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Biochemical parameters influenced groundnut (*Arachis Hypogaea* L.) yield under water stress at different flowering phases

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Abstract
The study was conducted to evaluate the variations in biochemical parameters and yield among groundnut genotypes under drought condition. Four genotypes *viz.,* CO 7, COGn 4, TMV 7 and TMVGn 13 were used for this investigation with water stress at different flowering phases *viz.*, Pre Flowering Drought - PDF (15-30 DAS), Flowering Drought – FD (35-50 DAS) and Post Flowering Drought - POFD (75-90 DAS) by withholding irrigation and also control maintained with irrigation to field capacity for comparison. Nitrate reductase activity-NR, proline content, peroxidase activity-POX and Superoxide dismutase activity-SOD were observed during stress period and after recovery of stress. Under stress condition, proline, POD, SOD levels were increased, but NR activity got decreased. After rewatering, flowering and post flowering stressed plants are failed to recover NR activity but it was completely recovered in pre flowering drought imposed plants. PFD recorded more kernel yield, than flowering and post flowering stage stress imposed plants.

Keywords: groundnut, pre-flowering drought, nitrate reductase activity, proline, peroxidase, superoxide dismutase

Introduction

Groundnut (*Arachis hypogaea* L.) is an economically important food and oil seed crop and it also known as peanut, which is mainly grown as a rainfed crop (Wright and Nageswara Rao, 1994 [21] and Songsri *et al.*, 2008) [19]. It is one of the world's most popular and universal crops. More than half of the production area, which accounts for 70% of the peanut growing area fall under arid and semi-arid regions, where peanuts are frequently subjected to drought stresses for different duration and intensities (Wright and Nageswara Rao, 1994 [21] and Reddy *et al.*, 2003 [18] and Songsri *et al.*, 2008) [19]. Due to erratic rainfall and frequent drought during the crop growth period groundnut yields are generally low and unstable under rainfed conditions. Drought during reproductive stages like flowering and pod filling stage is crucial for yield in groundnut varieties (Paungput *et al.*, 2013) but tolerant genotypes may give better yield due to biochemical changes that were triggered during drought. NR is a very sensitive enzyme for any abiotic stress but specially under water stress condition. NR activity will get reduced (Jharna *et al*. 2001), proline is the primary indicator of any stress which acts as osmoprotetant to give tolerance to the plants under water stress conditions (Delauney and Verma, 1993) [5]. Peroxidase and Superoxide dismutase are the Reactive Oxygen Species (ROS) scavengers which scavenge the peroxidase and superoxide radical and minimize the damage to the plants and helps to yield more even under water stress (Chakraboarty *et al.*, 2015). The objective of the present research program was to assess the impact of water stress at different flowering stages on biochemical and yield performance of popularly cultivated groundnut genotypes in Tamil Nadu.

2. Materials and methods

A pot culture study was conducted in Rain out Shelter (ROS), Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with three replications. Uniform watering was given up to 15 days to all treatments. Thereafter, control plots received watering on every third day to maintain the normal soil moisture percentage. The water was with held between 15-30 days for PFD, 35-50 days for FD, 75-90 days after sowing (DAS) for PoFD.
Once in two days soil moisture content was observed by using ML2 Theta Probe moisture meter (Delta T. Sensor type). Observations on and bio-chemical aspects were studied during stress period *viz.*, PFD (25-30 DAS), FD (45-50 DAS) and PoFD (85-90 DAS) and after stress recovery *viz*., PFD (40-45 DAS), FD (60-65 DAS) and PoFD (100-105 DAS).
NR activity in young leaves was estimated as per the method described by Nicholas *et al.* (1976) [14] and expressed as µg NO_2 g^{-1} h^{-1} fresh weight. The estimation of proline content was done by adopting the method of Bates *et al.*, (1973) [1] and expressed as µg g^{-1}. Peroxidase activity was determined by adopting the method of Mallick and Singh (1980) [13]. SOD activity was determined by using nitroblue tetrazolium (NBT) salt as described by Beau-champ and Fridovich (1971) [2] and expressed in enzyme unit mg $protein^{-1} min^{-1}$. Kernel yield was determined by taking kernel weight of three plants randomly selected from each treatment and replication and mean value was expressed as g $plant^{-1}$. Haulm yield was determined by taking haulm weight of two plants randomly selected from each treatment and replication and the mean value was expressed as g $plant^{-1}$. Oil content was estimated using Sochs-let plus (SCSO 08 AS, Pelican Pvt. Ltd., India) instrument and expressed in per cent. The data collected from this experiment were statistically analyzed in a FCRD (Factorial Completely Randomized Design) as suggested by Gomez and Gomez (1992) [8]. The critical difference (CD) was computed at five per cent probability.

