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Sequence of physiological responses in groundnut (*Arachis hypogaea* L.) subjected to soil moisture deficit

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Abstract

Responses of drought-tolerant (DT) and drought-susceptible (DS) pot-grown groundnut (*Arachis hypogaea* L.) varieties to changes in leaf relative water content (RWC) were studied. Water stress (WS) was imposed on 30-day-old plants for 2 weeks. Leaf RWC decreased significantly under WS conditions with simultaneous decrease in net photosynthetic rate (P_N) and stomatal conductance (g_s). Even though no significant difference was observed between DT and DS varieties with regard to RWC, DT varieties were able to maintain significantly higher P_N than DS varieties. Higher values of water use efficiency (WUE) were also observed in DT varieties during WS conditions. The decline in P_N due to WS could be attributed to both reduction in g_s (*i.e.* stomatal limitation) and to reduction in chlorophyll content (Chl). No significant difference in leaf area index (LAI) was found between DT and DS types and LAI was not reduced by WS. Significant differences were found among the studied groundnut varieties, but not between DT and DS types, in terms of root, aboveground, and total dry mass. These growth parameters significantly decreased under WS conditions. Based on the results, a sequence of physiological responses in groundnut crop subjected to WS was postulated.

Additional key words: drought susceptibility; drought tolerance; net photosynthesis; peanut; physiological mechanism; water-use efficiency.

Introduction

Groundnut (*Arachis hypogaea* L.) also known as peanut, is an important crop used for food and oil production (Smith 1995). It is grown on 19.3 million ha of land area in about 82 countries (Reddy *et al.* 2003). Generally, field crops including groundnut undergo WS under field conditions (Ndunguru *et al.* 1995), which causes reduction in a pod yield. WS or drought is the most devastating, abiotic stress and the most resistant to breeders' efforts (Tuberosa and Salvi 2006). In the past, breeding efforts to improve drought tolerance have been hindered by its quantitative genetic basis and our poor understanding of the physiological basis of the yield under WS conditions (Blum 1988, Passioura 2002). For this reason, advancement of the current understanding of plant responses to drought stress and the mechanisms involved has become a major target of research and investment, with the ultimate goal of developing crops with improved WUE and minimized drought-induced loss of the yield (Somerville and Briscoe 2001, Zhang *et al.* 2004). More DT species control stomatal function to allow carbon fixation under stress, thus improving WUE (Yordanov *et al.* 2000).

Plants perceive and respond rapidly to even small alterations in water status *via* a serie of physiological, cellular, and molecular events developing in parallel (Chaves *et al.* 2009). WS can trigger a variety of plant responses, which include decrease in RWC and water potential (Lawlor and Cornic 2002), reduction of stomatal aperture (Dubey 1997) and P_N (Leport *et al.* 1999). RWC ranging between 40 and 50% due to severe WS caused

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Abbreviations: ADM – aboveground dry mass; C – control, irrigated plants; Chl – chlorophyll; DS – drought-susceptible; DT – drought-tolerant; *E* – transpiration rate; g_s – stomatal conductance to water vapour; LAI – leaf area index; P_N – net photosynthetic rate; RDM – root dry mass; RLA – relative leaf area; RWC – leaf relative water content; SLM – specific leaf mass; TDM – total dry mass; WS – water stress; WUE – water-use efficiency.

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the decrease in g_s, E, P_N, and WUE in groundnut (Lauriano *et al.* 2004). Photosynthesis is one of the key processes to be affected by WS *via* decreased CO_2 diffusion to the chloroplast and metabolic constraints (Pinheiro and Chaves 2011). Reduction in P_N can be attributed in part to the reduced intercellular CO_2 concentration due to stomatal limitation (Lawlor and Cornic 2002) and also to perturbations of biochemical processes (Lauer and Boyer 1992). Nonstomatal limitation of photosynthesis has been attributed to a reduced carboxylation efficiency (Jia and Gray 2004), reduced ribulose-1,5-bisphosphate regeneration, and reduced amount of functional ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Kanechi *et al.* 1995). Critical reviews on responses of plants in general (Yordanov *et al.* 2000), and groundnut in particular (Boote 1983, Reddy *et al.* 2003), to drought stress provide more information on physiological characteristics associated with drought stress. Vu (2005) inferred that in the absence of other environmental stresses, groundnut leaf photosynthesis would perform well under rising atmospheric CO_2 and temperature as predicted for this century.

The mechanism of drought response has been extensively investigated in the model plant, *Arabidopsis thaliana,* and a resurrection plant, *Craterostigma plantagineum* (Yamaguchi-Shinozaki *et al.* 1995, Shinozaki and Yamaguchi-Shinozaki 1996). Assessment of the regulatory mechanisms of groundnut photosynthesis in response to future changes in climatic conditions, including drought, is limited (Clifford *et al.* 2000). Although impressive advances have been made in the last decade with respect to the nature of events occurring in plants subjected to drought, an integrated picture of the metabolic regulation is still missing (Rolland *et al.* 2006, Shinozaki and Yamaguchi-Shinozaki 2007). In groundnut, limited literature is available about the physiological changes under drought conditions. Matching physiological responses of the crop to WS with molecular studies requires further elucidation, because it is hard to relate molecular events to plant physiological status and to WS intensity (Pinheiro and Chaves 2011).

