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Variability in Drought Stress Induced Responses of Groundnut (*Arachis hypogaea* L.) Genotypes

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Abstract

Drought stress is one of the important abiotic stresses which can limit the crop growth and yield by altering various physiological and biochemical processes. Groundnut (*Arachis hypogaea* L.) is an important oil seed cash crop and can be affected by dry spells during critical pheno-phases. A field trial was conducted with six genotypes- JL-24, ICGV 91114, Narayani, Abhaya, Dharani and Greeshma in order to identify genotypic variability in physiological and biochemical changes that are triggered during drought stress. Drought stress imposition at flowering stage reduced Anet, gs, Tr and WUE. Reduction of Anet ranged from 60% (Abhaya) to 77% (ICGV 91114) whereas the reduction in Tr was lower. The genotypes Dharani and Abhaya with higher Anet and better intrinsic WUE at leaf level during stress period along with highest membrane stability index (MSI), higher accumulation of proline, FAA and total soluble proteins with better yield potentials proved to be tolerant to drought stress. The results indicated that response of groundnut genotypes to drought stress differed significantly and genotypes Dharani and Abhaya are likely to be tolerant to drought stress.

Keywords: Drought stress; Groundnut genotypes; Anet; gs; Tr; WUE; Biochemical parameters

Introduction

Abiotic stresses are an integral part of 'climate change' which can change soil-plant-atmosphere continuum thereby influencing the productivity of crops [1]. 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 percent [2]. Drought triggers a wide range of physiological [3] and biochemical processes [4] 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 [5], accumulation of osmoprotectants like proline, FAA in the cell [6]. 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. Due to erratic rain fall and frequent drought during the crop growth period groundnut yields are generally low and unstable under rain-dependent conditions [7] due to poor adaptation of improved varieties and the influence of drought stress depends on the magnitude of stress, its duration, growth stage and type of genotype [8]. Drought stress during reproductive stages like flowering and pod filling stage is crucial for yield in groundnut and this reduction of crop yield depends on groundnut varieties [9] and tolerant genotypes will be able to give better yield considerably [10] due to physiological and biochemical changes that were triggered during drought stress. There are significant genotypic variations in response to drought and their tolerance levels in groundnut [5], it is necessary to screen the selection of tolerant groundnut lines for breeding purposes [10] and better understanding of the stress induced responses of physiological and biochemical traits can prove to be very useful to screen drought tolerant genotypes [6]. The objective of the present research program was to assess the impact of drought stress at flowering stage on the physiological, biochemical and yield performance of popularly cultivated groundnut genotypes in order to quantify their tolerance levels.

Materials and Methods

A field trial was conducted with six groundnut genotypes- JL-24, ICGV 91114, Narayani, Abhaya, Dharani at Central Research Institute for Dryland Agriculture Research Farm, Hyderabad (situated at 17°18- N latitude, 78°36- E longitude and an elevation of 515 m above mean sea level) during summer season in 2014. The average temperature was 26.2˚C during crop growth period where minimum and maximum temperatures recorded during crop growth period was 13.6˚C and 41.7˚C.The pod materials of JL-24 and ICGV 91114 genotypes were obtained from ICRISAT, Hyderabad and genotypes Narayani, Abhaya, Dharani from Regional Research Centre, Thirupathi. Three field replications were maintained in RBD for control and stress treatment i.e. 1) Control (CN) treatment with regular irrigation schedules to maintain stress free condition, and 2) Drought stress (DS) treatment in which stress was imposed at flowering stage by withholding irrigation till wilting symptoms (twelve days) appeared. Recommended agronomic practices and plant protection measurements were followed, except for the irrigation schedules.