3. Result and Discussion

3.1. Nitrate Reductase (NR) activity (µg NO_2 g^{-1} h^{-1})

There was a decreasing trend in Nitrate Reductase Activity (Table 1) was observed. In the present study, all the water stress treatments reduced the NR activity significantly in all the genotypes during stress and even after recovery compared to control. More recovery percent was observed in PFD (21.2 %) and FD (19.0 %) and PoFD was not recovered even after irrigation. Among the genotypes, CO 7 recorded less reduction in NR activity under PFD was 23.9 % followed by FD 30.6 % and PoFD 35.4% compared to control. Jharna *et al*. (2001) explained that, the decrease in NR activity range from 4.97 to 6.69 per cent in groundnut genotypes under drought. The interaction of CO 7 along with treatment PFD recorded higher recovery per cent than FD and PFD and genotypes. In the drought susceptible variety, NR activity is reduced drastically under water stressed condition. The reduction in NR activity might be due have to less protein synthesis and thus reduced protein pools including key enzymes. In the present study, there was a decrease in NR activity during stress and recovered by re-watering.

3.2. Proline content

A general increase in proline accumulation was observed in plants those under stress condition. In the present study, there was a gradual accumulation of proline upto 55 DAS with the remarkable reduction in the subsequent stages. In Table 2 all the genotypes, 15 days drought improved the accumulation of proline by 38, 15, 34 and 32 per cent in CO 7, COGn 4, TMV 7 and TMVGn 13 respectively at PFD but in FD and PoFD causes less increase in proline content. More accumulation was observed at vegetative stage might be due to active young leaves was observed by Johari- Pireivatlou *et al*. (2010) [11]. Vurayai *et al*. (2010) [20] discussed that, the concentration of free proline accumulation in stressed bambara groundnut leaves were higher by up to four times than in non-stressed plant leaves. Reduced in mean proline content was observed and also accumulation of proline is more in recovery of PFD (351.5 µg g^{-1}) compared to control (326.2 µg g^{-1}). High significant variation was observed within the genotypes and between the treatments not only in stress and also in recovery period. Several investigators (Ramanjulu and Sudhakar, 2000) [16] have established a positive correlation between the accumulation of proline and its osmoprotective role at the whole plant level and in cell cultures due to synthesis or accumulation of proline is depending on the activities of two enzymes, pyrroline-5-carboxylate synthetase (P-5-C synthetase) and pyrroline 5-carboxylate reductase from glutamate. These two enzyme activities were influenced by drought. The present investigation also corroborated these views.