In a previous study (Jeyaramraja and Thushara 2011), we have categorized selected groundnut varieties into DT and DS varieties based on their drought-response index values, *i.e.* ratio of the pod yield during drought and crop seasons under field conditions. In the present investigation, we tested the hypothesis that the DT and DS varieties of groundnut were different in their physiological responses to WS. Hence, this investigation was aimed to study the effect of WS on various physiological parameters in groundnut varieties varying in their drought tolerance, as this is useful in understanding the drought-tolerance mechanisms and also in suggesting a sequence of physiological responses in groundnut crop subjected to WS.

Materials and methods

Plants: The experiments were carried out at Karpagam Arts and Science College, Coimbatore during April 2008–May 2009. Plant materials include 9 groundnut varieties. Among these, six varieties came from the Regional Research Station of Tamilnadu Agriculture University (TNAU), Vridhachalam, and three varieties from the Regional Research Station of TNAU, Aliyar. These varieties were already categorized into DT and DS varieties based on their drought response index values (Jeyaramraja and Thushara 2011).

Variety	Origin	Drought response
VRI-2	Vridhachalam	DT
VRI-3	Vridhachalam	DS
VRI-4	Vridhachalam	DS
VRI-5	Vridhachalam	DT
VRI-6	Vridhachalam	DT
TMV-13	Vridhachalam	DS
ALR-1	Aliyar	DT
ALR-3	Aliyar	DS
CO-2	Aliyar	DS

Plant water status: RWC was determined gravimetrically using samples of 10 leaf discs of 0.5 cm diameter according to Clavel *et al.* (2006) from the third leaf from the top of the main shoot using the equation: RWC = [(FM – DM)/(TM – DM)] × 100, where FM was fresh mass of the leaf, TM was turgid mass after 4-h rehydration of the leaf in distilled water, under dark conditions, at room temperature, and DM was the dry mass after drying at 85°C for 24 h.

WS imposition: WS treatment was conducted according to Jain *et al.* (2001) with slight modifications. The experiment was designed to determine the physiological responses of DT and DS types of groundnut to drought stress. All varieties were grown in pots filled with sandy-clay-loam soil with pH 8.7. The seeds were treated with with a fungicide, Mancozeb, at 4 g kg^{-1}(seed) just before sowing. This was done to protect the young seedlings from root-rot and collar-rot infection. The seeds were also treated with 600 g ha^{-1} of rhizobial culture, TNAU 14 (*Tamilnadu Agriculture University*, Vridhachalam, India; 600 g ha^{-1}), using rice congee as a binder.

There was one plant in each pot and there were 18 pots for each variety (9 for control (C) and 9 for WS treatment). Therefore, there were 162 pots in total (81 for C and 81 for WS treatment). Plants were grown in a greenhouse under natural irradiance (PPFD up to 1,500 μmol m^{-2} s^{-1}), daily temperatures of 18–32°C, and relative humidity between 75 (morning) and 45% (late afternoon).

The plants were irrigated by saturating the soil every other day. Soil-drying techniques are generally regarded as the most practical means of approximating field drought conditions for laboratory-based research (Woo *et al.* 2008). Hence, in the present study, 30-day-old plants were drought-stressed for 2 weeks by withholding the irrigation. For C, the plants were irrigated until 45th d. For each variety receiving the irrigation or WS treatment, all measurements were taken randomly from 3 plants.

Physiological studies: Fully developed, expanded leaves (5th–9th leaves from the shoot tip) were used for physiological measurements. P_N [μmol(CO_2) m^{-2} s^{-1}] and g_s [mol(H_2O) m^{-2} s^{-1}] were monitored using infrared gas analyzer (*ADC*, UK) with leaf chamber and porometer, respectively. Since high light intensity and temperature reduced P_N, measurements were made between 08:30 and 11:30 h, when the greenhouse temperature ranged from 25 to 28°C and photosynthetically active radiation ranged from 900 to 1,000 μmol m^{-2} s^{-1}. During P_N measurement, the leaf chamber had flow rate of 360 μl(CO_2) l^{-1}(air), 60% humidity, and ~1199 Pa vapour-pressure deficit. Each leaf was equilibrated for 15 to 20 s before a single P_N or g_s record, to obtain a constant value of P_N or g_s. WUE is the capacity of a plant economizing the use of moisture for the production of dry matter (Handique 1992). It was calculated as the ratio between P_N and g_s.

Pigment estimation: Chl in the second, fully expanded leaf from the top was analyzed spectrophotometrically at 645 nm and 663 nm following the method of Sadasivam and Manickam (1996). Acetone was used as solvent.

Plant growth parameters: Root dry mass (RDM), aboveground dry mass (ADM), and total dry mass (TDM) were estimated according to Clavel *et al.* (2005). Plants were carefully removed from the pots and the roots were washed with water. Thereafter, RDM and ADM were measured after 48-h drying at 80°C. TDM is the sum of ADM and RDM.

Leaf area was measured using allometric model given by Kathirvelan and Kalaiselvan (2007) and LAI was computed as the ratio of leaf area to ground area (the area within the pot). RLA (Clavel *et al.* 2005) was estimated by the following formula: RLA = ADM/SLM, where SLM [g cm^{-2}] was the specific leaf mass of the third leaf of each plant and ADM [g $plant^{-1}$]. SLM was estimated as the ratio of leaf dry mass per unit leaf area.

