Physiological impact of moisture deficit stress on Relative Water Content (RWC) and Membrane Stability Index (MSI) of groundnut (*Arachis hypogea* L.) at different stages of growth

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Abstract
Physiological responses of moisture deficit stress on seven ground nut genotypes (TAG 24, TG 37A, TG 39, Dh 256, GNH 804, ICGV 07041 and CSMG 2010-28) have been observed to evaluate the drought-tolerant (DT) and drought-susceptible (DS) groundnut (Arachis hypogaea L.) genotypes through phenotyping screening tools, Relative Water Content (RWC) and Membrane Stability Index (MSI). All the genotypes have been assessed in terms of Relative Water Content and Membrane Stability Index (MSI) of leaf under both irrigated and drought stress conditions during different growth stages (at 30, 60, 90DAS and at harvest). Results indicated that there was a significant difference in the average value of RWC and MSI which reduced with the progression of rapid water deficit stress.

The RWC and MSI progressively increase with the advancement of leaf growth stages up to 60 DAS and thereby decrease. During 60 DAS, highest RWC and MSI was obtained in all the genotypes. Significantly extreme differences in all genotypes under water stress became more and more protruding from 60 DAS onwards. It was observed that those ground nut plants relish with no stress or zero stressed condition exhibiting higher maintenance of RWC (%) as well as MSI throughout the growth period comparatively than mild to sturdy stressed and severe stressed conditions. Some of the genotypes like TG 37A and TAG 24 showed higher maintenance of RWC and MSI by confirming better hydration as well as better degree of membrane protection and more favorable intracellular water relations whereas CSMG-2010-28 followed by ICGV 07041 and TG 39 are more susceptible to drought (DS).

Keywords: Groundnut, Relative Water Content (%), Membrane Stability Index (MSI), genotypic variation, drought susceptible (DS) and drought tolerance (DT).

Introduction

Groundnut (*Arachis hypogea* L.) also known as peanut, is an important legume oilseed crop as its seed contain 44–56% oil and 22–30% protein on a dry seed basis[18]. It is grown mostly for its high quality edible oil and protein. India and China are the largest producers of groundnut. Groundnut is the largest source of edible oils in India and constitutes roughly about 50% of the total oilseeds production. It is largely grown under rain-fed conditions in the tropics and sub-tropics semi-arid regions of the world where production and productivity are usually affected by soil moisture stress.

Rainfall is a major constraint in groundnut cultivation and low rainfall as well as prolonged dry spells during the crop growth period as the main reason for low average yields in India. In India, rainfall accounts for over 50% of variance in yield[5] and the average groundnut yield in our country is very low because of moisture stress faced at various growth stages, irrespective of the other factors of the crop production package[20]. In India, groundnut yields fluctuated from 550 to 1100 kg ha–1 in different years and consequently the total production of the country also varied from 4.3 to 9.6 million tons[14].

Groundnut is frequently subjected to drought stress of various duration and intensity. The severity of drought stress greatly depends on the stage of the crop, duration of moisture stress and magnitude of the stress. Drought is generally accompanied by low relative humidity, high temperature and high wind speed which also influence groundnut. Drought stress has an adverse influence on the water relations[2], photosynthesis[3], mineral nutrition, metabolism, growth and yield of groundnut[22]. Leaf water potential, transpiration rate and photosynthetic rate decreased progressively with increasing duration of water stress indicating that plants under mild stress were postponing tissue dehydration[21].

Drought-resistant varieties typically showed a smaller decrease in Relative Water Content (RWC) per unit decrease in leaf water potential compared to susceptible cultivars. Osmotic adjustment has been suggested as a mechanism that leads to smaller changes in RWC per unit decrease in leaf water potential and consequently helps to maintain positive turgor potential during water stress.

The RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and the leaf matures[9]. Obviously, stressed plants have lower RWC than non-stressed plants. The RWC of non-stressed plants ranges from 85 to 90%, while in drought-stressed plants it may be as low as 30%[2]. There was a significant reduction in Membrane Stability Index (MSI), Relative Water Content (RWC) and chlorophyll content in different wheat genotypes under water stress[23] and also there may be a link existing in between the capacity for osmotic adjustment and the degree of membrane protection from the effect of dehydration[10].

Membrane stability index (MSI) reduced due to imposition of water deficit stress showing significant interaction effect between the stress and groundnut genotypes at different growth stages. Maintenance of membrane integrity and function under given level of dehydration stress has been used as a measure of drought tolerance by various workers.[7,15]

Therefore, present investigation has been undertaken to investigate the genotypic variability of seven groundnut genotypes to water deficit stress at different growth stages and to ascertain the role of RWC and MSI in imparting the sensitivity to drought stress.

