RESEARCH ARTICLE

Photosynthetic characteristics of peanut genotypes under excess and deficit irrigation during summer

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Abstract In a field experiment three irrigation treatments were given to twelve peanut genotypes through drip. At 80 days after sowing (DAS) the amount of irrigation applied was 20 % higher than the evaporative demand (ET) in T_1, 25 % less than ET in T_2 and 48 % less than ET in T_3 against the cumulative evaporative demand of 412 mm. The relative water content (RWC) of peanut leaves reduced by cutting irrigation from 93.5 % in T_1 to 91.1 % in T_2 and 77.2 % in T_3 but, net photosynthetic rate (P_N) was higher in T_2 (29.6 μmol m^{-2} s^{-1}) than T1 (28.6 μmol m^{-2} s^{-1}) and T3 (24.3 μmol m^{-2} s^{-1}) at 75–80 DAS. Peanut genotype ICGV 91114 showed the highest P_N (30.9 μmol m^{-2} s^{-1}) which was statistically at par with GG 20, ICGV 86590, TAG 24, SB XI, TMV 2 and TPG 41. The non-photochemical quenching (NPQ) varied with different irrigation treatment with lowest in T_2 and highest in T_3. The de-epoxidation state (DeS) was 38 % in T_1 and T_2 but, increased to 47 % in T_3 due to the sever water deficit stress. Applying 20 % higher irrigation than the ET demand (T_1) does not warrant any extra benefits in terms of higher photosynthesis in peanut at 75–80 DAS. Further, a reduction of 25 % of the ET (T_2) in peanut seems to be the ideal condition for photosynthesis and desirable chlorophyll fluorescence parameters at 80 DAS. Girnar 3 and ICGV 91114 showed NPQ value above 2.2 and higher de-epoxidation state, maintained least deviation in Fv/Fm and Fv'/Fm' under severe water deficit condition are promising peanut genotypes.

Introduction

Peanut (*Arachishypogaea* L.) is an important food legume as well as an oilseed crop being grown in 112 countries of the world on about 25 million ha of land. The production of peanut is about 41.1 m ton and is grown mostly in tropics and subtropics of arid and semi-arid regions where the availability of water is a major constraint. Frequent drought of various spells and intensities in these areas results in the productivity of peanut being less than 1000 kg ha^{-1} in more than 35 % of peanut growing countries. About 5.7 million ha in India is under the cultivation of peanut with productivity of about 1300 kg ha^{-1}. Due to suitable environment and photoperiod matching to the growing season, the commercial peanut cultivation is possible mainly in Asia (47 % of the world peanut cultivation area contributing 60 % of the total world production), Africa (47 % area, 27 % production) and America (4.4 % area and 8 % production) (FAO 2012).

Photosynthesis is the most important process influencing crop production and the high P_N is one of the most important breeding strategies for crop improvement (Richards 2000). The simultaneous occurrence of water deficit stress coupled with heat and high irradiance leads to severe photo-oxidative damage to the photosynthetic apparatus which often aggravates the amount of excess excitation energy and this excess excitation energy, when not dissipated harmlessly, would be transformed to O_2 to form reactive oxygen species (ROS) which could damage the photosynthetic apparatus. Plants have developed many protective mechanisms to balance absorbed light energy with photosynthesis; the most important one is the xanthophyll cycle-dependent thermal energy dissipation, measured as the non-photochemical quenching (NPQ) of chlorophyll fluorescence. Chlorophyll *a*

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fluorescence is a very sensitive probe of physiological status of leaves and plant performance in a wide range of situations (Daniele et al. 2006). Recently, mini-core germplasm of peanuts has been evaluated and grouped for variability in various physiological traits including P_N, g_s, E and F_v/F_m in field during dry season (Singh et al. 2014).

For plant improvement, information on photosynthetic performance cannot just be obtained by measurements of gaseous exchange (Dwyer et al. 1992). Therefore, evaluation of physiological state based on the time dependent changes of the chlorophyll *a* fluorescence along with photosynthetic activities is required to be studied under stress environments. Such studies in peanuts are in limited numbers with special focus on heat and light stress. Further, there are no reports on association of de-epoxidation state with the surrogates of WUE (SCMR and SLA) and WUE_{int} in peanuts under water deficit stress. Thus, a study was aimed to unravel the changes in chlorophyll fluorescence parameters, net photosynthetic rate and relative de-epoxidation state and its association with WUE_{int} at 75–80 DAS under limited water supply in 12 peanut genotypes during dry season in open field condition.

Materials and methods

A field experiment taking 12 genotypes of peanut in split plot design giving first two flood irrigations of 60 mm followed by three irrigation treatments *viz.* T_1: well watered in which the amount of irrigation water supplied replenish the cumulative PAN evaporation, T_2- 50 % of T_1 and T_3-25 % of T_1 through drip during dry season (January- June) in year 2013 was conducted at the research farm of ICAR-Directorate of Groundnut Research, Junagadh, Gujarat (lat. 21^0 31′N, Long $70^°36′E$) in the Vertic Ustochrept soil with pH of 8.5 and electrical conductivity of 0.16 dSm^{-1}. A total of 12 rows of peanut were sown in plot of 5×4 m size in three replications with four rows of each genotype in each replication. Before sowing, fertilisers (40 N: 50 P: 50 K) were applied in furrows. A set of 12 peanut genotypes, including three from Spanish bunch (AK 265, GG 20, Girnar 2) and nine from Virginia bunch (Girnar 3, ICGV 86590, ICGV 91114, JL 24, SB XI, SG 99, TAG 24, TMV 2 and TPG 41) were sown during last week of January in year 2013 maintaining a population density of 22 plants m^{-2}. Recommended agronomic and plant protection measures were followed, except for the irrigation treatments. The total quantity of irrigation water calculated from total hours of irrigation multiplied by the discharge rate of the drippers in addition to first two flood irrigations of 60 mm each showed that at 80 DAS, treatments T_1, T_2 and T_3 received 498, 309 and 215 Lm^{-2}, respectively (Table 1). Thus, at 80 DAS, the actual quantity of water given in T_1 was 20 % higher than the evaporative demand (ET), in T_2 it was 25 % less than ET and in T_3 the supplied irrigation quantity was 48 % less than

ET against the cumulative evaporative demand of 412 mm. The observations on RWC were recorded during 75–80 DAS.