Statistical analysis: Two-way analysis of variance (*ANOVA*) was performed and critical difference (CD) values were calculated at 0.05 level to find out whether statistically significant difference existed within types, varieties, and treatments. The regression analysis tool of *Microsoft® Office Excel® 2007* was used to perform linear regression analysis by using the "least squares" method to study the relationships among different parameters analyzed in this study.

Results

Leaf RWC decreased significantly in all the pot-grown groundnut varieties under WS conditions. In the present study and relatively to leaf RWC, there was no significant difference between DT and DS types both under C and WS conditions (Table 1).

Gas-exchange parameters and pigments: P_N and g_s decreased significantly under WS condition both in DT and DS varieties (Table 1). Nevertheless, DT varieties were found to maintain significantly higher P_N and WUE than the DS types both under C and WS conditions. No significant difference between DT and DS types could be found in Chl content (Table 1). Although the DT varieties had significantly higher g_s than DS varieties under C conditions, WS led to statistically insignificant difference in g_s between DT and DS varieties.

Growth parameters: No significant difference in LAI was found between DT and DS types and LAI was not reduced by WS (Table 2). Significant differences were observed among the groundnut varieties, but not between DT and DS types, in RDM, ADM, and TDM, all of them significantly decreased under WS conditions (Table 2). ADM and RLA correlated positively (Table 3) with a correlation coefficient of 0.976 ($P<0.001$).

Discussion

In this work, RWC was used to study the water content of the leaves, because this parameter is considered to be a more useful integrator of plant water balance than the leaf water potential (Sinclair and Ludlow 1985, Wright and Nageswara Rao 1994). The reduction of RWC in plants under WS might be associated with the decrease in plant vigour as it was observed in many plant species (Lopez *et al.* 2002, Halder and Burrage 2003). While screening groundnut cultivars for drought tolerance, Boote (1983) pointed out a close relationship between RWC of leaves and transpiration.

P_N of groundnut varieties observed in the present study were in the same range as those observed by Pallas *et al.* (1974). In groundnut, relatively high g_s values were obtained by us similar to the findings of Pallas (1980), which was likely the result of high P_N observed in this crop (Bennet *et al.* 1993). Light and temperature did not vary much during P_N measurements inside the

Table 1. Influence of water stress (WS) on physiological and pigment parameters; relative water content (RWC), net photosynthetic rate ($P_{N,}$ [μmol(CO_2) m^{-2} s^{-1}]), stomatal conductance to water vapour ($g_{s,}$ [mol(H_2O) m^{-2} s^{-1}]), water-use efficiency (WUE), and chlorophyll content (Chl, [mg g^{-1}(FM)]) in selected groundnut varieties. Values are the means of three separate measurements from the leaves of 3 plants in the case of RWC and Chl, while for P_N, g_s, and WUE, the values are means of three separate measurements from the leaves of 3 plants and in each plant, the measurement was carried out 3 times. Each value is followed by ± standard deviation. C – control; CD – critical difference; FM – fresh mass.

Varieties	RWC [%]		P_N		g_s		WUE		Chl	
	C	WS	C	WS	C	WS	C	WS	C	WS
Drought-tolerant										
VRI-5	87 ± 1.58	80 ± 1.53	15.5 ± 0.29	14.3 ± 0.42	1.53 ± 0.03	1.44 ± 0.01	10.13 ± 0.08	9.93 ± 0.24	1.23 ± 0.02	0.93 ± 0.02
VRI-2	89 ± 1.53	81 ± 1.15	15.4 ± 0.15	12.5 ± 0.06	1.55 ± 0.08	1.43 ± 0.02	9.94 ± 0.17	8.74 ± 0.10	1.20 ± 0.04	0.92 ± 0.05
VRI-6	90 ± 1.55	79 ± 2.65	14.9 ± 0.38	12.5 ± 0.31	1.48 ± 0.03	1.42 ± 0.04	10.07 ± 0.37	8.80 ± 0.33	1.27 ± 0.03	0.84 ± 0.03
ALR-1	86 ± 2.08	78 ± 1.55	15.1 ± 0.40	12.8 ± 0.15	1.52 ± 0.02	1.45 ± 0.03	9.93 ± 0.30	8.83 ± 0.26	1.23 ± 0.02	0.89 ± 0.05
Mean	88	80	15.2	13.0	1.52	1.44	10.02	9.08	1.23	0.90
Drought-susceptible										
VRI-3	86 ± 3.21	81 ± 1.00	14.1 ± 0.30	11.1 ± 0.26	1.45 ± 0.01	1.42 ± 0.01	9.72 ± 0.35	7.82 ± 0.18	1.24 ± 0.02	0.96 ± 0.04
VRI-4	84 ± 1.00	80 ± 1.53	14.5 ± 0.25	11.3 ± 0.25	1.49 ± 0.02	1.45 ± 0.03	9.73 ± 0.26	7.79 ± 0.26	1.29 ± 0.05	0.87 ± 0.02
TMV-13	87 ± 2.04	79 ± 1.63	13.8 ± 0.31	11.7 ± 0.40	1.49 ± 0.03	1.44 ± 0.04	9.26 ± 0.30	8.13 ± 0.29	1.30 ± 0.06	0.82 ± 0.03
ALR-3	86 ± 2.00	80 ± 1.51	13.5 ± 0.15	11.3 ± 0.21	1.37 ± 0.04	1.29 ± 0.02	9.85 ± 0.07	8.76 ± 0.12	1.19 ± 0.03	0.86 ± 0.02
CO-2	89 ± 1.53	77 ± 1.59	14.8 ± 0.51	12.5 ± 0.25	1.51 ± 0.02	1.48 ± 0.04	9.80 ± 0.30	8.45 ± 0.15	1.21 ± 0.05	0.90 ± 0.03
Mean	86	79	14.1	11.6	1.46	1.42	9.67	8.19	1.25	0.88
C.D. (5%)										
Between varieties	2.93		0.43		0.07		0.13		0.06	
Between types	7.94		0.63		0.05		0.31		0.17	
Between treatments	5.07		1.20		0.03		0.85		0.10	
Interaction (type × treatment)	6.48		0.95		0.05		0.51		0.13	