Physiological and biochemical parameters were assessed in both irrigated and drought stress treatments when the wilting symptoms appeared in DS. Net photosynthetic rate (Anet) of fully expanded young leaves was measured with a portable photosynthesis system (LI-6400, LI-COR). Photosynthetic rate (Anet) along with stomatal conductance (gs), transpiration rate (Tr) measurements were recorded between 10:00 and 12:00hrs, with irradiance set at 1200 μ mol $m^{-2}s^{-1}$. Water use efficiency (WUE) was calculated as the ratio of Anet and Tr. Leaf Membrane Stability Index (MSI) was determined according to the method of Premachandra [11] modified by Sairam [12]. MSI was calculated using the formula MSI = [1- (C1 /C2)] x100 where, C1 and C2 are the electric conductivities recorded at 40°C and 100°C respectively. Accumulation of proline [13], free amino acids (FAA) [14], and total soluble proteins [15] were estimated in leaf extractions of control and drought stress treated plants. At harvest, yield was taken in terms of seed weight. The data was statistically analyzed using two-way analysis of variance (ANOVA) for physiological and biochemical parameters to test the significance of genotypes, drought stress and their interactions.

Results and Discussion

Physiological parameters

The response of Anet, gs, Tr were highly significant ($p<0.01$) for genotypes, drought stress and interaction of drought stress×genotype. The ANOVA and mean performance of these parameters are presented in Table 1-2.

Drought stress reduced all the physiological parameters of all six groundnut genotypes and the reduction was high with Anet (70%) and gs (78%) as compared with Tr (59%), hence the reduction was less for intrinsic WUE (24%). However, the magnitude of reduction was different with genotype. Similar genotypic variability in response to drought stress was reported in groundnut [16,17] soyabean [3] and cowpea [18].

Among the genotypes assessed, Anet values ranged from 28.0 μmoles $CO_2/m^2/s$ (Abhaya) to 35.9μmoles $CO_2/m^2/s$ (ICGV 91114) in control where as it reduced to 7.4 μmoles $CO_2/m^2/s$ (Narayani) to 11μmoles $CO_2/m^2/s$ (Abhaya and Dharani) under drought stress conditions. It was interesting to observe that genotype ICGV 91114 with highest Anet under control condition recorded highest reduction with drought stress whereas genotype Abhaya with lowest Anet under control condition recorded highest Anet with lowest reduction under drought stress.

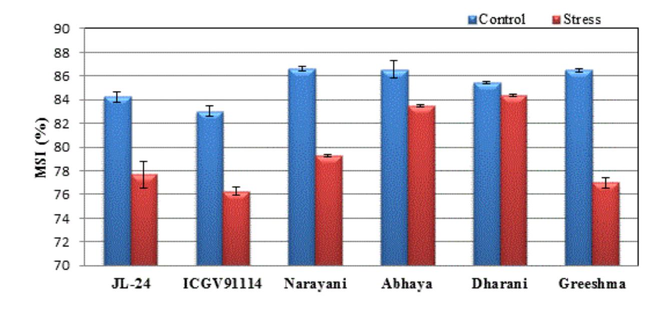

Figure 1: Membrane stability index (MSI) under control and drought stress conditions in groundnut genotypes

The results agrees with the reports of Kalariya et al. [19] that there is a decrease in the Anet of groundnut genotypes with different magnitude due to drought stress. The reduction of Anet due to drought stress may be due to several coordinated events, such as stomatal closure which reduces CO_2 availability in the leaves. This in turn inhibits carbon fixation and reduced activity of photosynthetic enzymes [5] such as rubisco. The genotypes with higher Anet and low reduction with drought stress is expected to perform better and in the present investigation, the genotypes Abhaya and Dharani can perform better under drought stress conditions as they recorded higher Anet.