To determine soil moisture content (SMC) by gravimetric method, soil samples were drawn from the upper layer (0–15 cm) and lower layer (15–30 cm) soil depths. The RWC was measured by the formula RWC (%)=[(FW−DW)/(TW−DW)]*100, where, FW fresh weight, DW dry weight and TW is turgid weight.

Maximum efficiency of PSII (F_v/F_m) of the dark adapted leaves were recorded after 30 min dark adaptation by leaf clips and actual quantum yield of PSII (Fv'/Fm') and net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were recorded between 08:00 and 10:00 h by LI-COR 6400, portable photosynthesis system (LI-COR Inc. Lincon, Nebraska, USA) with modulated fluorescence measurement as described by Maxwell and Johnson (2000). The third leaf from the main axis was kept in the chamber by ensuring the thermocouple touching it from the underside. Temperature was set at ambient and giving a stable T_{leaf} reading. The artificial light was given at 1650 PAR including 10 % of blue light. The ambient CO_2 was supplied to the chamber at the flow rate of 300 m mol s^{-1} and reading was considered when all 3 factors *viz.* flow, CO_2 and H_2O were stable.

To investigate the relative de-epoxidation state, the xanthophyll cycle pigments were extracted from leaf samples by crushing with liquid nitrogen in chilled mortar and pestle. The sample was homogenised in 1 mL of acetone and collected in a 2 mL Eppendorf tube. One milliliter of acetone was used to clean the mortar and pool with the extract, the volume in the Eppendorf was adjusted to 2 mL. The extracts in the tubes were centrifuge for 5 min at a speed of 5000 rpm and the supernatant was collected. The extract was filtered through a 0.22 μm PTFE filter. The first drop that passed through the filter was discarded to avoid contamination. The HPLC vial was filled and closed with a cap and these extracts were injected in the HPLC. The separation of the xanthophyll pools was carried out as per the modified method of García-Plazaola and Becerril (1999) used for Shimadzu, HPLC system. Detection was performed with a PAD 996 detector in the range 250–700 nm.

Chromatographic conditions included HPLC solvents with the mobile phase consisting of two components: solvent A; acetonitrile: methanol: Tris buffer (0.1 M; pH 8) (84:2:14) and solvent B; methanol: ethyl acetate (68:32) (Polle et al. 2001). The injection volume was 15 μl and the solvent flow rate was 0.8 ml min^{-1} with working pressures below 1000 psi. After pigments separation, identification and quantification was done on the basis of the absorption spectra and the standards with known concentration and peaks were detected and integrated at 445 nm for xanthophylls content. The relative de-epoxidation state was calculated as (A+Z)/(V+A+Z) (%).

All the data recorded were the mean values of at least three independent observations with three repetitions in each replication. The data was subjected to analysis of variance

Table 1 Temperature (°C), relative humidity (RH %), solar radiation (SR W/m2), evaporative demand (ET mm), quantity of irrigation water applied (L m²) and soil moisture content (%) at different soil depth during peanut crop growth period

| | Weather parameters | | | | | | Irrigation quantity applied | | | Soil moisture content (%) | | | | | |
| | Temperature | | | RH (%) | SR | ET | T_1 | T_2 | T_3 | T_1 | | T_2 | | T_3 | |
	T. Max.	T. Min.	T. Mean		(W/m2)	(mm)	(L m²)			0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
0–10 DAS	30.9	15.2	23.1	45.3	2346	35.9	120[a]	120[a]	120[a]	17.3	18	12.7	15.6	9.1	10.3
11–20 DAS	34.2	15.5	24.9	39.3	2489	35.1	36	18	9	18.7	19.9	18.5	19.3	18.9	20.1
21–40 DAS	36.8	16.3	26.6	27.9	5649	88.8	90	45	23	16.5	19.3	12.3	15.7	8.7	11.2
41–80 DAS	37.3	21.8	29.6	47.2	12,285	253.3	252	126	63	18.1	18.6	13.2	14.4	7.2	9.8
Mean/total till 80 DAS	34.8	17.2	26.1	39.9	22,769	413.1	498	309	215	17.7	19.0	14.2	16.3	11.0	12.9

[a] indicates flood irrigation

appropriate to the experimental design using DSAASTAT (Onofri 2007) and the least significant differences were calculated to assess the significance of treatment means where the "F" test was found significant at 5 %. Principal component analysis was carried out PAST (ver. 2.17c) statistical software

Results and discussion

Soil moisture status

The soil moisture content (SMC) of the control field at 0–15 cm and 15–30 cm soil depth remained 17.7 % and 21.0 % respectively till 80 days. The corresponding SMC in 0–15 cm soil layer was 14.2 and 11.0 in T_2 and T_3 respectively, whereas in 15–30 cm soil layer it was 16.3 in T_2 and 12.9 in T_3 (Table 1).