Table 2. Growth parameters in selected groundnut varieties under irrigated (C) and water-stress (WS) conditions; leaf area index (LAI), root dry mass (RDM, [g plant^{-1}]), aboveground dry mass (ADM, [g plant^{-1}]), total dry mass (TDM, [g plant^{-1}]), and relative leaf area (RLA, [cm^2]). Values are the means of three separate measurements. Each value is followed by ± standard deviation.

Varieties	LAI		RDM		ADM		TDM		RLA	
	C	WS	C	WS	C	WS	C	WS	C	WS
Drought-tolerant										
VRI-5	1.30 ± 0.10	0.97 ± 0.12	1.182 ± 0.04	0.683 ± 0.01	1.251 ± 0.01	0.546 ± 0.02	2.434 ± 0.06	1.229 ± 0.01	268.81 ± 12.20	118.52 ± 0.85
VRI-2	1.37 ± 0.15	1.23 ± 0.15	1.268 ± 0.06	0.693 ± 0.03	1.359 ± 0.05	0.553 ± 0.01	2.627 ± 0.10	1.245 ± 0.02	284.74 ± 10.50	119.94 ± 1.12
VRI-6	1.47 ± 0.21	1.37 ± 0.13	0.963 ± 0.01	0.667 ± 0.01	1.105 ± 0.02	0.522 ± 0.04	2.068 ± 0.02	1.189 ± 0.02	232.44 ± 03.85	113.77 ± 0.82
ALR-1	1.50 ± 0.10	1.40 ± 0.10	1.523 ± 0.07	0.718 ± 0.02	1.692 ± 0.03	0.581 ± 0.02	3.216 ± 0.04	1.299 ± 0.03	302.52 ± 22.52	107.66 ± 7.00
Mean	1.41	1.24	1.234	0.690	1.352	0.550	2.586	1.240	272.13	114.97
Drought-susceptible										
VRI-3	1.33 ± 0.15	1.40 ± 0.10	0.876 ± 0.04	0.645 ± 0.04	0.984 ± 0.01	0.503 ± 0.03	1.860 ± 0.04	1.148 ± 0.01	234.57 ± 35.91	119.13 ± 06.93
VRI-4	1.40 ± 0.10	1.37 ± 0.12	0.961 ± 0.02	0.657 ± 0.02	1.090 ± 0.05	0.518 ± 0.02	2.051 ± 0.05	1.175 ± 0.01	229.38 ± 09.60	114.38 ± 01.89
TMV-13	1.47 ± 0.15	1.43 ± 0.06	1.121 ± 0.01	0.675 ± 0.01	1.176 ± 0.05	0.532 ± 0.01	2.298 ± 0.06	1.207 ± 0.02	263.55 ± 35.66	122.91 ± 11.91
ALR-3	1.67 ± 0.14	1.60 ± 0.09	1.365 ± 0.16	0.709 ± 0.01	1.530 ± 0.03	0.567 ± 0.04	2.894 ± 0.14	1.276 ± 0.03	308.63 ± 09.47	119.23 ± 13.47
CO-2	1.33 ± 0.18	1.47 ± 0.06	0.874 ± 0.02	0.642 ± 0.05	0.987 ± 0.01	0.497 ± 0.01	1.861 ± 0.03	1.139 ± 0.01	244.12 ± 61.62	114.15 ± 07.30
Mean	1.44	1.45	1.039	0.666	1.154	0.523	2.193	1.189	256.05	117.96
C.D. (5%)										
Between varieties	0.21		0.076		0.041		0.082		34.47	
Between types	1.14		1.081		1.089		2.171		121.1	
Between treatments	0.36		0.131		0.071		0.141		59.71	
Interaction (type × treatment)	0.73		0.652		0.724		1.252		85.63	

Table 3. Linear regression analysis by using the "least squares" method giving certain relationships among net photosynthetic rate (P_N, [µmol(CO_2) m^{-2} s^{-1}]), chlorophyll content (Chl, [mg g^{-1}(FM)]), total dry mass (TDM, [g $plant^{-1}$]), Aboveground dry mass (ADM, [g $plant^{-1}$]), and relative leaf area (RLA, [cm^2]) in different varieties of groundnut. The relationships are given by the formula y = a + b x. $^*P<0.001$. y – dependent variable; x – independent variable; R – correlation coefficient; a, b – constants.

y	x	R	R^2	a	b
Chl	P_N	0.812^*	0.660	–0.29	0.1
TDM	P_N	0.747^*	0.557	–2.756	0.338
RLA	ADM	0.976^*	0.953	20.38	190.7

greenhouse and hence their effect on P_N was considered negligible based on the light- and temperature-response curves available in literature for groundnut (Pallas *et al.* 1974, Pallas 1980).

