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Complete List of Authors:	Nautiyal, Prakash; IARI, Seed Science and Technology Rathnakumar, A; DGR, Plant Breeding
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Evaluation of wild *Arachis* species for cultivation under semi–arid tropics as fodder crop

P.C. NAUTIYAL *[+], A. L. RATHNAKUMAR[*], GANESH KULKARNI[**], and M. S. SHESHSHAYEE[***]

Directorate of Groundnut Research, Post bag 5, Junagadh– 362001, Gujarat, India.*

Department of Botany, Junagadh Agricultural University, Junagadh–362001, Gujarat, India[**].

Division of Crop Physiology, College of Agriculture, GKVK, UAS, Bangalore–560065, Karnataka , India[***].

Present address: Division of Seed Science and Technology, IARI, Pusa Campus, New Delhi–110012, India[+].

Abstract

Wild *Arachis* genotypes were analysed for chlorophyll a fluorescence, carbon isotope discrimination (ΔC), specific leaf area (SLA) and SPAD–readings. Associations between different traits i.e. SLA and SPAD–readings (r = –0.76), SLA and ΔC (r = 0.42), and ΔC and SPAD–readings (r = 0.30) were established. The ratio of F_v/F_m showed wide variability under water-deficit (WD) than after irrigation (IR). Genotypes were grouped as: F_v/F_m ratio between 0.80 and 0.85 efficient, 0.79 and 0.75 moderately–efficient and <0.74 inefficient. Selected genotypes were evaluated for green fodder yield, ranging between 3.0 and 3.8 in efficient, 2.6 and 2.7 in moderately–efficient and, 2.3 and 2.5 t ha^{-1} $year^{-1}$ in inefficient genotypes in 2008 and 2009, respectively. Leaf water relation traits studied in WD and IR showed that efficient genotypes are superior in maintenance of leaf water relation traits, especially under WD. Potential genotypes identified in this study may enhance biomass productivity in the semi–arid–tropics.

Additional key words: water use efficiency, efficiency of photosystem II, leaf water relation traits, green biomass, water scarcity environments

Abbreviations

ANOVA – analysis of variance

DM – dry mass

SD – standard deviation

Chl – chlorophyll (a, b)

E – transpiration rate

FM – fresh mass

F_0 – minimal fluorescence yield of the dark-adapted state

F_m – maximal fluorescence yield of the dark-adapted state

F_s – steady-state fluorescence yield

F_v – variable fluorescence

F_v/F_m – maximal quantum yield of PSII photochemistry

F_0/F_m – thylakoid membrane stability

g_s – stomatal conductance

ICRISAT – international crop research institute for semi–arid tropics

IR – irrigated (after irrigation)

LA – leaf area

PCA– principal component analysis

PDB – PeeDee belemnite

PAR – photosynthetic active radiation

PS II – photosystem II

RWC – relative water content

RCBD – completely randomized block design

SPAD – soil plant analysis development

TM – turgid mass

WD – water-deficit (before irrigation)

ψ_w – water potential

Introduction

The genus *Arachis* has evolved in some unusual niches, ranging from semi–arid areas of north–eastern Brazil to Carrado pockets in the Amazon forest, to low, deep–soil alluvial plains and humus clay swamps of the Gran Pantanal. Under such a wide ecological diversity these species have been acclimatized for climate prevailing in the tropical and subtropical regions, especially water scarcity environments and poor soil conditions. In addition, these species could easily establish effective association with *Bradyrhizobium* in root nodules which increase soil fertility (Valls 1983). Wild *Arachis* species are considered best legumes for pasture improvement or forage crop. The most valuable attribute that several wild species possess is persistence under grazing, which makes them special in development of permanent pastureland (Simpson 1991). On the other hand, in groundnut cultivars genetic base has become quite narrow and wild *Arachis* species are only source for variability in morphological, physiological and genetic traits leading to detectable differences in isoenzyme level, under normal irrigation conditions (Lu and Pickersgill 1993). Moreover, these species have been identified as donor source for various biotic (Subrahmanyyam *et al.* 1985, Bera *et al.* 2014, Michelotta *et al.* 2015) and abiotic (Nautiyal *et al.* 2008, Upadhyay *et al.* 2011, Bera *et al.* 2013) stresses. The climate change scenario also demands to increase biomass production by developing pastureland or cultivation of wild *Arachis* species for fodder purpose (Nautiyal *et al.* 2008).

