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RESEARCH PAPER

Effect of moisture stress on leaf protein, proline, peroxidise activity and yield in released and pre-released genotypes of groundnut (*Arachis hypogaea* L.)

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Abstract : The performance of crops need to be assessed for their production under erratic rain fall pattern, increased temperatures, and enhanced atmospheric CO_2 concentration. In the present study groundnut was chosen as test crop and selected genotypes [four released (GPBD-4, G2-52, Dh-86 and TMV-2) and four pre-released (Dh-245, Dh-232, Dh-256 and Dh-257)] were studied to quantify the impact of moisture deficit stress at critical growth stages *i.e.*, 40 to 80 DAS and 80 DAS to harvest. Leaf protein and proline increases in tolerant genotypes at higher moisture stress levels than susceptible genotypes as they acts as osmolytes and maintains the turgidity of the cell and hence, checks the water loss and peroxidase enzyme activity which in turn scavenges ROS produced due to stress as a result there was reduction in yield. The genotypes, GPBD-4, Dh-257 and Dh-256 recorded higher per cent increase in leaf soluble protein, leaf proline and peroxidase enzyme activity at all the stages. Increase was higher at 80 DAS to harvest stressed plants than 40 to 80 DAS stressed plants.

Key Words : Moisture stress, Leaf soluble protein, Leaf proline, Peroxidase enzyme activity, Yield

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INTRODUCTION

Abiotic stresses are an integral part of 'climate change',which can change soil-plant-atmosphere continuum thereby influencing the productivity of crops. Drought is one of the important abiotic stresses and two thirds of India's agricultural land is susceptible to drought stress of various intensities and the probability of occurrence of drought is over 35 per cent. Drought triggers a wide range of physiological and biochemical processes and some of these responses will enable the plants to tolerate and adapt to such conditions with less reduction in economic yield of different crops. The adaptations include decreased stomatal conductance to prevent the transpirational water loss, reduced photosynthesis, accumulation of

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osmoprotectants like proline, FAA in the cell and anti-oxidant enzyme activity. These changes vary within the genotypes of same crop and environmental conditions and it is vital to study physiological and biochemical traits in order to identify tolerant genotype of important crops like groundnut.

Groundnut (*Arachis hypogaea* L.) is an important legume and oilseed cash crop which is mainly grown as a rainfed crop. Drought is recognized as one of the major constraints limiting groundnut productivity in semiarid regions. Drought stress during reproductive stages like flowering and pod filling stage is crucial for productivity in groundnut and this reduction depends on physiological and biochemical changes that were triggered during drought stress. The present work was taken upto evaluate the released and pre-released groundnut genotypes for yield and biochemical responses to irrigated and water stressed conditions.

MATERIAL AND METHODS

The experiment was laid in split plot design with three replications and three treatments in groundnut crop. The seeds were sown on 16th November, 2018 by manually and to a depth of 2 to 3 cm. A spacing of 30 cm between rows and 10 cm from plant to plant was maintained. The observations were recorded at 30 DAS, 60 DAS and 90 DAS and at harvest to evaluate the effect of water stress at different growth stages on various biochemical characters and pod yield.

The estimation and quantification of protein was done by using Lowry's method and estimation of free proline adopting the method described by Bates *et al.* (1973) was followed. For estimation of antioxidative enzymes, extraction of leaf sample was done by homogenising one gram of leaf sample in 10 ml of 0.1M potassium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 1 % (w/v) polyvinyl pyrrolidone in prechilled mortar and pestle. The extract was centrifuged at 15,000 rpm for 20 min at 4°C and the supernatant was used as enzyme source. The activity of peroxidase was measured as per the method described by Sadasivam and Manickam (1996). The statistical analysis was done as described by Gomez and Gomez (1984). The levels of significance used in 'F' and 't' tests was P = 0.05. The least significant differences (LSD) values were calculated wherever the 'F' test was significant by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Accumulation of soluble compounds in cells increases osmotic potential and reduces water loss from cells. Proline and amino acids accumulates whenever there is moisture stress. Accumulation of proline is greater in the later stages of drought stress (Table 1). Therefore, increase in its concentration is considered a good indicator of moisture. At 60 DAS, M_2 (4.54 µmol g^{-1} fr. wt.) recorded significantly highest proline content, while control recorded (1.10 µmol g^{-1} fr. wt.). At 90 DAS. among genotypes, GPBD-4 (2.49 µmol g^{-1} fr. wt.) recorded significantly highest proline content, while G2-52 (1.77 µmol g^{-1} fr. wt.) recorded lowest. The results were on par with the findings of Ranganayakulu *et al*. (2015); Vaidya *et al*. (2015) and Shinde *et al*. (2017).