Genotypes	Treatment	Parameters			
		Anet	gs	Tr	WUE
JL-24	Control	28.2±0.82	0.34±0.009	9.9±0.08	2.86±0.06
	Drought stress	9.4±0.25	0.126±0.021	5.8±1	1.86±0.04
	Decrease (%)	61.4	62.9	40.8	34.7
ICGV 91114	Control	35.9±0.83	0.522±0.007	12.5±0.18	2.87±0.08
	Drought stress	8.3±0.45	0.077±0.01	3.3±0.44	2.52±0.04
	Decrease (%)	76.8	85.1	73.5	12.4
Narayani	Control	28.2±0.22	0.321±0.004	10.1±0.17	2.79±0.07
	Drought stress	7.4±0.64	0.077±0.002	3.9±0.06	1.9±0.01
	Decrease (%)	73.8	75.8	61.4	32.1
Abhaya	Control	28±0.12	0.362±0.049	9.4±0.71	3.01±0.02
	Drought stress	11.1±0.18	0.125±0.006	5.7±0.28	1.95±0.07
	Decrease (%)	60.24	65.5	39.1	34.7
Dharani	Control	35.1±0.07	0.575±0.023	12.8±0.53	2.72±0.12
	Drought stress	11±0.29	0.099±0.008	4.72±0.38	2.28±0.02
	Decrease (%)	69.3	82.7	63.3	16.4
Greeshma	Control	34.3±0.73	0.643±0.078	13.43±0.78	2.55±0.11
	Drought stress	10.2±0.53	0.103±0.021	4.7±0.94	2.23±0.05
	Decrease (%)	69.3	83.9	64.8	12.8

Table 1: Mean+SE and percentage of reduction of physiological parameters of groundnut genotypes under control and drought stress conditions in groundnut genotypes

First response to drought stress is closing of stomata which prevents the rate of water loss with reduced gs, and Tr. Higher gs was recorded in Greeshma (0.64 mmol/m^2/s) under control condition and lowest in Narayani (0.32 mmol/m^2/s), whereas highest gs was in JL-24 (0.127 mmol/m^2/s) and lowest in Narayani (0.078 mmol/m^2/s) under stress conditions. There are previous reports that there was a genotypic variation and also reduction of gs in groundnut in response to drought stress [17]. Tr also reduced under drought stress from 39.13% (Abhaya) to 73.52% (ICGV 91114) to prevent the rate of water loss. With high transpiration rates under control treatment, the genotypes Greeshma (13.4 mmol/m^2/s) and Dharani (12.8 mmol/m^2/s) showed moderate Tr (4.7 mmol/m^2/s) whereas under stress conditions ICGV 91114 recorded lowest Tr (3.3 mmol/m^2/s) under stress conditions with highest reduction (73.52%) from its control values. Earlier reports on groundnut also showed similar trend and variation within genotypes for Tr in response to drought stress [19]. In the present study, it is evident that in groundnut, the reduction of gs was higher than Tr clearly indicating that gs was more impacted by drought stress than Tr [20].

Intrinsic water use efficiency (WUE) decreased under drought stress conditions and it was non-significant for genotypes and highly significant ($p<0.01$) for drought stress and significant ($p<0.05$) for drought stress×genotype interaction (Table 2). Among the genotypes, Abhaya recorded highest WUE (3.0 µmoles CO_2/mmol H_2O) under control treatment and reduced under stress conditions (2.0 µmoles CO_2/mmol H_2O) due to higher reduction of Anet than Tr. The genotypes ICGV 91114 and Dharani could maintain better WUE under stress treatment though registered moderate WUE under control conditions (Table 1). It was interesting to observe that genotype Dharani was able to maintain better Anet under stress as higher reduction of Tr resulted better WUE whereas the genotype ICGV 91114 recorded higher reduction of Anet and Tr. This was also in line with the pattern of WUE observed by Songsri et al. [16] in groundnut genotypes due to drought stress.

A major impact of drought stress is usually on cellular membrane modification, which results in total dysfunction and it is generally accepted that the maintenance of integrity and stability of membranes under drought stress is a major component of drought tolerance in plants. In the present study, MSI was highly significant for genotypes, drought stress and drought stress × genotype interactions. MSI was ranged from 83% (ICGV 91114) to 86.6% (Abhaya and Narayani) in controls and whereas under stress it was 77% (Greeshma) to 84.4% (Dharani). Under drought stress conditions higher MSI was recorded in Dharani (84.4%) and Abhaya (83.5 %) with less reduction 1.2% and 3.4% respectively over controls (Figure 2). Earlier records on groundnut also showed significant effect of drought stress on MSI, the groundnut varieties WEST- 44, showed higher MSI with low decrease in MSI over control under drought stress conditions [9]. The genotypes with higher MSI have better adaptations under drought stress [21] so in the present investigation, genotypes Dharani and Abhaya with better MSI are likely to have better adaptations under drought stress conditions.