The decrease of P_N and g_s due to WS, found in this work, could be related to the observed decrease in RWC. P_N and g_s correlated with RWC in significantly positive relationships at 0.001 level (r = 0.782) and at 0.05 level (r = 0.502), respectively. Reduction in P_N and g_s due to WS was reported by Lauriano *et al.* (2004) in groundnut. Indeed, as referred by Ritchie *et al.* (1990), metabolic changes in response to WS include a reduction in photosynthetic activity. Flexas *et al.* (1999) reported that when WS became stronger, it induced a drastic downregulation of photosynthesis. On the other hand, stomatal control of water loss has been recognized as an early response for conditioning the leaf water status of plants in the field, but it severely limits carbon uptake and biomass production (Chaves 1991) as it was shown in the present work by the decrease in RDM, ADM, and TDM.

Although no significant difference was observed between DT and DS varieties with regard to RWC in the present study, DT types were found to maintain significantly higher P_N than the DS varieties both under C and WS conditions. The hypothesis that DT plants at the cellular level are often able to keep their stomata open under severe WS was established in a study involving four cultivars of another leguminous plant, *i.e. Phaseolus vulgaris* (Costa França *et al.* 2000). RWC data of Clavel *et al.* (2005) showed that DS cultivar had always higher water contents than the other cultivars, particularly until 35 days of applied WS on 2-week-old groundnut seedlings. This indicated that ability to conserve leaf water during WS is not a mechanism of drought tolerance in groundnut. However, drought tolerance means the ability of a plant to utilize the water effectively. This hypothesis was supported by significantly higher values of WUE observed in this study in DT types under WS conditions. The decrease in g_s would allow the DT varieties to use less water and to become more efficient in their water usage (Hetherington and Woodward 2003). An increase in WUE results in the reduction of transpiration (Vu 2005). If transpiration rate decreases more than P_N, an increase on WUE can occur. It was clarified by Krishnamurthy *et al.* (2007); they suggested that improving WUE would be the best strategy to cope with episodes of intermittent drought in groundnut, which is usually grown in rain fed conditions.

Based on the physiological data obtained, the reduction in P_N due to WS could be attributed both to reduction in g_s (stomatal limitation) and to reduction in Chl. Stomatal limitation of photosynthesis during WS has been reported by Ludlow and Muchow (1990), which is in contrary to the findings of Kicheva *et al.* (1994), who reported that decrease in CO_2 assimilation rate could occur because of decrease in Chl under severe drought. Moreshat *et al.* (1996) has reported that Chl increases in groundnut due to mild drought stress.

Comparing P_N with LAI indicated that reduction in leaf area did not contribute to drought susceptibility in DS types of groundnut during WS. Instead of reducing the leaf area, the DS types might alter the leaf angle during dehydration to diminish total intercepted radiation and therefore carbon assimilation by a plant (Pinheiro and Chaves 2011). Our data suggested that it was the ability of the DT types to efficiently utilize the available leaf RWC and leaf area to maintain significantly higher P_N during WS, which conferred them the drought tolerance.

The reduction in P_N should have contributed to reductions in RDM, ADM, and TDM. Long-term consequence of WS seems to be a reduction of growth (Lauriano *et al.* 2004), which is usually measured in terms of biomass. The question, how ADM could decrease without any reduction in LAI, was answered by the values of RLA, which were influenced by leaf dry masses. RLA also followed the same trend of ADM and hence, our data proved that leaf dry mass decreased without any effect on the leaf area during WS, thus contributing to low ADM with high LAI. SLM, which expresses leaf thickness (Jun and Imai 1999), decreased due to WS in most of the groundnut varieties studied (data not shown). An increase in the root/shoot ratio (RDM/ADM) due to WS observed in the present study (data not shown) was attributed to the reason that decline observed in leaf net carbon uptake was followed by an alteration in partitioning of the photoassimilates at the whole plant level as referred by Pinheiro and Chaves (2011). This is the result of the decline in shoot growth and the maintenance of root growth under decreasing water in the soil (Sharp 2002). The changes in the root/shoot ratio as well as the temporary accumulation of reserves in the stem occur in several species under WS (Blum *et al.* 1994, Chaves *et al.* 2002).

Although we hypothesized that ability to conserve water during WS was not a mechanism of the drought tolerance in groundnut, it was found that leaf RWC had positive relationships with P_N (r = 0.782), Chl (r = 0.908), TDM (r = 0.788), and RLA (r = 0.891), which were significant at $P<0.001$. Hence, we put forward that leaf RWC is an index of various physiological parameters,