It is well known that plants under stress may accumulate small molecular mass proteins that could be used as a source of storage nitrogen that could be mobilized after stress relief or removal. Increase in leaf protein content during stress may be a result of increase in amino acid content (Table 1). At 60 DAS, M_2 (62.29 mg g^{-1} fr. wt.) recorded statistically higher leaf protein content over control M_1 (48.48 mg g^{-1} fr. wt.), which was on par with M_3 (48.51 mg g^{-1} fr. wt.). At 90 DAS, M_3 (74.78 mg g^{-1} fr. wt.) recorded significantly highest protein content, while M_1 (56.58 mg g^{-1} fr. wt.) recorded lowest. Among genotypes, Dh-232 (71.96 mg g^{-1} fr. wt.) recorded significantly highest protein content, whereas Dh-86 (58.35 mg g^{-1} fr. wt.) recorded significantly lowest protein content. These results were on par with Vaidya *et al*. (2015) and Shinde *et al*. (2017).

Peroxidase is an antioxidant enzyme which exhibits higher activity under water and high temperature stress. The peroxidase activity of groundnut genotypes increases significantly due to imposition of stress compared to control plants (Table 2). It is observed that peroxidase may prevent the degradation of membrane integrity of the cells against the free radicles formed under moisture stress (Mandal and Singh, 2000). The tolerant genotypes shows higher increase of peroxidase activity under water stress. In the present study, M_2 (0.37) recorded significantly higher peroxidase activity at 60 DAS over control M_1 (0.23 µ mol. min^{-1}), which was on par with M_3 (0.24 µ mol. min^{-1}). At 90 DAS, M_3 (1.31 µ mol. min^{-1}) recorded significantly highest peroxidase activity, while M_1 (0.35 µ mol. min^{-1}) recorded lowest. Among genotypes, Dh-257 (1.38 µ mol. min^{-1}) recorded significantly highest peroxidase activity, whereas TMV-

Table 1: Effect of soil moisture stress at different growth stages on soluble protein content (mg g^{-1} fr. wt.) and proline content (µmol. g^{-1} fr. wt.) in leaf of groundnut genotype