Phenotyping for water use or photosynthetic efficiency require a high throughput screening technology which is still in developing stage. So far, in groundnut, water use efficiency has been determined by analysing various traits for example ΔC (Hubick et al. 1986), specific leaf area (SLA) (Nautiyal *et al.* 2002) and SPAD–readings (Nageswara Rao and Wright 1994). Basically ΔC is tendency of tolerant genotype to fix carbon molecule irrespective of its isotope form; hence tolerant genotypes do not discriminate between ^{12}C and ^{13}C present in the ambient air. Thus lower values of ΔC are the indicative of photosynthetic efficiency (Hubick *et al.* 1986, Nageswara Rao *et al.* 1994).

The surrogate traits often used for measuring ΔC are SLA and SPAD–reading, these are basically indicative of leaf thickness and total leaf nitrogen content, respectively, which are ultimately measuring chlorophyll (Nigam and Aruna 2008). These traits were also found associated with water use efficiency in groundnut cultivars (Varshney *et al.* 2009). In addition, chlorophyll (Chl) *a* fluorescence is widely accepted as an indication of the energetic behavior of photosynthetic system. Since, Photosystem II (PS II) emits energy in the range of 680–740 nm spectra region, and considered as an intrinsic probe of

the fate of excitation energy and indicative of various light reactions occurring in thylakoid membranes (Govindjee 2004). It helps in maintaining balance between energy supply *via* photochemistry and energy consumption *via* photosynthetic carbon reduction in leaf (Franks and Beerling 2009). Different parameters of Chl fluorescence have been used for investigations on various crops under diverse growth condition, such as, barley (Guo *et al.* 2008), maize (O'Neil *et al.* 2006), groundnut (Lauriano *et al*. 2006, Singh *et al.* 2014) and broad-bean (Stefano and Terashima 2008). Change in the state of PS II is related with a decrease in the value of F_v/F_m. In most of the plant species the optimal value of F_v/F_m varies between 0.79 and 0.83 and lower values indicate that plant is lacking an optimal health state (Bjorkman and Demming 1987). In addition to F_v/F_m, other parameters such as F_0 and F_m measured during grain filling stage of wheat under drought stress showed higher genetic correlation with grain yield. Recently, full–length DNA of the chloroplast Cu/Zn-SOD gene (AhCSD2) from allotetraploid groundnut cultivars and diploid wild *Arachis* species has been characterised for superoxide dismutase activity (Zhang *et al.* 2015). So far wlid *Arachis* species remained neglected, especially in search of genes responsible for maintaining higher photosynthetic rate under water-deficit condition. The aim of present study was to evaluate wild *Arachis* genotypes for photosynthetic efficiency which is basic requirement for cultivation in arid and semi–arid tropics where scarcity of water is main problem.

Materials and Methods

Experiments were conducted at the Directorate of Groundnut Research, Junagadh (*lat* 21°31′N, *long* 70°36′E), Gujarat, India. One experiment was conducted under greenhouse conditions and 54–wild *Arachis* species including their accessions were analysed for photosynthetic efficiency following various traits. After identification of genetic potential for photosynthetic efficiency, selected genotypes were evaluated for fodder yield and leaf water relation traits, under field conditions.

Greenhouse experiment: Genetic stocks of wild *Arachis* species and their accessions were procured from the International Crop Research Institute for Semi–Arid Tropics, India Centre, Patancheru (ICRISAT). These genotypes were propagated through rhizome or seed by transplanting in pot during rainy season (June–September) in 2000. Canopy of individual genotype was developed in cemented hollow bottom ring shaped pots of 0.60 m diameter and 0.75 m height. Pots were filled with soil and sand in 1:1 ratio (w/w). In each pot a single seed or rhizome was planted, since wild *Arachis* species are rhizomatous, it

could develop canopy that has covered whole pot up to 2006. During plants establishment irrigations were provided as and when required however before recording observations, irrigation was given to the field capacity on first September 2006 and observations were recorded from 26 September (WD). Second irrigation was provided on 30 September and same set of observations was recorded (IR). After recording observations, soil samples from each pot between 0 and 10 cm depths were collected and analyzed gravimetrically to determine moisture content.