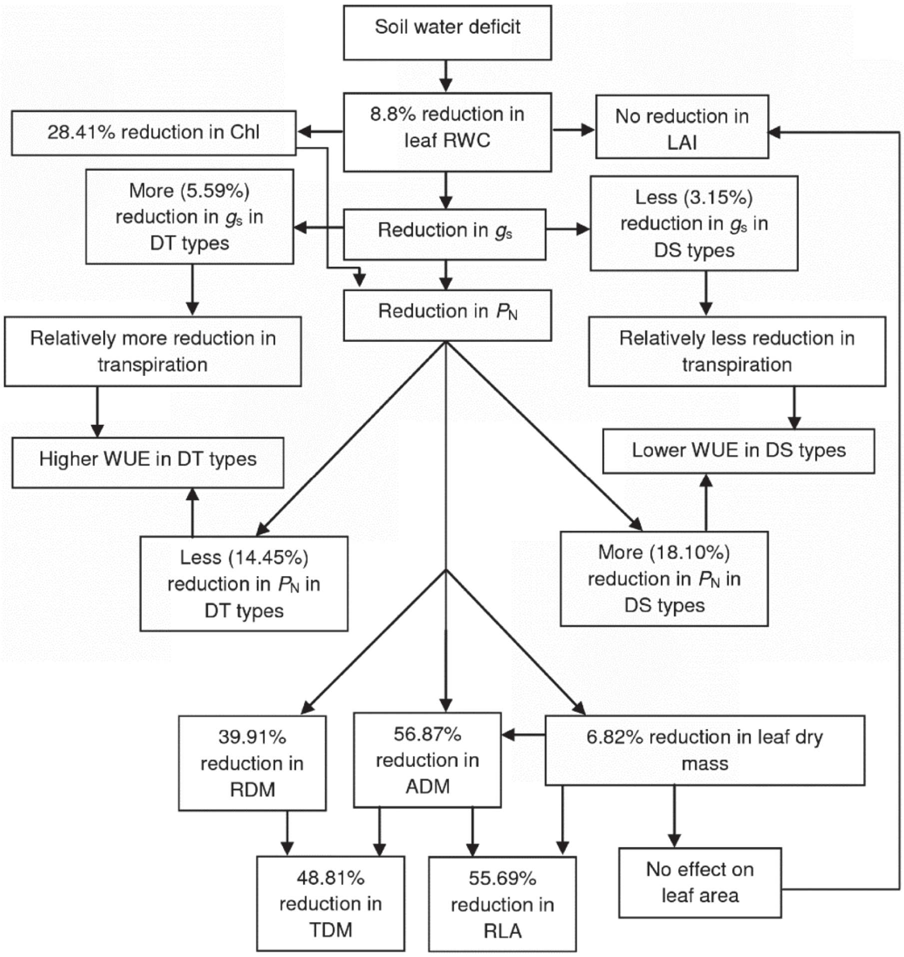

Fig. 1. Schematic representation of suggested sequence of physiological responses in groundnut owing to soil water deficit. Physiological responses are expressed in terms of leaf relative water content (RWC), stomatal conductance to water vapour (g_s), net photosynthetic rate (P_N), chlorophyll content (Chl), water-use efficiency (WUE), leaf area index (LAI), root dry mass (RDM), aboveground dry mass (ADM), total dry mass (TDM), and relative leaf area (RLA).

pigment concentration, and dry mass accumulation, but not that of the drought tolerance. Based on the observation and analysis of physiological data, we suggested a sequence of physiological responses in groundnut crop subjected to WS (Fig. 1). WS for 2 weeks in 30-day-old groundnut plants resulted in 8.8% reduction in leaf RWC compared with C plants. Consequently, the plants closed the stomata in order to reduce the transpiratory loss of water. Thus, it was a reduction in g_s, which was more pronounced in DT types (5.6%) than in DS types (3.2%). As a result, CO_2 diffusion into the leaf was also reduced. Hence, there was a reduction in P_N. Chl reduction (28.4%) due to WS also contributed to reduction in P_N. However, the reduction in P_N was found to be lower (14.5%) in DT types and higher (18.1%) in DS types. Lesser reduction of P_N coupled with higher reduction of g_s resulted in high WUE values in DT types and *vice versa*. Reduction in P_N caused reductions in growth parameters, such as RDM and ADM, and therefore, reductions in TDM and RLA. No significant reduction in LAI due to WS was attributed to the reduction (6.8%) in leaf dry mass without any effect on leaf area.

Groundnut suffers usually from terminal drought stress and thus, the yield decreases to a large extent. The varieties that yield well during drought are therefore considered to be DT and *vice versa*. In a previous study (Jeyaramraja and Thushara 2011), such a categorization of recently used groundnut varieties into DT and DS varieties was done based on their yield during drought and non-drought seasons. Comparison of yield data of our previous study with the physiological data of the present investigation showed some interesting relationships. The pod yield showed significant positive relationships with leaf RWC ($r = 0.523$), WUE ($r = 0.550$), and Chl ($r = 0.492$), and it also showed highly significant positive relationships with P_N (r = 0.652) and g_s (r = 0.601). However, no significant relationships could be noticed between the yield and any of the physiological parameters using the data obtained only during non-drought conditions. This is due to the fact that DS varieties yielded more than the DT types during non-drought conditions (Jeyaramraja and Thushara 2011), which did not coincide with the higher values of P_N, g_s, and WUE in DT types compared with DS types. On the other hand, a significant positive relationship between the yield and P_N was observed (r = 0.754) using the data obtained only during drought conditions. This was because of higher yield in DT varieties compared to that of the DS types during drought (Jeyaramraja and Thushara 2011) that matched with the higher values of P_N in DT types than DS types. Hence, being the DT variety is useful, because groundnut is usually grown under drought conditions and usually faces terminal drought stress. Although the DS varieties give higher yield than DT types during nondrought conditions, the DS varieties could be useless to the farmers, because most of the groundnut is cultivated as a rainfed crop, which faces drought under field conditions. There are a very few places, where this crop is cultivated as an irrigated crop.