Relative Water Content (RWC), Specific leaf area (SLA), soil plant analytical development chlorophyll meter reading (SCMR)

The RWC of peanut leaves decreased with decreasing irrigation quantity. It was 93.5 % in T_1, 91.1 % in T_2 but had drastically decreased to 77.2 % in T_3. Genotypes also differed significantly with the highest RWC in Girnar 2 (92.5 %) and the lowest in Girnar 3 (84 %). The interaction was significant with the highest RWC in AK 265 (96.7 %) in T_1 and the lowest in SG 99 (72 %) in T_3. The SLA had decreased whereas the SCMR had increased due limiting irrigation quantity (Table 2). The mean SLA was 184, 171 and 162 cm² g⁻¹ dw in T_1, T_2 and T_3 respectively. Genotype SG 99 had the lowest SLA and the highest SCMR whereas the highest SLA was in SB XI (Table 2).

Total chlorophyll content, Net photosynthetic rate (P_N) and stomatal conductance (g_s)

The total chlorophyll content increased due to water deficit treatment. It was 6.84 mg g⁻¹ dw in T_1, 7.21 mg g⁻¹ dw in T_2 and 8.35 mg g⁻¹ dw in T_3. Among the genotypes, GG 20 topped for the highest total chlorophyll whereas it was lowest in SG 99 (Table 3). The photosynthetic rate was 28.6 μmol m⁻² s⁻¹ in T_1 and 29.6 μmol m⁻² s⁻¹ in T_2 which decreased to 24.3 μmol m⁻² s⁻¹ in T_3 during 75 to 80 days after sowing (DAS). The peanut genotype ICGV 91114 showed the highest P_N (30.9 μmol m⁻² s⁻¹) which was statistically at par with GG 20, ICGV 86590, TAG 24, SB XI, TMV 2 and TPG 41. The interaction was significant with the highest P_N in ICGV 91114 (34.5 μmol m⁻² s⁻¹) in T_1. The significant decrease in stomatal conductance was due to irrigation treatments (Table 3). The g_s was higher in few genotypes in T_1 and in some of the genotypes in T_2. The g_s decreased in all the

Table 2 Relative water content (*RWC*), specific leaf Area (*SLA*) and SPAD chlorophyll meter reading (*SCMR*) in peanut genotypes grown at 75–80 DAS, grown at various soil moisture regimes

Genotype/Treatment	RWC (%)				SLA (cm^2 g^{-1} dw)				SCMR			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
AK 265	96.7a	92.9a	79.5abc	89.7a	180cd	175ab	188a	181ab	29.5d	38.5b	41.1bc	36.4bcd
GG 20	93.9a	90.9a	72.2d	85.7a	180cd	175ab	162cde	172abcd	37.9a	38.2b	42.6	39.6ab
Girnar 3	90.4a	88.1a	73.6cd	84a	200ab	175ab	158cde	178ab	31.9bcd	32.8de	43b	35.9bcde
Girnar 2	95.1a	92.5a	82.9a	90.1a	178cde	169ab	135f	161cd	30.6cd	38.7b	41.4bc	36.9bc
ICGV 86590	91.2a	91.2a	82.2ab	88.2a	171de	160bc	151def	161cd	31bcd	37bc	44.2ab	37.4bc
ICGV 9114	92.6a	90.6a	78.9abcd	87.4a	203a	172ab	147ef	174abc	29.5d	33.4cde	34.1ef	32.3ef
JL 24	93.3a	89.1a	76.9abcd	86.4a	185bcd	181a	166bcd	177abc	34abc	31.7e	31.4ef	32.4def
SB XI	95.7a	93a	74.2cd	87.6a	200ab	184a	179ab	188a	35ab	36bcd	35.5e	35.5cdef
SG 99	93.2a	90.6a	72.0d	85.3a	163e	162bc	147ef	157d	36.7a	45a	47.1a	42.9a
TAG 24	94.0a	90.1a	75.6bcd	86.6a	183cd	181a	160cde	175abc	31.4bcd	30.9e	32.4ef	31.6f
TMV 2	93.1a	91.3a	75.5bcd	86.6a	182cd	152c	174abc	169bcd	28.6d	32.9de	34.6ef	32ef
TPG 41	92.4a	93.1a	83.2a	89.6a	188abc	163bc	179ab	177abc	36.5a	37.6b	38.9cd	37.7bc
Mean	93.5	91.1	77.2	87.3	184	171	162	172	32.7	36.1	38.9	35.9
LSD $_{0.05}$												
Treatments	7.4				1.9				0.9			
Genotypes	3.3				9.6				2.36			
Interaction T × G	5.6				16.7				4.09			

genotypes except SB XI in T_3. There was a 15 % and 39 % reduction in stomatal conductance is reported in T_2 and T_3 respectively as compared to the T_1. The g_s in T_1 was 0.456 m sec^{-1} which decreased to 0.383 msec^{-1} in T_2 and further to 0.278 msec^{-1} in T_3 at 60 DAS. Genotypes differed significantly for g_s with the highest in GG20 (0.465 msec^{-1}) and the lowest in Girnar 2 (0.286 msec^{-1}).

Intra cellular CO_2 (*Ci*), transpiration rate (E) and intrinsic water use efficiency (WUE$_{intr}$)

The interaction being significant, the highest g_s is reported in GG 20 (0.607 msec^{-1}) under T_1. The intra cellular CO_2 concentration (*Ci*) increased due to irrigation treatment. It was highest at the severe water deficit condition. As per the reported genotypic difference lowest *Ci* was in Girnar 2 (Table 4). The irrigation treatment affected the transpiration rate (E) which reduced by 15 % and 43 % in T_2 and T_3over T_1. The E was highest in genotype GG 20 (10.5 mmol m^{-2} s^{-1}) followed by genotype AK 265 (10.4 mmol m^{-2} s^{-1}). The interaction was significant with the highest E in TMV 2 (14.6 mmol m^{-2} s^{-1}) under T_1 and the lowest in Girnar 2 (5.4 mmol m^{-2} s^{-1}) under T_3. The intrinsic water use efficiency (WUE$_{int}$) had increased due to limited water supply with the highest in T_3. It ranged between 2.6 and 3.5 in among the genotypes and the highest WUE$_{int}$ in T_3 was in genotype SG 99 (Table 4).