3.3. Peroxidase and Superoxide Dismutase activity

Water deficit stress increases the activities of all the antioxidant enzymes. In the present investigation revealed that water stress significantly increases POX and SOD activities in all the groundnut genotypes under PFD, FD and PoFD than control. Among the genotypes CO 7 increased 61 per cent of POX followed by TMV 7 (57%) and TMVGn 13 (55%). At the same time in COGn 4, only 26 per cent increase was obtained under PFD (Fig. 1). Mean SOD activity was significantly higher in FD (2.25 enzyme units mg $protein^{-1}$) followed by PoFD (2.15 enzyme units mg $protein^{-1}$) and PFD (2.09 enzyme units mg $protein^{-1}$) than control (1.61 enzyme units mg $protein^{-1}$). Irrespective of the stages of stress treatments, after recovery, it was reduced. All the genotypes except COGn 4 are statistically not significant and on par with each other and also between control and recovery treatments under FD and PoFD (Table 3). After re-watering, irrespective of the stress treatments, drastic reduction was obtained in POX and SOD than control. The results were in conformity with findings of Dhindsa and Motowa (1981) who reported that, drought induced damage was negated with increasing activities of SOD and POX. It was interesting to note that, irrespective of the genotypes, the POX and SOD activities increased with the progress of water stress to certain level and then stable in some genotypes and only very less activity in susceptible genotypes. Activities of these enzymes increased at a low rate in COGn 4. This observation indicated that these enzymes degraded at high rate in COGn 4 under dehydration, while their synthesis might have been inhibited (Zhang and Kirkham, 1994) [22]. Raheleh *et al.* (2012) [17] also reported that tolerant chickpea genotypes could decrease damaging effects of drought stress by an increase in antioxidant enzymes activity such as SOD and POX in the vegetative, flowering and pod formation stages. Sensitive genotypes showed less increase in SOD and POX activities that may be related to the low water potential of this cultivar to remove O_2^- and H_2O_2 under water deficit.

3.4. Kernal Yield and Halum Yield

Water stress is an important factor limiting the yield and quality of groundnut. Groundnut genotypes differed substantially in their pre flowering, flowering and post flowering phases in response to drought. Performance of groundnut genotypes on kernel yield was high in PFD stage. FD and PoFD reduced the kernel yield in all the genotypes over control revealed in table 5. The decrease was 67.63 per cent under FD and 71.17 per cent in PoFD of kernel yield respectively. All the genotypes were recorded more kernel yield under PFD compared to control except CoGn 4. More kernel yield of 19.72 in CO 7, 14.99 in TMV 7 and 13.54 g $plant^{-1}$ in TMVGn 13 at PFD than control. With respect to kernel yield, high significant variation was observed within the genotypes and between the treatments. The same trend was observed in halum yield also. With respect to halum yield, high reduction was observed in FD (14.99g $plant^{-1}$) and PoFD (16.63g $plant^{-1}$) compared to control (26.75g $plant^{-1}$) but PFD performed well in recording more halum yield of 29.66 gram per plant than control. The reduction in NR activity under water stress conditions might be due have to less protein synthesis and thus reduced protein pools including key enzymes. In the present study, there was a decrease in NR activity during stress and recovered by re-watering. Vurayai *et al.*, (2010) [20] depending on the stage of development, water stressed plants produced about four fold increases in the amount of proline compared to non-stressed plants. Also they have explained that, plants which were water stressed during the pod filling stage had the lowest increase in proline content while plants which were water stressed during the vegetative stage had the highest increase in proline content. Huang *et al*. (2012) [9] reported that, SOD is a one of the key enzymes in the antioxidant protection system and they suggested that, superoxide dismutase (SOD) play an important role in detoxifying ROS-induced stresses. Based on its metal cofactors, SOD can be categorized into three forms: copper/zinc-(Cu/Zn-SOD), manganese-(Mn-SOD), and iron-(Fe-SOD). Cu/Zn-SOD is located mainly in the chloroplast and cytoplasm. Li and Zhang *et al*. (2014) [12]; Zhang and Liu *et al*. (2013) [11] have noted that, chloroplast Cu/Zn-SOD is one of the most important antioxidant present in plant leaves. Li and Zhang *et al*. (2014) [12] strongly suggested that, SOD activity has a close relationship with drought resistance in groundnut. Under water stress the activity of SOD, POX in leaves and roots increased sharply at prophase, metaphase growth stages, such as male tetrad stage, but then declined (GE Ti-da *et al.*, 2006) [7] towards the physiological maturity. Previous findings were supported to this current study that, pre flowering drought imposed plants showed high kernel yield and halum yield due to high recovery percentage of NR activity after rewatering, high proline, POX and SOD activity under Pre Flowering Drought than plants under Flowering and Post Flowering Drought conditions.