Measurement of Chl a fluorescence: Before starting experiment, 54–genotypes were divided into two groups and observations were recorded for two consecutive days in each group, first in WD followed by IR on cloud free days. Chl a fluorescence parameters were recorded with the help of Hansatech, Fluorescence Monitoring System, FMS 2 (England) equipped with a fiber probe and leaf clip holder. Fully expanded 2 or 3 leaf from top of the canopy on main stem or branches of each genotype was selected and observations were recorded on three leaflets between 09:00 and 12:00 h local time. After completing observations, each pot was irrigated to the field capacity and same set of observations was recorded 1-day after irrigation. All the observations were recorded on adaxial side of the leaflet and photosynthetic active radiation (PAR) during this period was between 800 and 1,130 μ mole m^{-2} s^{-1}. The light level, run–time, and dark adaptation period for all the measurements were 400 μ mole m^{-2} s^{-1}, 5s and 30 minutes, respectively. Care was taken before and during measurement not to disturb the natural leaf orientation with respect to the sun or to shade. Steady state fluorescence (F_s) was determined under actinic light following Nogues and Baker (2000). An actinic photosynthetic photon flux of 3,000 μmol m^{-2} s^{-1} PAR, 800 ms duration was used for determination of fluorescence induction. The maximum fluorescence (F_m) and the minimal fluorescence (F_0) of sampled leaves were used to calculate the F_v/F_m ratio following Maxwell and Johnson (2000) i.e. $F_v/F_m=[(F_m–F_0)/F_m]$ this represents the maximum quantum yield of PS II presuming that all the PS II centers were open. In addition, the changes in variable ($F_v=F_m–F_0$) fluorescence, the absolute values F_0, F_m and the half time of the increase from F_0 to F_m ($t_{1/2}$) were determined. Thus, F_v/F_m provides a measurement of the intactness of the Light Harvesting Complex (PS II/LHC) unit and indicates the probability of a trapped photon within the reaction centre to cause a photochemical event such as the efficiency of excitation capture by open PS II centers. As such, it can give a measure of the rate of linear electron transport thus could be an indication of overall photosynthesis. Moreover, a linear plot of the

quantum yield of CO_2 assimilation and photochemistry allows the electron requirement per molecule of CO_2 fixed (Epron *et al.* 1995).

Measurement of specific leaf area: For the measurement of SLA leaves were collected after irrigation. Total 60 fully expended 2 or 3 leaves from top of the canopy on main stem or branches of each genotype were collected and arranged in three replicate having 20 leaves in each. Leaf area was measured with the help of leaf area meter (Model 3,000, LI-COR Inc., Lincoln, NE). Leaf samples were dried at 80°C until constant mass in a hot-air oven and leaf dry mass (DM) was recorded. SLA was calculated as: LA [cm^2] /dry mass (DM) [g].

Carbon isotope discrimination: For the measurement of ΔC ($^{13}C/^{12}C$) leaf samples were collected from each pot by selecting fully expended 2 or 3 leaf from top of the canopy on main stem or branches. Total 30 leaves were collected from each genotype and arranged in three replicates. Leaf samples were dried in open sun inside butter paper bags; this was followed by drying at 40°C for 2 h before grinding. ΔC was calculated by measuring difference in carbon isotope ratios of the air and of the leaf samples. The dried material was ground to pass through a 100–μM iron sieve. Isotope composition was measured by ratio mass spectrometry. The ratio of the air was taken as –7.6‰ on the PeeDee belemnite (PDB) scale (Hubik *et al.* 1986). For illustration, leaf samples of approximately 10 mg were combusted in an elemental analyser (Carlo Erba Instrumazione, Italy). The combustion products were moved in a stream of helium, and CO_2 in the effluent gas was separated from impurities chromatographically. Carbon dioxide gas was concentrated in a trap cooled with liquid N_2 and helium was pumped away. The trap was warmed and the CO_2 was allowed to inter the inlet of the ratio mass spectrometer for measurement of isotope ratio. The isotope ratios of the samples were estimated by comparison with a working standard of CO_2 with an isotope ratio of –35.08‰ relative to PDB. Carbon isotope discrimination differs from $\Delta^{13}C$ in that it describes only that change in isotopic composition induced by the plant, eliminating variation as a result of the starting value of the atmospheric CO_2 used for photosynthesis. ΔC was determined following Farquhar and Richards (1984) as quoted by Lucas *et al.* (2013):

$$\Delta = \frac{Ra - Rp}{Rp} = \frac{\delta a - \delta p}{1 + \delta p}, \qquad \text{(Eqn 1)}$$

where Ra is the $^{13}C/^{12}C$ ratio of CO_2 in air, and *R*p is that of plant carbon. In the second form of Eqn 1, δ a is $\Delta^{13}C$ of CO_2 in air and δp is that of plant carbon. The $\Delta^{13}C$ is defined with respect to a standard:

$$\delta^{13}C \text{ sample} = \frac{R \text{ sample} - R \text{ std}}{R \text{ std}}, \qquad \text{(Eqn 2)}$$

where $\Delta^{13}C$ sample is that of the sample of interest, *R* sample is its $^{13}C/^{12}C$ ratio, and *R* std is the $^{13}C/^{12}C$ ratio of a standard. The internationally accepted standard for expressing stable carbon isotope ratios is PDB, with a $^{13}C/^{12}C$ of 0.0112372 (Craig 1957) as quoted by Lucas *et al.* (2013). In order to avoid working with very small numbers, Δ and $\delta^{13}C$ sample are typically multiplied by 1000, and denoted as parts per thousand (‰). When Eqn 1 is multiplied by 1000, this does not affect terms in the denominator. Therefore, if Δp were 28‰ in the numerator, 1+Δp in the denominator would still be 1.028.