Biochemical parameters

Drought stress induced higher amount of proline, free amino acids, soluble protein in all genotypes and they are highly significant ($p<0.01$) for genotypes, drought stress and drought stress × genotype interaction; however the degree of increase varied with genotypes. Plants respond to different types of stresses by accumulating certain specific metabolites such as amino acids, proteins in general and proline in particular [6]. Proline content is used as an indicator of drought tolerance capacity of plant tissue as accumulation of proline helps in osmotic balance during drought stress. In the present study proline content of all genotypes increased as a result of drought stress. The genotypes Dharani and Abhaya recorded higher increase in proline content as well as registered higher content under drought stress (Figure 2).

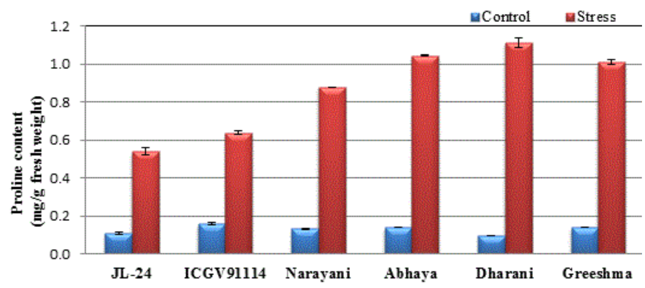

Figure 2: Proline content under control and drought stress conditions in groundnut genotypes

There are similar reports that there is an increase of proline content in cultivars of blackgram, green gram [4] and groundnut [22] due to drought stress. Genotypes Dharani and Abhaya in the present study with higher increment and proline content under drought stress are expected to have better stress tolerance capacity.

With drought stress the content of free amino acids (FAA) increased in all the genotypes and this increase was pronounced in Dharani (32%) with higher FAA (3.4 mg g^{-1} FW) whereas lower in Greeshma with lower FAA (2.6 mg g^{-1} FW) (Figure 3). Genotypic variability and an increase in the activity of FAA were also observed in pearl millet [6] and groundnut [5]. All the selected groundnut genotypes recorded increased amount of total soluble proteins (TSP) due to drought stress conditions. The increase was more conspicuous in Dharani and Abhaya as these two genotypes maintained higher protein (79 and 73 mg g^{-1} FW) whereas Greeshma had lower protein (59 mg g^{-1} FW) (Figure 4). These results are in tune with previous results of blackgram [23] and groundnut [5] genotypes. Usually under drought stress, increase of total soluble proteins was as a result of high amino acids contents as plants accumulate small molecular mass proteins as there may be increase of de novo synthesis or inhibition of amino acid degradation [5] and higher protein content might impart better drought tolerance [23] as it helps in osmotic balance. Among the genotypes, Dharani and Abhaya have higher protein content when exposed to drought stress and this may alleviate the stress impact.

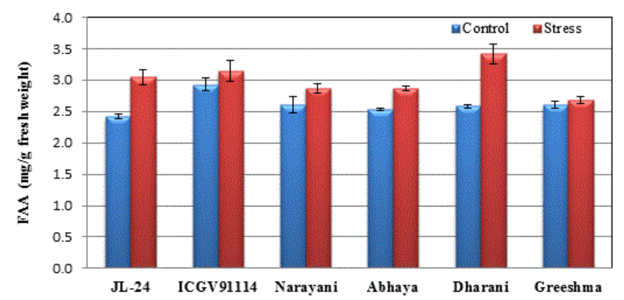

Figure 3: Free Amino Acid (FAA) content under control and drought stress conditions in groundnut genotypes