Maximum quantum yield of PSII (F_v/F_m), actual efficiency of photosynthesis (F_v'/F_m') and quantum yield of PS II (Φ_{PSII})

The maximum quantum yield of PSII (F_v/F_m) reduced to 0.831 in T_3 from 0.839 in T_1. Among genotypes, highest F_v/F_m was in TMV 2 (0.844) and the lowest in TAG 24 (0.822). The interaction for F_v/F_m was significant with the highest value in TMV 2 (0.847) in T_1 and the lowest in TAG 24 (0.799) in T_3. Interestingly, the actual efficiency of photosynthesis (F_v'/F_m') was significantly highest in T_2. Among the genotypes, the highest F_v'/F_m' was in ICGV 86590, which was statistically at par with JL 24, SG 99 and TAG 24. The quantum yield of PSII or the proportion of absorbed energy utilised for photochemistry (Φ_{PSII}) was not significantly affected due to irrigation quantity. Genotypic difference were observed for proportion of absorbed energy utilised for photochemistry and the highest Φ_{PSII} was in GG 20 (0.283) and the lowest in JL 24 (0.191). The interaction was significant for Φ_{PSII} with the highest in GG 20 (0.320) in T_1 and the lowest in JL 24 (0.181) in T_3 (Table 5).

Non-photochemical quenching (*NPQ*) and relative de-epoxidation state (*De*S)

The *NPQ* increased due to water deficit stress and was lowest in T_2 and the highest in T_3 (Table 6). Among the genotypes, the capacity to disseminate the extra absorbed energy in terms

Table 3 Total chlorophyll content (mg g^{-1} dw), net photosynthetic rate (P_N -μ mol m^{-2} s^{-1}) and stomatal conductance (g_S m sec^{-1}) in peanut genotypes at 75–80 DAS grown at various soil moisture regimes

Genotype/treatment	Total chlorophyll content (mg g^{-1} dw)				P_N (μ mol m^{-2} s^{-1})				g_S (m sec^{-1})			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
AK 265	5.68g	6.31e	9.37ab	7.12def	26.1bcd	27.9bc	26.1ab	26.7bc	0.478bcd	0.335cde	0.286abc	0.366abcd
GG 20	6.48ef	9.25c	8.91b	8.21a	31.2abc	32.6a	23.5bc	29.1ab	0.607a	0.292de	0.276abc	0.465a
Girnar 3	7.79ab	6.52e	7.28de	7.19cdef	29abcd	25.9c	23bcd	26bc	0.412cd	0.234e	0.253bc	0.319bcd
Girnar 2	8.03a	5.1f	9.21ab	7.45bcde	31.4ab	26.1c	19.4d	25.6bc	0.437cd	0.336cde	0.188c	0.286d
ICGV 86590	7.26bcd	5.92e	9.69a	7.63abcd	29.6abcd	30.4ab	25.5abc	28.5abc	0.515abc	0.449abc	0.306abc	0.386abcd
ICGV 9114	6.5ef	7.94d	8.99b	7.81abc	34.5a	34.1a	24.1bc	30.9a	0.574ab	0.338cde	0.266bc	0.43ab
JL 24	4.93h	10.23b	9.42ab	8.19a	24.3cd	27.3bc	22.0cd	24.5c	0.435cd	0.341cde	0.275abc	0.349abcd
SB XI	6.2fg	11.56a	6.84e	8.2a	28.8abcd	26.0c	29.0a	27.9abc	0.471bcd	0.437abc	0.39a	0.4abcd
SG 99	7.59abc	4.32g	7.86cd	6.59fg	23.2d	32.4a	23.4bcd	26.3bc	0.244e	0.369bcd	0.238bc	0.306cd
TAG 24	6.8def	6.07e	8.07c	6.98efg	30.2abcd	31.3ab	25abc	28.8ab	0.432cd	0.479ab	0.286abc	0.362abcd
TMV 2	7.9ab	8.8c	6.86e	7.85ab	29abcd	30.3ab	22.8bcd	27.3abc	0.486bcd	0.474ab	0.259bc	0.408abc
TPG 41	6.97cde	4.47fg	7.65cd	6.36g	26.5bcd	30.7ab	28.2a	28.5abc	0.383d	0.513a	0.319ab	0.392abcd
Mean	6.84	7.21	8.35	7.47	28.6	29.6	24.3	27.5	0.456	0.383	0.278	0.373
LSD $_{0.05}$												
Treatments	0.97				1				0.07			
Genotypes	0.18				2.4				0.07			
Interaction TxG	0.3				4.2				0.12			

Table 4 Intra cellular CO_2 (Ci,) transpiration rate (E mmol m^{-2} s^{-1}) and intrinsic water use efficiency (WUE$_{Int}$) in peanut genotypes at 75–80 DAS, grown at various soil moisture regimes