Soil plant analysis development (SPAD) readings: Soil Plant Analysis Development (SPAD) readings were recorded with the help of SPAD–meter (SPAD–502, Minolta Corp, Ramsey, NJ, USA). For recording observations three fully expanded 2 or 3 leaf from top of the canopy on main stem or branches of each genotype were selected. Observations were recorded on each leaflet and averaged. While taking observations care was taken to ensure that the SPAD meter sensor has fully covered the leaf lamina and that the interference from veins and midribs is totally avoided.

Evaluation of fodder yield: Based on ratio of F_v/F_m in WD genotypes were identified as efficient, moderately–efficient, and inefficient. Field trials were conducted to analyse six selected genotypes i.e. *A. prostrata* Benth. (section: *Extranervosae*; NRCG 11,847), *A. glabrata* Benth. (section: *Rhizomatosae*, NRCG 11,818) and *A. marginata* Gardner [section: *Extranervosae*; NRCG 17,206 (efficient)], *A. pintoi* Krapov. and W.C. Gregory [section: *Caulorhizae*, NRCG 12,990 (moderately–efficient)], and *A. hagenbeckii* Benth. (section: *Rhizomatosae*, NRCG 11,846) and *A. appressipila* Krapov. and W.C. Gregory [section: *Procumbentes*, NRCG 12,035 (inefficient)] for fodder yield during 2008 and 2009 in Completely Randomized Block Design (RCBD).

Plants of each genotype were multiplied through stem cuttings in polyethylene bags filled with soil and sand in 1:1 ratio during July 2006. After one year, fully grown cuttings were pit–planted in 15 x 15 m plot size with spacing of 7 m between rows and 1 m between plants with three replicates. Plants were allowed to establish and develop into dense foliage for one year. Agronomical practices such as application of fertilizers,

insecticides and pesticides were avoided, in spite of the low soil fertility of the experimental site. Crop after establishment in field received four irrigations to the field capacity between February and May at one month intervals, each year. Crop did not receive any irrigation between June and January and sustained on available soil moisture generated during rainy season (June–October). After one year of planting fodder yield was recorded by performing four cuttings at every 45-day intervals between July and January; fresh mass (FM) of the foliage was recorded and expressed as fresh mass yield t ha^{-1} $year^{-1}$.

Leaf water relation traits: Leaf relative water content (RWC) [%], water potential (ψ_w) [MPa] transpiration rate (*E*) [mmol (H_2O) m^{-2} s^{-1})] and stomatal conductance (g_s) [mol (H_2O) m^{-2} s^{-1})] were measured under water-deficit (WD) and fully irrigated (IR) conditions. Water-deficit was simulated by withholding irrigation for 26-days and observations were recorded for three consecutive days. Crop was irrigated to the field capacity and after 1-day same set of observations was recorded for three consecutive days. During this period maximum evapotranspiration was around 6 mm daily, as recorded by using Class A pan evaporation system and this period was free from rain–fall. Soil samples from 0−10 cm depth were collected from both IR and WD conditions immediately after recording observations and soil moisture content was determined gravimetrically.

Relative water content: For the measurement of RWC leaf samples were collected from fully expended 2 or 3 leaf from top of the canopy on main stem or branches, in an ice box, between 09.00 and 10.00 h local time. Sampling was performed from each genotype, replicate and water regime for three consecutive days. Leaf samples were arranged in laboratory in six replicates for each genotype, soil moisture regime and three days. Thus two leaves i.e. eight leaflets were arranged in each replicate and fresh mass (FM) was recorded. Leaflets were soaked in distilled water in petriplates, after 4-h of soaking leaf turgid mass (TM) was recorded. After recording turgid mass samples were dried at 80°C until constant weight in hot–air oven and dry mass (DM) was recorded. Relative water content was calculated following the formula as suggested by Barrs and Weatherly (1962) i.e. RWC [%] = [(FM – DM)/(TM – DM)] × 100.

Leaf water potential: For the measurement of ψ_w three fully expanded 2 or 3 leaf from top of the canopy on main stem or branches were collected in each genotype, replicate and water regime, in an icebox, between 11.00 and 12.00 h local time (mid–day) for three days. Leaf ψ_w was determined on 12 leaf discs collected from each leaflet of three leaves, thus

ψ_w is average of three leaves, 12 leaf disc and three replicates for three consecutive days. Each leaf disc was placed in the leaf chamber (C–2 Samples Chambers) of CR 7 Measurement and Control System (Campbell Scientific INC Logan, Utah) and ψ_w was recorded.

