L.). *Legume Research-An International Journal*, 41(1), 67-72.

Sofy, M. R., Elhindi, K. M., Farouk, S., & Alotaibi, M. A. (2020). Zinc and paclobutrazol mediated regulation of growth, upregulating antioxidant aptitude and plant productivity of pea plants under salinity. *Plants*, 9(9), 1-15.

Solanki, J. K., & Sarangi, S. K. (2014). Effect of drought stress on proline accumulation in peanut genotypes, *International Journal of Advanced Research*, 2(10), 301-309.

Solichatun, S., Khasanah, F. U., Pitoyo, A., Etikawati, N., &Mudyantini, W. (2021). Exogenous application of paclobutrazol promotes water-deficit tolerance in pepper (*Capsicum annuum*). *Cell Biology and Development*, 5(1), 1-6.

Sunitha, V., Vanaja, M., Sowmya, P., Razak, S. A., Kumar, G. V., Anitha, Y., & Lakshmi, N. J. (2015). Variability in response of groundnut (*Arachis hypogaea* L.) genotypes to moisture stress and stress release. *International Journal of Bio-resource and Stress Management*, 6(2), 240-249.

UNDER PEER REVIEW

Sunitha, V., Vanaja, M., Sowmya, P., Razak, S. A., Kumar, G. V., Anitha, Y., & Lakshmi, N. J. (2015). Variability in response of groundnut (*Arachis hypogaea* L.) genotypes to moisture stress and stress release. *International Journal of Bio-resource and Stress Management*, 6(2), 240-249.

Woodroof, J. G., (1966). Peanut production and processing products, *AV1 publication*, West Port, USA.

Yooyongwech, S., Samphumphuang, T., Tisarum, R., Theerawitaya, C., & Cha-Um, S. (2017). Water-deficit tolerance in sweet potato [*Ipomoea batatas* (L.) Lam.] by foliar aplication of paclobutrazol: role of soluble sugar and free proline. *Frontiers in Plant Science*, 8, 1400.

UNDER PEER REVIEW