Genotype/treatment	Ci				E (mmol m^{-2} s^{-1})				WUE$_{int}$			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
AK 265	220ab	209abc	247ab	225ab	13.7ab	10.1bc	7.4abc	10.4a	1.9c	2.8b	3.6bc	2.8bc
GG 20	216ab	225abc	215bc	219ab	13.1ab	11.9a	6.5bcde	10.5a	2.4abc	2.7b	3.6bc	2.9abc
Girnar 3	197ab	203bc	234abc	211ab	9.7f	9.9bc	5.6e	8.4b	3.0a	2.7b	4.1ab	3.3ab
Girnar 2	152c	204bc	210bc	189b	13.2ab	9.6c	5.4e	9.4ab	2.4abc	2.8b	3.6bc	2.9abc
ICGV 86590	220ab	220abc	257a	232a	12.7bc	9.5c	8.3a	10.2a	2.4abc	3.2ab	3.1cd	2.9abc
ICGV 9114	227a	198c	213bc	213ab	13.5ab	9.1c	6cde	9.6ab	2.6abc	3.7a	4.0b	3.4ab
JL 24	180bc	210abc	258a	216ab	10.0ef	10.4bc	8.4a	9.6ab	2.5abc	2.7b	2.6d	2.6c
SB XI	215ab	228abc	244ab	229ab	9.7f	9.5c	6.3bcde	8.5b	3a	2.7b	4.7a	3.5a
SG 99	205ab	219abc	195c	206ab	11.2def	10.5bc	7.5ab	9.7ab	2.1bc	3.1ab	3.1cd	2.8bc
TAG 24	187abc	228abc	249ab	221ab	11.3cde	10.4bc	8.4a	10a	2.7ab	3.0b	3.0cd	2.9abc
TMV 2	221ab	240ab	238ab	233a	14.6a	10.5abc	5.9de	10.3a	2c	2.9b	4ab	3.0abc
TPG 41	222a	246a	259a	242a	11.6cd	11.2ab	7.1abce	10a	2.3bc	2.7b	4.2ab	3.1abc
Mean	205	219	235	220	12	10.2	6.9	9.7	2.4	2.9	3.6	3.0
LSD $_{0.05}$												
Treatments	11.9				0.58				0.24			
Genotypes	23.4				0.84				0.39			
Interaction T × G	NS				1.46				0.68			

of NPQ was highest in Girnar 3 (1.88) and the lowest in JL 24 (1.10). The DeS was 38 % in T_1 and T_2 but, increased to 47 % in T_3. The highest de-epoxidation state was found in ICGV86590 (48.7 %) followed by SG 99 (47.2 %) whereas the lowest de-epoxidation state was found in TMV 2 (33.8 %). Genotypes performed differently for the state of de-epoxidation under different irrigation treatment. The per cent de-epoxidation was higher in genotypes GG 20, ICGV 86590, JL 24, SG 99, Girnar 3, ICGV 91114 and TMV 2 in T_2 as compared to the T_1. But, under T_3 the per cent de-epoxidation had increased in all the genotypes.

Genotype by trait biplots and trait relationship analysis

The genotype-by-trait (GT-biplot) for each of the three treatments explained more than 85 % variation. The GT-biplot of first five PCs with eigen values more than one explained 86.8 % variation observed in T_1 (Fig. 1a). Among the components of the PCA of T_1 (Fig. 2a, b and c) the highest contribution was governed by g_s very closely followed by NPQ and F_v/F_m in PC 1. The PC 2 was governed by P_N and DeS whereas the PC 3 was governed by C_i and E. Overall the vectors of WUE$_{int}$, E and SLA explained maximum variation among genotypes. A fair contribution to the variation also comes from DeS and NPQ whereas the contribution of RWC, $\Phi_{PS\ II}$, and total chlorophyll content were relatively low in T_1. Genotype SG 99 out performed for de-epoxidation state, AK 265 for E, ICGV 91114 for P_N and

Girnar 3 for high WUE$_{int}$. An acute angle observed between P_N, g_s and $\Phi_{PS\ II}$ indicated positive associations among these traits. Genotypes ICGV 91114 and GG 20 were best for this group of traits. This further explains that increasing g_s can improve the P_N under well watered condition.

PCA of T_2 explains that the variation was derived from five PCs (Fig. 2d, e and f) reaching 87.7 % of total variation observed. PC 1 was dominated by WUE$_{int}$ and SLA, PC 2 was dominated by $\Phi_{PS\ II}$ and PC 3 was again dominated by WUE$_{int}$. Over all, among the components of the PCA (Fig. 1b), the highest contribution was governed by g_s, very closely followed by NPQ and F_v/F_m in PC 1. Higher proportion to the variation due to $\Phi_{PS\ II}$, C_i, NPQ, WUE$_{int}$ and DeS is observed in GT-biplot of T_2. This was the condition when WUE$_{int}$ was closely and positively associated with DeS and $\Phi_{PS\ II}$ whereas SCMR, F_v/F_m and DeS were very closely associated with the P_N. GG 20 was the best for higher stomatal conductance and low NPQ whereas Girnar 3 tops for high NPQ and low g_s. The first PCA component in GT-Biplot of the T_2 explains the contribution by the P_N very closely followed by the DeS (Fig. 2b).