Measurement of transpiration rate: For measuring E and g_s leaf Porometer (AP 4, Leaf Porometer, Delta–T Devices, England) was used. The measurements were made on three fully expended 2 or 3 leaves from top of the canopy on main stem or branches of each genotype, replicate and soil moisture regime. Both abaxial and adaxial surfaces of single leaflets were used to record observations. Observations were recorded during between 09.00 and 10.00 h, 12.00 and 13.00 h, and 15.00 and 16.00 h local time. Thus values presented are average of three different times, three leaflets of different leaves, two leaf surfaces and three days.

Leaf protein: For the measurement of protein fully expended 2 or 3 leaf from top of the canopy on main stem or branches were sampled from each genotype and replicate. Leaves were dried in oven at 80°C to constant mass. Micro–Kjeldahl method was followed to measure nitrogen content and values were multiplied by 5.46 to convert it into total protein contents.

Statistical analysis: Statistical analysis was conducted following Gomez and Gomez (1984). Data collected in greenhouse experiment and field trial for fodder yield were analysed following one–way ANOVA. Standard deviation (SD) was calculated and used at p=0.05 to explain genotypic variations. Principal component analysis (PCA) was performed following Davis (1986) by using correlations method. Number of significant PCs was identified based on "Screen plot" as suggested by Jackson (1993) and PC 1 and PC 2 with eigenvalue >1 and per cent variance between 43 and 27, respectively, were used to explain their contribution.

Results

Photosynthetic efficiency: Photosynthetic efficiency was measured by following different traits i.e. F_v/F_m ratio, ΔC, SLA and SPAD–readings and genotypic response for these traits varied (Table 1). For example, F_v/F_m ratio ranged from 0.83−0.85 in IR and 0.85–0.69 in WD indicating higher variability in WD while F_0/F_m exhibited considerable degree of variability both in IR and WD (Table 1). Based on F_v/F_m ratio in WD i.e. average (0.80) plus least standard deviation and average minus least standard deviation, genotypes were identified as F_v/F_m ratio between 0.80 and 0.85 efficient, 0.79 and 0.75 moderately–

efficient while less than 0.74 inefficient (Table 1). Soil moisture during observation period in IR and WD from 0−10 cm depths ranged between 19 and 20%, and 15 and 16%, respectively. Association between F_v/F_m and F_0/F_m was inverse (r = –0.85, *e.g. p=0.05*). In addition, chlorophyll fluorescence parameters did not show any significantly associated with rest of the traits measured in this study. Further, ΔC ranged from 19.0−24.5 being lower in *A. monticola* 11,800 and *A. duranensis* 11,809 and higher in *A. glabrata* 12,046 (Table 1). This range of ΔC in wild *Arachis* species was slightly higher than the range recorded in groundnut cultivars and germplasm (data not presented). In addition, ΔC in about 50% of genotypes ranged between 19 and 22, and association between ΔC and SLA was not strong enough (r = 0.42, *e.g. p=0.05*). While SLA which is surrogate trait for ΔC ranged between 103 in *A. duranensis* 11,809 and 310 in *A. glabrata* 11,835 (Table 1) however such a wide range also was not able to indicate photosynthetic efficiency due to poor association with main trait. SPAD–readings also followed more or less same trend as shown in SLA, it ranged between 16 and 41. This range also indicated about total Chl concentration and aassociations between SPAD–readings and SLA (r = –0.76, *e.g. p=0.05*) was strong however SPAD–readings and ΔC (r = –0.30, *e.g. p=0.01*) was weakly associated.

Principal component analysis: All traits studied were analysed for genotype–by–trait (GT–biplot) interaction following PCA and only two PCs i.e. PC 1 and PC 2 showing eigenvalues more than 1 were used. Among the traits analysed loadings of components were higher in F_v/F_m followed by F_0/F_m and correlation between loading and trait was also higher in these two parameters i.e. r = –0.55, *e.g. p=0.05* and r = 0.52, *e.g. p=0.05*, respectively, in WD. Further, "scatter plot analysis" indicated variability among genotypes for the value of trait. In addition, some of the variables were correlated with each other at a higher degree indicating that they were measuring the same content, for example, SPAD–readings and SLA, and F_v/F_m and F_0/F_m. In bi–plot analysis vector length of trait showed that each parameter is contributing variedly and their association with each other varying significantly due to genotype–by–trait, and trait–by–trait interactions. In WD, vector length in F_v/F_m was longer than all the other traits, indicating that most of the variations are represented by this trait.

Field trials: The efficient, moderately–efficient and inefficient genotypes identified based on F_v/F_m ratio indicated significant variations in leaf water relation traits (Table 2) and fodder yield (Table 3). This vindicated that selection based on F_v/F_m ratio is true representation of the measurement of photosynthetic efficiency.