Yield

Reduction of yield recorded due to drought stress in all groundnut genotypes that were studied and it was highly significant ($p<0.01$) for genotypes, drought stress and their interaction (Table 2). The genotypes Dharani and Abhaya performed better in terms of *per se* seed weight under control (29 g $plant^{-1}$, 26.5 g $plant^{-1}$) as well as stress conditions (22 g $plant^{-1}$, 17 g $plant^{-1}$) (Figure 5). Under stress conditions lower reduction of yield was recorded in Dharani (25%) and ICGV 91114 (6%), and but the *per se* seed yield was lower in ICGV 91114 (13.7 g $plant^{-1}$) and higher in Dharani (22 g $plant^{-1}$). According to Ratnakumar and Vadez, [10] the seed weight reduced significantly under drought stress in groundnut genotypes and tolerant genotypes were able to maintain better yield.

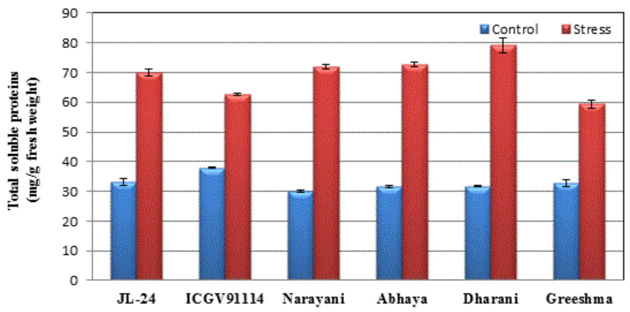

Figure 4: Total soluble protein content under control and drought stress conditions in groundnut genotypes

Parameter	Mean sum of squares				
	Genotypes (G) df(5)	Drought stress (D) df (1)	G × D df (5)	Error df (22)	CV (%)
Anet	27.4**	4382**	23.6**	0.805	4.4
gs	0.027**	1.2**	0.03**	0.002	15.1
Tr	3.6**	399**	8.7**	0.68	10.3
WUE	0.164 NS	4.7**	0.29 *	0.102	12.9
MSI	28.2**	295**	13.9**	0.46	0.8
Pr	0.08**	4.9**	0.085**	0.005	3
FAA	0.163**	1.4**	0.12**	0.011	3.8
TSP	54.9**	11871**	121.5**	1.87	2.7
SW	113.2**	439.2**	13.9**	3	9.2

Table 2: ANOVA for physiological and biochemical parameters under control and drought stress conditions in groundnut genotypes [*significant at $p<0.05$, **significant at $p<0.01$.Anet-Photosynthetic rate (μmol $CO_2/m^2/s$), gs-Stomatal conductance $mmol/m^2/s$, Tr-Transpiration rate ($mmol/m^2/s$), WUE- Water use efficiency (μmol CO_2/mmol H_2O) MSI-Membrane Stability Index (%), Pr-Proline (mg/gm FW), FAA-Free amino acids (mg/gm FW), TSP-Total soluble protein (mg/gm FW), SW-Seed weight (g/plant)]

Conclusions

The results are clearly indicating that two groundnut genotypes Dharani and Abhaya have higher Anet, lower gs and Tr recorded better WUE under control as well as drought conditions. This coupled with greater MSI and biochemical parameters such as proline, FAA and protein content helped them to efficiently overcome the ill effects of drought stress and perform better even in terms of yield. These physiological and biochemical parameters help to screen the genotypes tolerance to drought stress and is useful in selecting genotype or developing new varieties with tolerant mechanisms in predicted future climatic conditions.

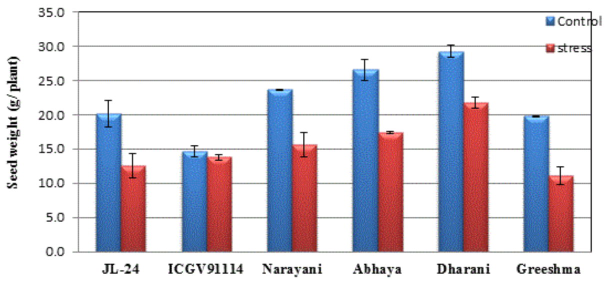

Figure 5: Seed weight under control and drought stress conditions in groundnut genotypes

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