Similarly as T_1 and T_2, the variation in T_3 also from major first five PCs contributing to a total of 87.7 % variation (Fig. 2g, h and i). PC 1 of the loadings in the T_3 showed that the highest contribution to the variation was governed by the chlorophyll content whereas in PC 2 the variation was mainly contributed by F_v/F_m and total chlorophyll content whereas in PC 3, the major variation was governed by decreased F_v'/F_m'

Table 5 Maximum efficiency of photosynthesis (F_v/F_m), actual efficiency of photosynthesis (F_v'/F_m') and quantum yield of PS II (Φ_{PSII}) grown at various soil moisture regimes

| Genotype/treatment	F_v/F_m				F_v'/F_m'				Φ_{PSII}			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
AK 265	0.845a	0.831abc	0.825c	0.834ab	0.571abcd	0.615abcde	0.565abcd	0.584abc	0.219ef	0.242abcd	0.235ab	0.232bc
GG 20	0.837ab	0.839ab	0.825c	0.833ab	0.538d	0.621abcd	0.513cd	0.557abc	0.361a	0.259ab	0.23ab	0.283a
Girnar 3	0.84a	0.845a	0.837abc	0.841a	0.54d	0.592bcde	0.572abcd	0.568abc	0.32ab	0.281a	0.214abc	0.272ab
Girnar 2	0.842a	0.823c	0.838abc	0.834ab	0.545d	0.562de	0.549bcd	0.552bc	0.297bc	0.235abcd	0.209bc	0.247ab
ICGV 86590	0.843a	0.838ab	0.841ab	0.841a	0.636a	0.673a	0.579abc	0.629c	0.224ef	0.234bcd	0.228ab	0.229bc
ICGV 9114	0.846a	0.836abc	0.826bc	0.836ab	0.562bcd	0.579cde	0.506d	0.549bc	0.287bcd	0.259ab	0.227abc	0.258ab
JL 24	0.844a	0.834abc	0.831abc	0.836ab	0.585abcd	0.665a	0.634a	0.628a	0.193f	0.199d	0.181c	0.191c
SB XI	0.838ab	0.829bc	0.841a	0.836ab	0.590abcd	0.555de	0.58abc	0.575abc	0.242de	0.218bcd	0.245ab	0.235bc
SG 99	0.824b	0.839ab	0.841a	0.835ab	0.619abc	0.666a	0.579abc	0.621a	0.218ef	0.246abc	0.218abc	0.227bc
TAG 24	0.834ab	0.833abc	0.799d	0.822b	0.627ab	0.649ab	0.573abcd	0.616ab	0.225ef	0.234abcd	0.258a	0.239ab
TMV 2	0.847a	0.845a	0.838abc	0.844a	0.553cd	0.546e	0.547bcd	0.548c	0.25cde	0.218bcd	0.21bc	0.226bc
TPG 41	0.824b	0.834abc	0.834abc	0.831ab	0.603abcd	0.647abc	0.586ab	0.612abc	0.237ef	0.21cd	0.239ab	0.228bc
Mean	0.839	0.836	0.831	0.835	0.581	0.614	0.565	0.587	0.256	0.236	0.224	0.239
LSD $_{0.05}$												
Treatments	0.004				0.024				NS			
Genotypes	0.009				0.041				0.026			
Interaction T × G	0.015				NS				0.045			

Table 6 Non-photochemical quenching (*NPQ*) and relative de-epoxidation state (*De*S) in peanut genotypes at 75–80 DAS, grown at various soil moisture regimes

Genotype/treatment	NPQ				DeS			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
AK 265	1.77a	1.3bcde	1.85ab	1.64ab	34.8e	30.8f	48.0de	37.9d
GG 20	1.61a	1.17bcde	2.19a	1.66ab	35.1d	43.0bc	44.4f	40.8c
Girnar 3	1.44a	2.06a	2.13a	1.88a	31.8g	37.7e	38.8g	36.1de
Girnar 2	1.67a	2.03a	1.58abc	1.76ab	45.9a	40.4d	54.3b	46.8b
ICGV 86590	1.14a	0.78e	1.78ab	1.23ab	40.9c	44.4b	60.7a	48.7a
ICGV 9114	1.72a	1.54abc	2.17a	1.81ab	29.9h	38.1e	44.4f	37.5d
JL 24	1.22a	0.82de	1.25bc	1.1b	33.1ef	38.1e	51.0c	40.8c
SB XI	1.35a	1.79ab	1.73abc	1.63ab	30.1h	25.2g	49.2cd	34.9ef
SG 99	1.30a	0.96cde	1.54abc	1.26ab	45.2a	49.2a	47.2e	47.2ab
TAG 24	1.43a	0.9cde	1.06c	1.13b	45.9a	37.7e	54.5b	46.0b
TMV 2	1.75a	1.53abcd	1.88ab	1.72ab	32.3fg	32.6f	36.5h	33.8f
TPG 41	1.11a	0.97cde	1.28bc	1.12b	43.2b	41.5cd	55.5b	46.8b
Mean	1.46	1.32	1.7	1.5	37.4	38.2	48.7	41.4
LSD $_{0.05}$								
Treatments	0.26				0.54			
Genotypes	0.41				1.07			
Interaction T × G	NS				1.85			

and $\Phi_{PS\ II}$. Overall, four most important traits that has contributed heavily are WUE_{int}, P_N, Ci, E and total chlorophyll content in T_3 (Fig. 1c). Two prominent groups of traits are visualised from the GT-by plot of the T_3 (Fig. 1c). The first group is comprised of P_N, SLA, $\Phi_{PS\ II}$ and g_S and the second one comprising of RWC, DeS and F_v'/F_m'. Both these groups were associated with the Ci which in turn was plotted almost at right angle with the WUE_{int} indicating a weak relationship. From the GT-biplot, it is clear that genotypes behaved differently under different soil moisture regimes. Most of the traits contributed for the observed variation in T_1 and T_2 independently, but in T_3, these traits were divided in group of traits making the relationship more complex.