Material and Methods

The experiment was laid out in Randomized Block Design with three replication for seven genotypes of groundnut (TAG 24, TG 37A, TG 39, Dh 256, GNH 804, ICGV 07041 and CSMG 2010-28) with a maturity period of 115–125 days at different irrigation interval (irrigation after 7 days for non-stress and 15 days for stress) with corresponding non-stressed controls.

The field study was carried out during summer season of 2018 at the adjacent experimental field of Bidhan Chandra Krishi Viswavidyalaya (Bankura Campus), Susunia, West Bengal. The net plot size was 5 × 4 m with 10 rows per plot at 50 cm row to row and 10 cm plant to plant spacing.

Relative Water Content (RWC): Relative water content was measured at 30, 60, 90 DAS and at harvest to evaluate plant water status using four leaflets of the second fully expanded leaves from the top of the main stem of five plants from each plot. For evaluation of RWC for seven genotypes under moisture stress and non-stress conditions, twenty leaves from each plot were harvested and weigh the fresh weight (FW) immediately after harvest.

The leaf samples were then soaked in distilled water for 8 hrs and blotted for surface drying and then turgid weight (TW) was measured at water saturated condition. Then the leaf samples were oven dried at 80 °C for 24 h to obtain their constant dry weight (DW). RWC was calculated based on the formula suggested by Gonzalez and Gonzalez[8] as follows:

$$RWC (\%) = (FW-DW) / (TW-DW) \times 100$$

where FW is the sample fresh weight, TW is the sample turgid weight and DW is the sample dry weight.

Membrane stability index (MSI): MSI was estimated by measuring the electrical conductivity of 100 mg of leaf discs with uniform diameter taken in 10 ml double distilled water in two sets. Test tubes in one set were kept at 40^0C in a boiling water bath for 30 min and electrical conductivity of the sample was measured (C1) using a conductivity meter. Test tubes in another set were incubated at 100^0C in the boiling water bath for 15 min and their electrical conductivity was measured (C2). MSI was calculated according to the method proposed by Premchandra et al[15] that was further updated and modified by Sairam[17] using the formula given below:

$$MSI = [1-C1 /C2] \times 100$$

Results and Discussion

Relative Water Content (RWC): Relative Water Content (RWC) of the seven genotypes has been shown in table 1 and table 2 for comparative analysis among them under non-stress and stress conditions respectively. During 30 DAS, the highest RWC was observed in GNH-804 (87.74) followed by TG 37A (87.26) and TAG 24 (86.53) under zero stress or irrigated condition whereas under stress TG 37A (49.15) showed maximum RWC followed by TAG 24 (48.20) and GNH-804 (46.68). The lowest RWC was found in genotype CSMG-2010-28 [78.33 in zero-stress and 35.65 in stress] that was significantly lowest as compared to all the genotypes.

During 60 DAS, the maximum RWC was detected in TAG 24 (88.46) followed by GNH-804 (88.04) and TG 37A (88.0) under zero stress or irrigated condition whereas under stress TG 37A (44.55) showed maximum RWC followed by TAG 24 (43.0) and GNH-804 (42.68).

During 90 DAS, the highest RWC was observed in GNH-804 (89.06) followed by TAG 24 (88.32) and TG 37A (88.03) under zero stress or irrigated condition whereas under stress TG 37A (39.55) showed maximum RWC followed by TAG24 (38.0) and GNH-804 (37.68). During harvest, the highest RWC was observed in TG 37A (76.26) followed by TAG 24 (74.12) and Dh 256 (72.83) under zero stress or irrigated condition whereas under stress TG 37A (33.55) showed maximum RWC followed by TAG 24 (32.90) and Dh 256 (32.68). The lowest RWC was also found in genotype ICGV 07041 [68.80 in zero stress and 29.68 in stress] during harvest.

Among all the genotypes, TG 37A showed maximum content of RWC in both zero and stress conditions followed by TAG 24 and hereby exhibited high yield whereas CSMG-2010-28 found least content of RWC and less yield in both the situations.

Therefore, from the observation shown in both the tables 1 and 2, it may be concluded that TAG 37A had the greater maintenance in terms of RWC and exhibited less reduction in RWC under moderate to severe moisture stress i.e. TG 37A to some extent drought tolerant (DT) among the other genotypes.