Treatments	Protein content			Proline content		
M: Moisture stress levels	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
M_1: Control	16.05	48.48^{b}	56.58^{c}	0.07	1.10^{b}	5.14^{c}
M_2: Pegging stage	15.75	62.29^{a}	71.18^{b}	0.07	4.54^{a}	9.07^{b}
M_3: Pod Dev. stage	25.27	48.51^{b}	74.78^{a}	0.08	1.11^{b}	11.59^{a}
LSD @ 5%	NS	2.50	2.48	NS	0.11	0.11
V: Genotypes						
V_1- GPBD-4	20.21^{b}	55.19^{b}	71.56^{a}	0.15^{a}	2.49^{a}	9.72^{a}
V_2- G2-52	18.85^{bc}	50.41^{f}	61.48^{d}	0.05^{bc}	1.77^{e}	7.74^{f}
V_3- Dh-86	16.84^{c}	47.34^{g}	58.35^{e}	0.05^{bc}	2.11^{c}	7.62^{g}
V_4- TMV-2	17.95^{c}	51.14^{e}	63.77^{c}	0.05^{bc}	1.97^{d}	7.24^{h}
V_5- Dh-245	17.47^{c}	54.01^{d}	70.38^{b}	0.12^{a}	2.45^{ab}	9.21^{c}
V_6- Dh-232	18.71^{bc}	55.99^{a}	71.96^{a}	0.08^{b}	2.42^{ab}	8.92^{e}
V_7- Dh-256	18.86^{bc}	54.78^{c}	70.76^{b}	0.05^{bc}	2.39^{ab}	9.05^{d}
V_8- Dh-257	23.29^{a}	55.87^{a}	71.86^{a}	0.04^{c}	2.38^{b}	9.32^{b}
LSD @ 5%	1.97	0.13	0.70	0.03	0.09	0.10
M×V: Interaction						
M_IV_1	18.34^{d}	49.74^{hi}	57.84^{k}	0.15^{a}	1.14^{gh}	5.22^{l}
M_IV_2	15.42^{d-f}	48.93^{l}	57.03^{kl}	0.04^{d}	1.03^{l}	5.11^{l}
M_IV_3	14.02^{ef}	47.48^{pq}	55.58^{m}	0.04^{d}	1.13^{gh}	5.11^{l}
M_IV_4	13.88^{ef}	48.58^{m}	56.68^{k-m}	0.05^{cd}	1.04^{kl}	5.11^{l}
M_IV_5	16.12^{d-f}	46.05^{r}	54.15^{n}	0.11^{a-c}	1.18^{fg}	5.18^{l}
M_IV_6	16.68^{d-f}	49.59^{ij}	57.69^{k}	0.08^{b-d}	1.07^{i-l}	5.15^{l}
M_IV_7	16.50^{d-f}	48.08^{o}	56.18^{lm}	0.07^{cd}	1.06^{j-l}	5.14^{l}
M_IV_8	17.42^{de}	49.37^{jk}	57.47^{kl}	0.04^{d}	1.12^{hi}	5.11^{l}
M_2V_1	14.86^{d-f}	66.51^{d}	75.84^{g}	0.16^{a}	5.11^{a}	9.51^{g}
M_2V_2	13.83^{ef}	52.42^{f}	61.75^{j}	0.06^{cd}	3.15^{e}	7.68^{k}
M_2V_3	13.27^{f}	48.38^{mn}	57.71^{k}	0.04^{d}	4.19^{c}	8.72^{i}
M_2V_4	13.68^{ef}	56.63^{e}	65.96^{i}	0.07^{cd}	3.78^{d}	8.32^{j}
M_2V_5	14.20^{ef}	68.66^{b}	77.99^{ef}	0.13^{ab}	5.08^{a}	9.54^{g}
M_2V_6	14.78^{d-f}	69.47^{a}	77.62^{f}	0.05^{cd}	5.00^{b}	9.61^{g}
M_2V_7	14.32^{ef}	68.56^{b}	76.73^{fg}	0.04^{d}	4.99^{b}	9.52^{g}
M_2V_8	27.05^{ab}	67.67^{c}	75.84^{g}	0.03^{d}	4.98^{b}	9.65^{g}
M_3V_1	27.43^{a}	49.33^{k}	81.00^{b}	0.14^{a}	1.20^{f}	14.41^{a}
M_3V_2	27.31^{a}	49.89^{h}	65.66^{i}	0.06^{cd}	1.12^{hi}	10.41^{f}
M_3V_3	23.24^{bc}	46.15^{r}	61.76^{j}	0.07^{cd}	1.03^{l}	9.04^{h}
M_3V_4	26.27^{ab}	48.19^{no}	68.66^{h}	0.02^{d}	1.09^{h-k}	8.28^{j}
M_3V_5	22.09^{c}	47.32^{q}	78.99^{de}	0.11^{a-c}	1.10^{h-j}	12.92^{c}
M_3V_6	24.66^{a-c}	48.91^{l}	80.58^{bc}	0.11^{a-c}	1.18^{fg}	11.99^{e}
M_3V_7	25.77^{a-c}	47.70^{p}	79.37^{cd}	0.05^{cd}	1.12^{hi}	12.47^{d}
M_3V_8	25.41^{a-c}	50.59^{g}	82.26^{a}	0.05^{cd}	1.03^{l}	13.22^{b}
LSD @ 5%	3.42	0.23	1.22	0.05	0.07	0.16

Note: Alphabets in the column followed by the same letter do not differ significantly as per the DMRT NS= Non-significant

Table 2: Effect of soil moisture stress at different growth stages on peroxidase enzyme activity (µmol. min^{-1}) and pod yield (kg ha^{-1}) of groundnut genotypes