Discussion

The RWC, stomatal resistance, rate of transpiration and canopy temperatures are important parameters that influence water relations in peanut (Nautiyal et al. 1995, 2012). The RWC of the leaf is more stable and sensitive than water potential in peanut limited soil moisture availability (Clavel et al. 2005). The significant reduction in RWC in this study was in close agreement with soil water availability under different irrigation treatments. Daniele et al. (2006) found that in peanut, the genotypic discrimination of RWC trait depends on the water regime and also genetic background which seems to be the reason behind genotypic difference in RWC.
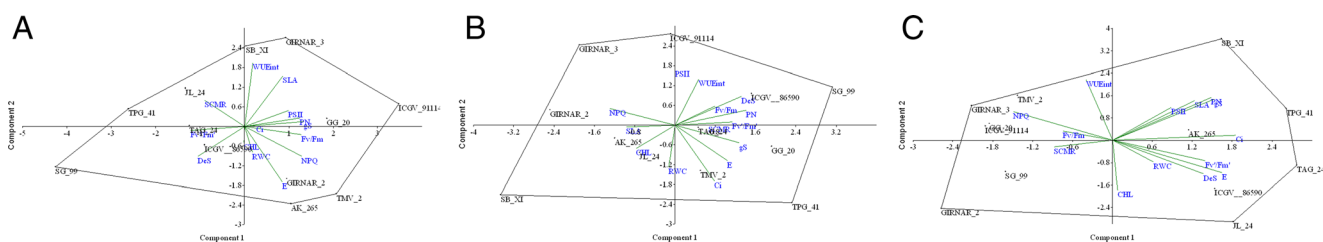
Fig. 1 Vector view of genotype × trait biplot summarising the interrelationship among the traits under various irrigation treatments (**a**) T_1 (**b**) T_2 and (**c**) T_3. *RWC* relative water content, *SLA* specific leaf area, P_N net photosynthesis rate, *E* transpiration rate, *WUE* intrinsic WUE, g_s stomatal conductance, F_v/F_m Maximum quantum yield of PSII F_v'/F_m'= actual efficiency of photosynthesis, Φ_{PSII} Energy utilized for photochemistry, *NPQ* Non-photochemical quenching, *De*S relative de-epoxidation state, *Chl* total chlorophyll content, *SCMR* SPAD chlorophyll meter reading, *Ci* intracellular CO_2 concentration
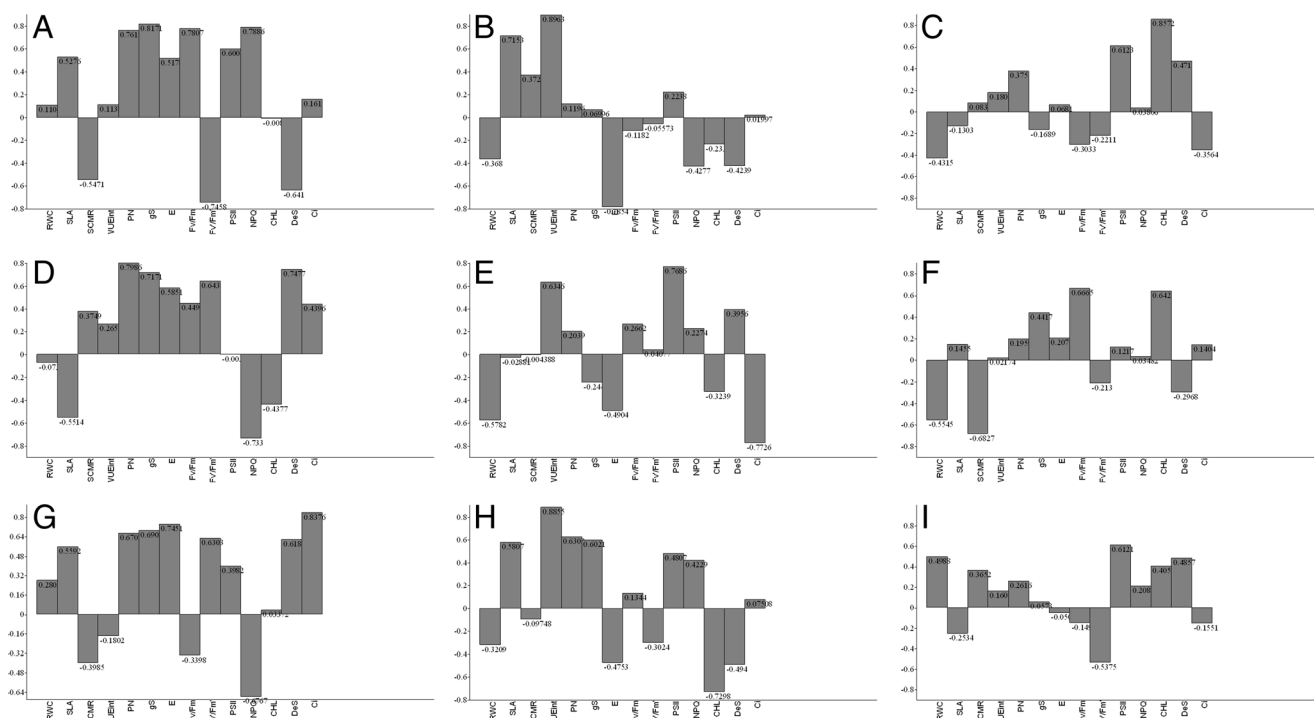
Fig. 2 Principal component s of various treatments (**a**) PC 1 T_1, (**b**) PC 2 T_1, (**c**) PC 3 T1, (**d**) PC 1 T_2, (**e**) PC 2 T_2, (**f**) PC 3 T_2, (**g**) PC 1 T_3, (**h**) PC 2 T_3 and (**i**) PC 3 T_3

Decrease in SLA or more appropriately the thickening of leaves usually have higher chlorophyll content per unit leaf area and hence had a greater photosynthetic capacity compared with thinner leaves The photosynthetically active light-transmittance characteristics of the leaf is dependent on the unit amount of chlorophyll content per unit leaf area (chlorophyll density) (Richardson et al. 2002). The SCMR is the indirect measure of the chlorophyll density. The decrease in SLA and increase in SCMR and total chlorophyll content are closely associated in this study. Significant and positive correlations between SCMR and chlorophyll content (Akkasaeng et al. 2003) and chlorophyll density (Arunyanark et al. 2008) have been reported.