Table 1
Comparative study of Relative Water Content (%) in seven genotypes under zero or non-stress condition i.e. irrigation at 7 days interval.

Genotypes	Relative Water Content (%)			
	30DAS	60DAS	90DAS	At Harvest
TG 37A	87.26	88.0	88.03	76.26
TAG 24	86.53	88.46	88.32	74.12
GNH-804	87.74	88.04	89.06	71.65
Dh-256	85.21	86.78	87.0	72.83
TG 39	83.54	84.05	85.54	71.24
ICGV 07041	80.59	83.07	83.16	68.80
CSMG 2010-28	78.33	79.57	79.80	70.0
Mean	84.17	85.42	85.84	72.12
LSD	2.31	2.78	2.88	3.16
CD (0.05)	3.198	2.885	3.046	2.236

Least Significant Difference (LSD) was applied to compare the means.

Table 2
Comparative study of Relative Water Content (%) in seven genotypes under drought stress condition i.e. irrigation at 15 days interval.

Genotypes	Relative Water Content (%)			
	30DAS	60DAS	90DAS	At Harvest
TG 37A	49.15	44.55	39.55	33.55
TAG 24	48.20	43.0	38.0	32.90
GNH-804	46.68	42.68	37.68	32.52
Dh-256	46.56	45.78	37.0	32.68
TG 39	42.66	38.05	35.84	30.24
ICGV 07041	40.59	37.07	35.16	31.80
CSMG 2010-28	35.65	34.0	32.70	29.68
Mean	44.21	40.73	36.56	31.91
LSD	2.12	2.02	1.77	1.63
CD (0.05)	2.086	1.055	2.012	2.124

Least Significant Difference (LSD) was applied to compare the means

TG 37A was also followed by another drought tolerant (DT) genotype, TAG 24 showed the least reduction in RWC compared to non-stressed plants and may be placed in second rank. However, it might be further concluded that severe damaging effect in terms of RWC shown by CSMG 2010-28 revealed least maintenance regarding RWC and may be reckoned as most drought susceptible (DS) among the other genotypes. Regarding the drought susceptibility, ICGV 07041 might be placed in second drought susceptible (DS) genotype.

Relative water content allows direct phenotypic as well as genotypic comparison among the genotypes of various crops because it expresses directly in the crop. Similar study was demonstrated by Puangbut et al[16] that RWC in stressed plants ranged between 68-83% compared with 90-96% in non-stressed plants. The average reduction in leaf RWC at pod developmental stage was 6.5%, which resulted in reduced growth and partial wilting of leaves in stressed plants[4]. The RWC, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important parameters that influence water relations in groundnut.[11-13,19] The RWC of leaves is reported to be higher during the initial stages of development and declines as the dry matter accumulates and leaf matures[9].

In non-stressed groundnut plants, the RWC in leaves ranges from 85 to 90 %, while under drought stress it goes down heavily[2]. Some objections have been raised about the similar findings that in groundnut, the genotypic discrimination of RWC trait depends on the water regime and genetic background and this trait has no value as selection criteria but serves to characterize finely the water status of plants[6].

Another similar finding as well as contradictory reported by Awal and Ikeda[1] shows that RWC of non-stressed plants ranges from 85-90%, whereas RWC in stressed plants could be as low as 30%. RWC in the range of 68-83% in this study was not too severe compared with RWC of 30% in the most severely stressed plants and the stress in the rainy season was slightly more severe than in the dry season. This could be due to higher air temperature during drought period causing the rapid depletion of stored soil moisture.

However, peanut genotypes were not statistically different in relative water content for both soil moisture levels in both the seasons.

The results indicated that relative water content might be a useful tool for discriminating water status of stressed and non-stressed plants, but its discriminating power is not enough to distinguish the differences among peanut genotypes. RWC completely recovered within 1-3 days of re-watering and some peanut genotypes tended to have higher RWC compared to non-stress treatment. RWC recovered within 1-2 days of re-watering suggesting that stomatal conductance of peanut responded very vigorously during recovery following the stress period[1].

Membrane Stability Index (MSI): Table 3 and 4 demonstrated that the % of Membrane Stability Index (MSI) under non-stress and stress conditions respectively on the seven genotypes and showed that there was a significant reduction of MSI due to burden of moisture stress in groundnut crop. MSI was higher at non-stressed conditions and maximum MSI was observed during 60DAS in TG 37A that was reduced into the leaves age and showed 75.06 at harvest. However, under stress condition it was severely reduced to 65.33 at harvest.