3.4. Oil content

Oil content of the groundnut genotypes varied significantly among the genotypes under stress and control condition (Table 5). Among the genotypes, COGn 4 (51.3 %) recorded the highest oil content followed by TMVGn 13 (49.1 %), TMV 7 (48.5 %) and CO 7 (51.0 %) in control. CO 7 maintained its superiority in recording oil content of, 50.9 and 50.0 per cent in FD and PoFD respectively but in PFD COGn 4 recorded high oil content (49.5 %). Other genotypes were reduced oil content drastically especially under FD and PoFD.

Statistical parity was observed in mean of control and PFD. Highly significant variation is observed under FD and PoFD with control. Chakraborty *et al.*, 2013, suggested that prolonged moisture deficit stress reduced kernal yield, fodder/halum yield and oil content, this previous study was supported to this present study.

Table 1: Effect of water stress on Nitrate reductase activity (µg NO_2 g^{-1} h^{-1}) of groundnut genotypes

Genotypes	At Stress						At Recovery					
	Pre flowering drought		Flowering drought		Post flowering drought		Pre flowering drought		Flowering drought		Post flowering drought	
	Control	Stress	Control	Stress	Control	Stress	Control	Recovery	Control	Recovery	Control	Recovery
CO 7	86.11	69.5	99.51	76.22	88.32	65.23	94.48	88.41	126.48	98.73	42.00	25.54
COGn 4	84.22	51.38	97.38	47.15	83.76	27.40	94.35	58.24	119.98	73.23	40.71	14.16
TMV 7	83.37	66.83	98.59	72.77	79.65	49.57	93.81	82.47	122.62	89.27	38.42	22.82
TMVGn 13	78.99	57.97	96.04	68.39	76.27	42.31	91.33	79.37	118.25	81.17	35.08	20.13
Mean	83.17	61.42	97.88	66.13	82.00	46.13	93.49	77.12	121.83	85.60	39.05	20.66
	G	S	T	GxS	SxT	GxT	G	S	T	GxS	SxT	GxT
SEd	0.700	0.606	0.495	1.213	0.858	0.990	0.834	0.722	0.589	1.445	1.021	1.179
CD (0.05)	1.408	1.220	0.996	2.440	1.725	1.992	1.677	1.452	1.186	2.905	2.054	2.372

Table 2: Effect of water stress on proline content (µg g^{-1}) of groundnut genotypes

Genotypes	At Stress						At Recovery					
	Pre flowering drought		Flowering drought		Post flowering drought		Pre flowering drought		Flowering drought		Post flowering drought	
	Control	Stress	Control	Stress	Control	Stress	Control	Recovery	Control	Recovery	Control	Recovery
CO 7	325.9	451.1	423.1	535.2	381.4	416.5	354.8	381.0	463.6	497.9	251.4	263.8
COGn 4	321.1	332.9	316.1	418.5	231.4	252.0	251.7	276.5	321.9	332.1	157.3	147.0
TMV 7	318.7	428.7	418.1	528.7	366.9	396.3	350.2	375.8	458.1	471.7	234.7	236.8
TMVGn 13	312.4	413.8	412.4	519.0	358.7	384.0	348.4	372.4	452.1	464.8	218.9	219.7
Mean	319.5	398.6	392.4	500.3	334.6	362.2	326.3	351.5	423.9	441.6	215.6	216.8
	G	S	T	GxS	SxT	GxT	G	S	T	GxS	SxT	GxT
SEd	3.477	3.011	2.459	6.023	4.259	4.918	2.651	2.295	1.874	7.429	3.714	4.289
CD (0.05)	6.992	6.055	4.944	12.111	8.564	9.888	5.330	4.616	3.76	14.939	7.469	8.625

Table 3: Effect of water stress on superoxide dismutase (SOD) activity (enzyme units mg $protein^{-1}$ min^{-1}) of groundnut genotypes