Reduction in canopy photosynthesis by imposing moisture stress is found mainly due to reduction in stomatal conductance and leaf area. As moisture stress increases, stomata start closing as a mechanism to reduce transpiration, as a consequence, the entry of carbon dioxide is also reduced. The P_N in peanut leaves decreases as relative water content (RWC) and water potential (Ψ) decreases due to water deficit stress condition (Kalariya et al. 2013). The photosynthesis is fundamental in both biomass accumulation and productivity and it could be best utilized in identifying the efficient genotypes and to understand the physiological traits of productivity both under normal and stress conditions. Under increasing moisture deficit, the low SLA type peanut genotypes were able to maintain higher RWC, P_N and g_s (Nautiyal et al. 2002). In general, the limitation of net photosynthetic rate in low moisture stressed plant is mainly through stomatal closure (Cornic and Massacci 1996) and/or by metabolic impairment (Flexas and Medrano 2002), however, the severity of drought decides the relative magnitude of stomatal and non-stomatal factors limiting photosynthesis. Plants transpire less under limited soil water status, as a result decreased rate of transpiration was observed in this study. Subramaniam and Maheswari (1990) reported that leaf water potential, transpiration rate and photosynthetic rate decreased progressively with increasing duration of water stress in peanut. The gas exchange variables at 60 DAS were affected by various frequency of irrigation and the best results for gaseous exchange characteristics were gained by highest irrigation frequency tested (every 2 days) in peanuts (Sousa et al. 2014). The transpiration efficiency (TE) is the WUE_{int} (the ratio of instantaneous CO_2 assimilation (A) to transpiration at leaf level. A high photosynthetic efficiency consumes more CO_2 and ultimately decreases C_i value. The increased value of C_i in T_3 seems to be because of in-efficiency of the photosynthetic incorporation of CO_2. A tight link has been found between large differences in TE in several crops and attributes of plants that make them restrict water losses under high vapour-pressure deficits (Vadez et al. 2014).

The GT-biplot shows the negative relationship between the E and WUE_{int} across the treatments and a very strong and

positive association between the P_N and the g_s under severe water deficit stress condition. The g_s was positively correlated with the Ci during T_2 and T_3. Reduced g_s had resulted in restricted influx of CO_2 and efflux of water however, a low P_N resulted in increased the Ci.

As per the reports of Baker and Horton (1987), decrease in Fv/Fm indicates a chronic photoinhibition due to photoinactivation of PSII centres. Reduced Fv/Fm is previously reported in duram wheat (Bogale et al. 2011) and Sapnish peanut genotypes (Kalariya et al. 2013). Most of the plants adapt themselves to water stress by dissipating the excess excitation energy thermally with the down regulation of PSII activity to protect photosynthetic apparatus from photo-damaging effect under water deficit stress often coinciding with high leaf temperature (Bilger and Bjorkman 1990). The level non-radiative energy dissipation in the LHC II of PSII helps prevent the over reduction of the electron transfer chain and thereby provides protection against the photo-damage is indicated by NPQ (Krause and Weis 1991, Finazzi et al. 2006). This is achieved by the xanthophyll cycle through inter conversions of three carotenoid pigments: violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z), which are catalysed by two enzymes: violaxanthin de-epoxidase (VDE: EC1.10.99.3) and zeaxanthinepoxidase (ZE: EC1.14.13.90). The accumulation of Z and A, along with the trans-thylakoid pH gradient, mediates non radiative dissipation of light energy in the antennae (Björkman and Demmig-Adams 1994). This non radiative dissipation of light energy is an alternative energy path that diverts energy from PSII, effectively down-regulating PSII's efficiency which is dependent on the accumulation of de-epoxidation products (A+Z) of the xanthophyll cycle. Furthermore, Z may directly protect the thylakoid membrane against photo-oxidation as an antioxidant. Higher plants when exposed to photo-inhibition, the xanthophyll cycle dependent NPQ is the most useful mechanism to dissipate excess energy. Limited water supply has increased the NPQ in close accordance with the de-epoxidation state. Mishra et al. (2012) proposed that chlorophyll fluorescence emission responds to the level of water stress and thus, can be used to identify elevated drought tolerance in high-throughput screens for selection of resistant genotypes.

It is clear from the study that applying 20 % higher water than the ET (T_1) does not warrant any extra benefit in terms of higher photosynthesis in peanut at 80 DAS. Further, a reduction of 25 % of the ET through drip (excluding first two flood irrigations) in peanut seems to be the ideal condition for photosynthesis and desirable chlorophyll fluorescence parameters at 75–80 DAS. But, a reduction of nearly 50 % of the evaporative demand through drip leads to a drastic reduction in photosynthesis, stomatal conductance and transpiration in peanut at 75–80 DAS.

As per the principal component analysis, a straight negative association of the DeS and the NPQ indicated that an efficient de-epoxidation state is meant to photosynthetic machinery in peanuts. The WUE_{int}, Ci, P_N, and NPQ are major source of variation under water deficit stress condition in peanuts during dry season. Our results suggest that the genotypes Girnar 3 and ICGV 91114 which showed NPQ value above 2.2 and higher de-epoxidation state, maintained least deviation in F_v/F_m and F_v'/F_m' under severe water deficit condition are promising genotypes against water deficit stress induced photo-inhibition.

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