Numerous physiological traits have been shown to potentially contribute to the yield under stress, but the development of an efficient breeding method for drought resistance in groundnut is still a long-standing objective (Wright *et al.* 2002). For example in the present study, P_N was found to have positive relationships with Chl and TDM, which were significant at $P<0.001$ (Table 3). However, P_N alone could not be used as a marker for biomass/economic productivity. A simple and direct link between a particular trait and the maintenance of the yield under drought has never been proven (Clavel *et al.* 2005). However, further studies on identifying the fine regulation of biochemical metabolism that control the physiological traits are necessary before unraveling the drought tolerance mechanism at the molecular level.

References

Bennet, J.M., Sinclair, T.R., Ma, L., Boote, K.J.: Single leaf carbon exchange and canopy radiation use efficiency of four peanut cvs. – Peanut Sci. **20**: 1-5, 1993.

Blum, A.: Plant Breeding for Stress Environments. – CRC Press, Boca Raton 1988.

Blum, A., Sinmena, B., Mayer J., *et al.*: Stem reserve mobilization supports wheat-grain filling under heat stress. – Aust. J. Plant Physiol. **21**: 771-781, 1994.

Boote, K.J.: Peanut. – In: Teare, I.D., Peet, M.M. (ed.): Crop-Water Relations. Pp. 255-286. John Wiley & Sons, New York 1983.

Chaves, M.M.: Effects of water deficits on C assimilation. – J. Exp. Bot. **42**: 1-16, 1991.

Chaves, M.M., Flexas, J., Pinheiro, C.: Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. – Ann. Bot. **103**: 551-560, 2009.

Chaves, M.M., Pereira, J.S., Maroco, J.P. *et al.*: How plants cope with water stress in the field: photosynthesis and growth. – Ann. Bot. **89**: 907-916, 2002.

Clavel, D., Drame, N.K., Roy-Macauley, H. *et al.*: Analysis of early responses to drought associated with field drought adaptation in four Sahelian groundnut (*Arachis hypogaea* L.) cultivars. – Environ. Exp. Bot. **54**: 219-230, 2005.

Clavel, D., Diouf, O., Khalfaoui, J.L., Braconnier, S.: Genotypes variations in fluorescence parameters among closely related groundnut (*Arachis hypogaea* L.) lines and their potential for drought screening programs. – Field Crops Res. **96**: 296-306, 2006.

Clifford, S.C., Stronach, I.M., Black, C.R. *et al.*: Effects of elevated CO2, drought and temperature on the water relations and gas exchange of groundnut (*Arachis hypogaea*) stands grown in controlled environment glasshouses. – Physiol. Plant. **110**: 78-88, 2000.

Dubey, R.S.: Photosynthesis in plants under stressful conditions. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 859-875. Marcel Dekker, New York 1997.

Flexas, J., Escalona, J.M., Medrano, H.: Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. – Plant Cell Environ. **22**: 38-48, 1999.

França, M.G.C., Thi, A.T.P., Pimentel, C. *et al.*: Differences in growth and water relation among *Phaseolus vulgaris* cultivars in response to induced drought stress. – Environ. Exp. Bot. **43**: 227-337, 2000.

Halder, K.P., Burrage, S.W.: Drought stress effects on water relations of rice grown in nutrient film technique. – Pakistan J. Biol. Sci. **6**: 441-444, 2003.

Handique, A.C. : Some salient features in the study of drought resistance in tea. – Two Bud **39**: 16-18, 1992.

Hetherington, A.M., Woodward, F.I.: The role of stomata in sensing and driving environmental change. – Nature **424**: 901-908, 2003.

Jain, A.K., Basha, S.M., Holbrook, C.C.: Identification of drought-responsive transcripts in peanut (*Arachis hypogaea* L.). – Electron. J. Biotechnol. **4**: 59-67, 2001.

Jeyaramraja, P.R., Thushara, S.S.: Biometric parameters in certain peanut (*Arachis hypogaea* L.) varieties varying in drought tolerance. – Asian Austr. J. Plant Sci. Biotech. **5**: 22-26, 2011.

Jia, Y., Gray V.M.: Interrelationships between nitrogen supply and photosynthetic parameters in *Vicia faba* L. – Photosynthetica **41**: 605-610, 2004.

Jun, H., Imai, K.: Cultivar differences of photosynthesis and respiration in peanut leaves. – Bull. Fac. Agr., Meiji Univ. **119**: 21-25, 1999.

Kanechi, M., Kunitomo, E., Inagaki, N., Maekawa, S.: Water stress effects on ribulose-1,5,-biphosphate carboxylase and its relationship to photosynthesis in sunflower leaves. – In: Mathis, M. (ed.): Photosynthesis: from Light to Biosphere. Vol. IV. Pp. 597-600. Kluwer Academic Publisher, Dordrecht-London 1995.

Kathirvelan, P., Kalaiselvan, P.: Groundnut (*Arachis hypogaea* L.) leaf area estimation using allometric model. – Res. J. Agr. Biol. Sci. **3**: 59-61, 2007.

Kicheva, M.L., Tsonev, T.D., Popova, L.P.: Stomatal and nonstomatal limitation of photosynthesis in two wheat cultivars subjected to water stress. – Photosynthetica **30**: 107-116, 1994.

Krishnamurthy, L., Vadez, V., Devi, M.J. *et al.*: Variation in transpiration efficiency and its related traits in a groundnut (*Arachis hypogaea* L.) mapping population. – Field Crops Res.: doi:10.1016/j.fcr.2007.06.009, 2007.