Treatments	Peroxidase enzyme activity			Pod yield
M: Moisture stress levels	30 DAS	60 DAS	90 DAS	Harvest
M_1: Control	0.14	0.23^{b}	0.35^{c}	3033^{a}
M_2:Pegging stage	0.14	0.37^{a}	0.71^{b}	2644^{b}
M_3: Pod Dev. stage.	0.13	0.24^{b}	1.31^{a}	1037^{c}
LSD @ 5%	NS	0.08	0.09	124.2
G: Genotypes				
V_1- GPBD-4	0.12^{b-e}	0.30^{b}	1.01^{c}	2374^{d}
V_2- G2-52	0.07^{c-e}	0.13^{cd}	0.48^{f}	2016^{e}
V_3- Dh-86	0.05^{de}	0.14^{cd}	0.66^{e}	1671^{f}
V_4- TMV-2	0.16^{bc}	0.19^{cd}	0.37^{g}	1254^{g}
V_5- Dh-245	0.02^{e}	0.09^{d}	0.43^{fg}	2126^{e}
V_6- Dh-232	0.14^{b-d}	0.22^{bc}	0.83^{d}	2537^{c}
V_7- Dh-256	0.34^{a}	0.63^{a}	1.19^{b}	2845^{b}
V_8- Dh-257	0.20^{b}	0.55^{a}	1.38^{a}	3082^{a}
LSD @ 5%	0.09	0.10	0.09	162.4
M×G: Interaction				
M_IV_1	0.12	0.18^{cd}	0.30^{hi}	3083^{c}
M_IV_2	0.07	0.10^{d}	0.28^{hi}	2829^{c}
M_IV_3	0.05	0.12^{cd}	0.24^{hi}	2517^{c}
M_IV_4	0.16	0.18^{cd}	0.22^{i}	1980^{d}
M_IV_5	0.02	0.08^{d}	0.18^{i}	2857^{e}
M_IV_6	0.14	0.18^{cd}	0.23^{hi}	3394^{cb}
M_IV_7	0.34	0.53^{b}	0.72^{fg}	3683^{ab}
M_IV_8	0.20	0.48^{b}	0.62^{g}	3923^{a}
M_2V_1	0.12	0.52^{b}	0.82^{f}	2815^{c}
M_2V_2	0.07	0.18^{cd}	0.42^{h}	2274de
M_2V_3	0.05	0.16^{cd}	0.33^{hi}	2078^{e}
M_2V_4	0.17	0.20^{cd}	0.28^{hi}	1443^{fg}
M_2V_5	0.02	0.12^{cd}	0.42^{h}	2501^{d}
M_2V_6	0.15	0.30^{c}	0.72^{fg}	2995^{c}
M_2V_7	0.35	0.80^{b}	1.11^{e}	3417^{b}
M_2V_8	0.21	0.66^{ab}	1.61^{bc}	3627^{b}
M_3V_1	0.12	0.19^{cd}	1.91^{a}	1222^{gh}
M_3V_2	0.07	0.10^{d}	0.72^{fg}	944^{h}
M_3V_3	0.05	0.12^{cd}	1.42^{d}	419^{i}
M_3V_4	0.16	0.19^{cd}	0.61^{g}	341^{i}
M_3V_5	0.02	0.08^{d}	0.68^{fg}	1022^{h}
M_3V_6	0.14	0.19^{cd}	1.53^{cd}	1222^{gh}
M_3V_7	0.33	0.55^{b}	1.73^{b}	1434^{fg}
M_3V_8	0.19	0.50^{b}	1.91^{a}	1694^{f}
LSD @ 5%	NS	0.16	0.17	281.4

Note: Alphabets in the column followed by the same letter do not differ significantly as per the DMRT NS= Non-significant

2 (0.37 µ mol. min^{-1}) recorded significantly lowest peroxidase activity irrespective of growth stages.

Highest pod yield reduction was observed under M_3 (1037kg ha^{-1}) stress than at M_2 (2644 kg ha^{-1}) because of the inability of the crop to sustain the drought at later stages. Whereas, control M_1 recorded 3033 kg ha^{-1} of yield. Among genotypes, Dh-257 (3082kg ha^{-1}) recorded significantly highest pod yield mainly because of higher number of pods per plant, higher HI. Similar results were observed by Vadez and Ratnakumar (2016) and Carvalho *et al*. (2017) in groundnut. Genotype, TMV-2 (1254 kg ha^{-1}) recorded significantly lowest pod yield (Table 2).

Conclusion:

All the genotypes responded differently at various moisture stress levels at all the growth stages. The tolerant genotypes adapted to drought stress by biochemical mechanisms like increased levels of leaf proline, leaf protein and increased level of enzyme activity. The increased levels of proline and protein are the best known osmolytes. Proline is one of the best known osmo-protectant which maintains the osmotic potential of the cell during stress condition, which helps the plant to overcome stress adopt and to provide a higher and quality yield. Increase in proline was three to four times under pod development stage when compared to controlled plants in drought tolerant genotypes like Dh-257, GPBD-4 and Dh-256. Lower increase was recorded in susceptible genotypes like G2-52, Dh-86 and TMV-2. Peroxidase enzyme activity increases under drought stress to scavenge the reactive oxygen species (ROS).

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