Leaf water relation traits: Among genotypes distribution of leaf water traits such as RWC, ψ_w, *E* and g_s when values were averaged over IR and WD varied significantly. This indicated that maintenance of leaf water status was better in efficient than inefficient genotypes, under water-deficit (Table 2). For illustration, among genotypes distribution of each component of leaf water relation trait based on average values was in higher range in efficient, moderately–higher in moderately–efficient and lower in inefficient, genotypes. In general, RWC ranged between 94 and 98% in IR, and 84 and 91 in WD (Table 2). Genotypic response in RWC in efficient and inefficient under water-deficit was quite distinct i.e. it ranged between 87 and 91% in efficient and 84 and 85% in inefficient. Similarly, ψ_w ranged between –0.7 and –0.8 in IR and –0.9 and –1.2 in WD however in WD it was more negative in inefficient (–1.1 to –1.2) than efficient (–0.9 to –1.0) genotypes (Table 2). Transpiration ranged between 10.0 and 11.6 in IR and 9.2 and 10.5 in WD, in addition, *E* was higher in *A. appressipila* 12,035 in IR and thereafter it decreased in WD (Table 2). In efficient genotypes decrease in *E* was lower in WD. Similarly, g_s varied from 278–305 and 246–256 in IR and WD respectively, however efficient genotypes maintained higher g_s both in IR and WD than inefficient (Table 2). Thus leaf water relation traits in efficient genotypes exhibited potential in maintaining higher leaf water status, especially under water-deficit. Soil moisture during the period of recording the observations in IR and WD from 0–10 cm depths ranged between 18 and 20%, and 14 and 15%, respectively.

Green biomass production or fodder yield: During both the years, fodder yield was recorded higher in efficient than inefficient genotypes and decreased in linear fashion starting from efficient to moderately–efficient to inefficient (Table 3). Among genotypes, it ranged between 2.3 and 3.8 being higher in *A. glabrata* 11,818 in 2008 and *A. prostrata* 11,847 in 2009 (both efficient). Among various groups fodder yield ranged from 3.1–3.8 in efficient, 2.6–2.7 in moderately–efficient and 2.3–2.5 t ha^{-1} in inefficient in 2008 and 2009, respectively. In addition, leaf protein contents on DM basis were higher in efficient, ranging between 14.2 and 16.9% than inefficient (11.1–14.8%) genotypes (Table 3).

Discussion

This study demonstrated wide genotypic variations in photosynthetic efficiency as defined based on F_v/F_m ratio. Further, analysis of identified genotypes, belonging to different groups, for biomass production and leaf water relation traits, indicated important role of Photosystem II (PS II) in adaptation of photosynthetic machinery. This adaptation helped efficient genotype in production of higher biomass under limited water supply and poor soil conditions. Thus it is postulated that adaptation in PS II favoured higher fixation of CO_2 molecules per molecule of water loss *vis–à–vis* water use efficiency in genotypes i.e. *A. glabrata* 11,818, *A. prostrata* 11,874 and *A. marginata* 17,206. Their cultivation under water–scarcity environments could be an advantage. Therefore, use of these genotypes either in cultivation as fodder crop or development of pastureland may increase biomass in marginal production environments in sub–tropical regions, worldwide.

In addition, detailed analysis of traits by following PCA indicated that F_v/F_m under water-deficit is closely associated with photosynthetic machinery than any other trait explored in this study. The characteristic of water saving and tolerance of photosynthetic machinery under water-deficit have also been reported in drought tolerant groundnut cultivars (Nautiyal *et al.* 1995, Nautiyal *et al.* 1999, Nautiyal *et al.* 2012). This mechanism could be illustrated by mentioning details of PS II activity. For example, it is possible that in inefficient genotype, under water-deficit, an overcharge of photosynthetic apparatus is generated while in efficient genotype stability of carotene and dissipative cycle around PS II might be protecting reaction centres (Lauriano *et al.* 2000). Ultimately this activity in efficient and inefficient genotypes might be influencing biomass productivity. In groundnut cultivars there are reports mentioning that maintenance of F_v/F_m ratio was at the antennae level and this regulatory mechanism was reported to be effective in some cultivars while not in others (Lauriano *et al.* 2006). Thus in the process of photosynthesis under water-deficit, F_v/F_m plays a regulatory role in maintaining a balance between energy supply *via* photochemistry and energy consumption *via* photosynthetic carbon reduction (Franks and Beerling 2009). In this study, lower biomass production in inefficient genotypes under limited water supply and poor soil conditions could be ascribed to susceptibility in the state of PS II under water-deficit which has been indicated in decrease in the value of F_v/F_m leading to restriction in diffusion of CO_2 into chloroplast. Susceptibility for water-deficit thus is modifying primary photochemistry and ultimate carbon metabolism (Chaves *et al.* 2009, Franks and Beerling 2009) which has been

resulted into lower biomass production as compared to efficient genotypes. Thus variations in PS II system activity is playing important role in defining adaptation for photosynthetic efficiency which is easy to measure by F_v/F_m ratio, under water-deficit.