All the other genotypes showed linearly decreased in MSI with increased water stress level that also varies with the tolerance and susceptibility of the genotypes. On the other hand, CSMG 2010-28 exhibited maximum reduction in MSI (56.21) at harvest followed by ICGV 07041 (59.0) at harvest.

So from the results shown in tables 3 and 4, it might be concluded that maximum damaging effect was observed in CSMG 2010-28 with moderate to severe stress conditions and contrary the genotypes TG 37A followed by TAG 24 showed better maintenance or protection of membrane damage by showing higher MSI in all the duration and even with the onset of stress and also its severity.

Table 3
Comparative study of Membrane stability index (%) in seven genotypes under zero or non-stress condition i.e. irrigation at 7 days interval.

Genotypes	Membrane stability index (%)			
	30DAS	60DAS	90DAS	At Harvest
TG 37A	91.21	93.0	87.51	75.06
TAG 24	90.03	92.46	85.52	74.32
GNH-804	88.12	89.08	84.0	72.15
Dh-256	86.11	87.87	84.03	71.35
TG 39	85.25	85.05	83.24	70.78
ICGV 07041	84.04	84.26	83.16	70.54
CSMG 2010-28	83.44	79.99	79.80	71.0
Mean	86.88	87.39	83.89	72.17
LSD (≤ 0.05)	3.15	4.40	2.75	3.06
CD (0.05)	4.103	4.005	3.846	2.236

Least Significant Difference (LSD) was applied to compare the means

Table 4
Comparative study of Membrane stability index (%) in seven genotypes under drought stress condition i.e. irrigation at 15 days interval.

Genotypes	Membrane stability index (%)			
	30DAS	60DAS	90DAS	At Harvest
TG 37A	80.03	81.85	75.34	65.33
TAG 24	78.55	79.0	75.0	63.08
GNH-804	74.66	76.78	73.57	60.52
Dh-256	69.47	70.11	67.23	62.44
TG 39	66.48	67.09	62.58	61.63
ICGV 07041	63.48	65.0	63.78	59.0
CSMG 2010-28	60.68	62.89	58.84	56.21
Mean	70.48	71.82	68.05	61.17
LSD	3.14	2.98	2.32	1.95
CD (0.05)	1.688	2.954	3.002	1.936

Least Significant Difference (LSD) was applied to compare the means

Thus, these two genotypes, TG 37A and TAG 24 are to some extent more tolerant among the other and may be regarded as drought tolerance (DT). CSMG 2010-28 followed ICGV 07041 that showed least MSI due to the imposition of moisture stress and may be reckoned as drought susceptible (DS) i.e. these two are highly sensitive to drought stress.

Similar trends of results also have been observed by Chakraborty et al[4] that membrane stability index (MSI) was reduced due to imposition of water deficit stress and the interaction effect of stress and peanut cultivars was found to be significant for all cultivars at both pegging and pod development stages. Maintenance of membrane integrity and function under given level of dehydration stress has been used as a measure of drought tolerance by various workers.[15,17] Another finding reported that there was a significant reduction in MSI, RWC and chlorophyll content in different wheat genotypes under water stress[23].

Conclusion

In conclusion, the results indicated that water deficit in peanut imparted different physiological responses at various growth stages and obviously showed that some genotypes differed significantly in ability to recover from drought. However, significant genotypic variation in regards to MSI as well as RWC etc. could be useful for improving genotypic performance in breeding program of peanut for drought tolerance. In general response, the peanut cultivars showed decrease in RWC, membrane stability index but the extent of reduction varied with cultivars and developmental stage of the crop. The RWC and MSI progressively increase with the advancement of leaf growth stages up to 60 DAS and thereby decrease.

During 60 DAS, highest RWC and MSI was obtained in all the genotypes. Significantly extreme differences in all genotypes under water stress became more and more protruding from 60 DAS onwards. Higher percentage of both the RWC and MSI contributed to less scratch to moisture deficit stress in drought tolerant (DT) genotypes like TG 37A followed by TAG 24 showed better maintenance or protection of membrane damage by showing higher MSI and higher maintenance of RWC by confirming better hydration as well as better degree of favorable intracellular water relations that might be due to higher water potential as well as better capability of drought tolerance (DT) whereas CSMG-2010-28 followed by ICGV 07041 and TG 39 is more susceptible to drought (DS). Therefore, it is very important to evaluate newly developed peanut genotypes to drought stress and to perform extensive field studies and expression genotypes under diverse environments like moisture deficit stress to assess their stress tolerance level in such crop.

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