Lauer, K.J., Boyer, J.S.: Internal CO_2 measures directly in leave: absicisic acid and low leaf water potential cause opposing effects. – Plant Physiol. **98**: 1010-1016, 1992.

Lauriano, J.A., Ramalho, J.C., Lidon, F.C., Matos, M.C.: Peanut photosynthesis under drought and re-watering. – Photosynthetica **42**: 37-41, 2004.

Lawlor, D.W., Cornic, G.: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. – Plant Cell Environ. **25**: 275-294, 2002.

Leport, L., Turner, N.C., French, R.J. *et al*.: Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. – Eur. J. Agron. **11**: 279-291, 1999.

Lopez, C.M.L., Takahashi, H., Yamazaki, S.: Plant water relations of Kidney bean plants treated with NaCl and foliarly applied glycine betaine. – J. Agron. Crop. Sci. **188**: 73-80, 2002.

Ludlow, M.M., Muchow, R.: A critical evaluation of traits for improving crop yields in water limited environments. – Adv. Agron. **43**: 107-153, 1990.

Moreshat, S., Bridges, D.C., NeSmith, D.S., Huang, B.: Effects of water stress on competitive interaction of peanut and sicklepod. – Agron. J. **88**: 636-644, 1996.

Ndunguru, B.J., Ntare, B.R., Williams, T.H., Greenberg, D.C.: Assessment of groundnut cultivars for end-of-season drought tolerance in Sahelian environment. – J. Agr. Res. **125**: 79-85, 1995.

Pallas, J.E.: An apparent anomaly in peanut leaf conductance. – Plant Physiol. **65**: 848-851, 1980.

Pallas, J.E., Samish, Y.B., Willmer, C.M.: Endogenous rhythmic activity of photosynthesis, transpiration, dark respiration, and carbon dioxide compensation point of peanut leaves. – Plant Physiol. **53**: 907-911, 1974.

Passioura, J.B.: Environmental biology and crop improvement. – Funct. Plant Biol. **29**: 537-546, 2002.

Pinheiro, C., Chaves, M.M.: Photosynthesis and drought: can we make metabolic connections from available data? – J. Exp. Bot. **62**: 869-882, 2011.

Reddy, T.Y., Reddy, V.R., Anbumozhi, V.: Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: a critical review. – Plant Growth Regul. **41**: 75-88, 2003.

Ritchie, S.W., Nguyen, H.T., Holaday, A.S.: Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. – Crop Sci. **30**: 105-111, 1990.

Rolland, F., Baena-Gonzalez, E., Sheen, J.: Sugar sensing and signaling in plants: conserved and novel mechanisms. – Annu. Rev. Plant Biol. **57**: 675-709, 2006.

Sadasivam, S., Manickam, A.: Biochemical Methods. 2nd Ed. – New Age Int. Limited Publishers, Tamil Nadu Agricultural University, Coimbatore 1996.

Sharp, R.E.: Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. – Plant Cell Environ. **25**: 211-222, 2002.

Shinozaki, K., Yamaguchi-Shinozaki, K.: Molecular responses to drought and cold stress. – Curr. Opin. Biotech. **7**: 161-167, 1996.

Shinozaki, K., Yamaguchi-Shinozaki, K.: Gene networks involved in drought stress response and tolerance. – J. Exp. Bot. **58**: 221-227, 2007.

Sinclair, T.R., Ludlow, M.M.: Who taught plants thermodynamics? The unfulfilled potential of plant water potential. – Aust. J. Plant Physiol. **12**: 213-217, 1985.

Smith, C.W.: Crop Production: Evolution, History and Technology. – John Wiley & Sons, New York 1995.

Somerville, C., Briscoe, J.: Genetic engineering and water. – Science **292**: 2217, 2001.

Tuberosa, R., Salvi, S.: Genomics-based approaches to improve drought tolerance of crops. – Trends Plant Sci. **11**: 405-412,2006

Vu, J.C.V.: Acclimation of peanut (*Arachis hypogea* L.) leaf photosynthesis to elevated growth CO2 and temperature. – Environ. Exp. Bot. **53**: 85-95, 2005.

Woo, N.S., Badger, M.R., Pogson, B.J.: A rapid, non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. – Plant Methods **4**: 27-27, 2008.

Wright, G.C., Nageswara Rao, R.C.: Groundnut Water Relations. – In: Smartt, J.T. (ed.): The Groundnut Crop. Pp. 281-335. Springer, Dordrecht 1994.

Wright, G.C., Rachaputi, N.R., Nigam, S.N., Basu, M.S.: More efficient breeding of drought resistant groundnut in India and Australia. – IAN **22**: 20-24, 2002.

Yamaguchi-Shinozaki, K., Urao, T., Shinozaki, K.: Regulation of genes that are induced by drought stress in *Arabidopsis thaliana*. – J. Plant Res. **108**: 127-136, 1995.

Yordanov, I., Velikova, V., Tsonev, T.: Plant Responses to drought, acclimation, and stress tolerance. – Photosynthetica **38**: 171-186, 2000.

Zhang, J.Z., Creelman, R.A., Zhu J-K: From laboratory to field. Using information from *Arabidopsis* to engineer salt, cold, and drought tolerance in crops. – Plant Physiol. **135**: 615-621, 2004.