Among other traits, ΔC is reported to be closely associated with photosynthetic efficiency (Hubick *et al.* 1986, Nageswara Rao *et al.* 1994) however in this study, it ranged narrowly. In addition, SLA and SPAD–readings are often used as surrogate trait for ΔC (Nautiyal *et al.* 2002, Nigam *et al.* 2008) however both of these traits measure chlorophyll contents in relation to leaf thickness and nitrogen content, respectively. Therefore, association between SLA and SPAD–readings was strong (r = 0.76, *e.g. p=0.05*) while between ΔC and SLA was weaker (r = 0.46, *e.g. p=0.05*). Thus surrogate traits such as SLA and/or SPAD–readings may (Varshney *et al.* 2009) or may not (Vasfilov 2012) be true indication of photosynthetic efficiency. In addition, there are reports that photosynthetic rate and F_v/F_m are more closely associated with Rubisco as compared to SPAD–readings, and it was concluded that the PS II photochemical and CO_2 assimilation capacities are strongly influenced by the Rubisco activity (Kumagai *et al.* 2009). Therefore, F_v/F_m measures photosynthetic efficiency more accurately than any other traits used in this study.

Conclusions

This study generated knowledge on genotype–by–trait and trait–by–trait interactions which lead us to identify efficient genotypes by measuring F_v/F_m ratio. Thus use of identified genotypes in cultivation as fodder crop or development of pastureland will certainly enhance biomass productivity in semi–arid tropics where scarcity of water is serious problem. In addition, donor source identified in this study could be of immense value in developing new germplasm and designing ideotype for improving photosynthetic efficiency in groundnut cultivars. Moreover, large number of populations may be screened by F_v/F_m ratio, under water-deficit, which is easy to use, precise and rapid.

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Table 1. Quantum yield of PS II [F_v/F_m] and stability of thylakoid membrane [F_0/F_m] under irrigated (IR) and water-deficit (WD) conditions, and carbon isotope discrimination ($^{13}C/^{12}C$ or ΔC), specific leaf area (SLA), SPAD–readings, under irrigated condition (IR) in 54–wild *Arachis* species and their accessions.

Genotypes	Accession number	Section	IR [F_v/F_m]	WD [F_v/F_m]	IR [F_0/F_m]	WD [F_0/F_m]	IR ΔC	IR SLA [$cm^2\ g^{-1}$]	IR SPAD–readings
A. rigonii	12,031	Procumbentes	0.85	0.84	0.12	0.15	20.8	253	19
A. glabrata	11,847	Rhizomatosae	0.85	0.84	0.13	0.15	21.7	187	29
A. duranensis	12,038	Arachis	0.85	0.84	0.13	0.15	21.0	206	19
A.prostrata	11,847	Rhizomatosae	0.85	0.84	0.13	0.15	21.9	184	30
A. appressipila	11,786	Procumbentes	0.85	0.84	0.12	0.15	19.0	184	30
A. glabrata	11,838	Rhizomatosae	0.85	0.84	0.12	0.16	19.0	130	38
A. glabrata	11829	Rhizomatosae	0.85	0.84	0.13	0.18	22.4	166	30
A. glabrata	11,818	Rhizomat	0.84	0.83	0.12	0.16	23.1	138	35
A. glabrata	11,831	Rhizomatosae	0.84	0.83	0.12	0.16	22.6	146	33
A. glabrata	11,815	Rhizomatosae	0.85	0.83	0.13	0.16	21.8	124	32
A.paraguariensis	11,793	Erectoides	0.85	0.83	0.13	0.16	21.7	144	26
A. glabrata	11,833	Rhizomatosae	0.85	0.82	0.12	0.17	19.5	140	30
A. glabrata	11,842	Rhizomatosae	0.85	0.82	0.12	0.17	21.4	162	37
A. marginata	17,206	Rhizomatose	0.85	0.82	0.13	0.17	22.1	150	41
A. kempff-mercadoi	12,019	Arachis	0.84	0.82	0.12	0.17	22.2	242	29
A. glabrata	11,839	Rhizomatosae	0.84	0.82	0.12	0.17	23.0	300	21
A. glabrata	11,845	Rhizomatosae	0.85	0.82	0.12	0.17	21.5	148	31
A. glabrata	11,844	Rhizomatosae	0.85	0.81	0.13	0.18	21.7	173	39
A. glabrata	12,033	Rhizomatosae	0.85	0.81	0.13	0.18	21.9	166	30
A. glabrata	11,841	Rhizomatosae	0.85	0.81	0.12	0.18	22.7	137	31
A. glabrata	11,819	Rhizomatosae	0.85	0.81	0.13	0.18	22.5	131	41
A. duranensis	11,782	Arachis	0.84	0.81	0.12	0.18	19.6	128	40
A. glabrata	11,826	Rhizomatosae	0.84	0.81	0.12	0.18	23.0	284	14
A. glabrata	11,822	Rhizomatosae	0.85	0.81	0.12	0.18	20.4	129	30