Genotypes	At Stress						At Recovery					
	Pre flowering drought		Flowering drought		Post flowering drought		Pre flowering drought		Flowering drought		Post flowering drought	
	Control	Stress	Control	Stress	Control	Stress	Control	Recovery	Control	Recovery	Control	Recovery
CO 7	1.88	2.87	1.95	2.93	1.99	2.73	1.91	2.12	2.18	2.67	2.05	2.15
COGn 4	1.13	1.18	1.31	1.37	1.40	1.49	1.31	1.15	1.34	1.32	1.43	1.46
TMV 7	1.82	2.27	1.93	2.44	1.85	2.27	1.87	1.99	2.10	2.12	1.89	1.89
TMVGn 13	1.62	2.02	1.89	2.25	1.79	2.09	1.77	1.87	2.08	2.11	1.82	1.83
Mean	1.61	2.09	1.77	2.25	1.76	2.15	1.72	1.78	1.93	2.06	1.80	1.83
	G	S	T	GxS	SxT	GxT	G	S	T	GxS	SxT	GxT
SEd	0.015	0.013	0.011	0.027	0.019	0.022	0.015	0.013	0.011	0.027	0.019	0.022
CD (0.05)	0.031	0.027	0.022	0.055	0.039	0.045	0.031	0.027	0.022	0.054	0.038	0.044

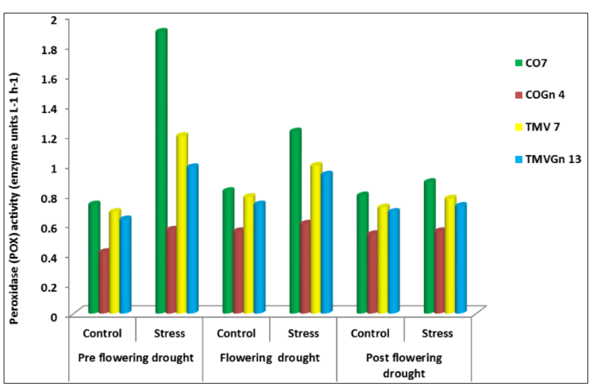

Fig 1: Effect of water stress on peroxidase (POX) activity (enzyme units L^{-1} h^{-1}) of groundnut genotypes

Table 4: Effect of water stress on kernel yield (g $plant^{-1}$) and Halum weight (g $plant^{-1}$) of groundnut genotypes

Genotypes	Kernel yield (g $plant^{-1}$)				Halum yield (g $plant^{-1}$)			
	Control	PFD	Control	PFD	Control	PFD	Control	PFD
CO7	16.81	19.72	16.81	19.72	16.81	19.72	16.81	19.72
COGn 4	16.52	8.85	16.52	8.85	16.52	8.85	16.52	8.85
TMV 7	12.50	14.99	12.50	14.99	12.50	14.99	12.50	14.99
TMVGn 13	10.63	13.54	10.63	13.54	10.63	13.54	10.63	13.54
Mean	14.12	14.28	14.12	14.28	14.12	14.28	14.12	14.28
	G	S	G		S	G	S	
SED	0.094	0.094	0.094		0.094	0.094	0.094	
CD (0.05)	0.192	0.192	0.192		0.192	0.192	0.192	

Table 5: Effect of water stress on oil content (%) of groundnut genotypes

Genotypes	Oil content (%)			
	Control	PFD	FD	PoFD
CO7	51.0	51.2	50.9	50.0
COGn 4	51.3	49.5	41.5	42.7
TMV 7	48.5	48.0	41.3	40.2
TMVGn 13	49.1	48.7	40.2	40.7
Mean	49.9	49.3	43.4	43.4
	G	S	GxS	
SED	0.289	0.289	0.578	
CD (0.05)	0.589	0.589	NS	

4. Conclusion

The study concludes that, vegetative water stress beneficial to groundnut yield. Decreased NR activity was observed during stress but after re watering, it was recovered in PFD imposed plants. Under FD, NR activity was recovered slowly but in PoFD, NR activity is not recovered even after irrigation. Proline, POX and SOD levels got increased at vegetative stage (PFD) but declined towards the physiological maturity and also proline plays osmoprotective role under water stress condition. POX and SOD plays a vital role in detoxifying ROS effects under water stress. This might be the reason for yield increment in PFD stressed plants but not in FD and PoFD stressed palnts. These biochemical parameters indirectly influenced high yield and oil content under PFD than other stages of drought. Among the genotypes, CO 7 performed better in all stages of water stress.

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