A. monticola	11,799	Arachis	0.84	0.81	0.13	0.18	20.8	152	30
A. glabrata	12,046	Rhizomatosae	0.85	0.80	0.14	0.19	24.5	233	19
A. glabrata	11,824	Rhizomatosae	0.85	0.80	0.14	0.19	23.5	231	24
A. glabrata	11,828	Rhizomatosae	0.85	0.80	0.12	0.19	21.2	133	39
A. batizocoi	11,795	Procumbentes	0.85	0.80	0.13	0.19	21.2	133	30
A.duranensis	11,803	Arachis	0.85	0.80	0.14	0.19	21.4	180	20
A. glabrata	11,837	Rhizomatosae	0.85	0.80	0.13	0.19	22.5	222	23
A. kretschmeri	12,029	Procumbentes	0.85	0.80	0.13	0.21	19.3	153	33
A. monticola	11,800	Arachis	0.84	0.80	0.12	0.17	19.0	140	35
A. glabrata	11,813	Rhizomatosae	0.85	0.80	0.14	0.22	21.3	137	32
A. glabrata	11,821	Rhizomatosae	0.85	0.80	0.14	0.19	20.4	111	31
A. stenosperma	12,026	Arachis	0.85	0.80	0.15	0.21	22.0	183	26
A.duranensis	12,045	Arachis	0.84	0.80	0.15	0.19	21.9	150	35
A. glabrata	11,835	Rhizomatosae	0.85	0.80	0.15	0.22	22.5	310	19
A. glabrata	11,834	Rhizomatosae	0.85	0.79	0.15	0.20	22.1	177	27
A. batizocoi	12,018	Procumbentes	0.84	0.79	0.14	0.20	22.0	136	33
A. batizocoi	11,810	Procumbentes	0.84	0.78	0.15	0.20	19.5	187	38
A. paraguariensis	ICG 8,903	Erectoides	0.85	0.78	0.16	0.21	20.0	230	15
A. glabrata	12,036	Rhizomatosae	0.85	0.78	0.17	0.21	22.5	225	26
A. glabrata	11,823	Rhizomatosae	0.85	0.78	0.16	0.22	21.9	117	38
A. diogoi	11,781	Arachis	0.84	0.78	0.14	0.22	22.1	187	27
A. duranensis	11,809	Arachis	0.85	0.78	0.15	0.29	19.0	103	30
A. duranensis	12,043	Arachis	0.85	0.78	0.15	0.30	22.0	211	22
A. pintoi	12,990	Caulorhizae	0.84	0.77	0.14	0.22	22.1	150	35
A. glabrata	11,832	Rhizomatosae	0.84	0.77	0.15	0.22	21.5	160	37
A. duranensis	11,801	Arachis	0.83	0.77	0.15	0.25	22.5	160	28
A. hagenbeckii	11,846	Rhizomatosae	0.83	0.70	0.16	0.23	21.2	155	31
A. batizocoi	12,030	Procumbentes	0.83	0.70	0.14	0.26	22.5	251	28
A. stenophylia	11,811	Erectoides	0.84	0.70	0.16	0.30	22.1	271	20
A. appressipila	12,035	Procumbentes	0.84	0.70	0.16	0.30	20.1	132	38
		SD (p=0.05)	0.005	0.035	0.013	0.038	2.96	49.4	6.88

Table 2. Leaf relative water content (RWC), water potential [MPa], stomatal conductance (g_s) and transpiration (E) under irrigated (IR) and water-deficit (WD) conditions in selected wild *Arachis* species belonging to efficient, moderately–efficient and inefficient groups.

Drought tolerance type	Species/Accession	IR RWC	WD RWC	IR Ψ_W [MPa]	WD Ψ_W [MPa]	IR g_s [mol (H_2O) m^{-2} s^{-1}]	WD g_s [mol (H_2O) m^{-2} s^{-1}]	IR E [mmol (H_2O) m^{-2} s^{-1}]	WD E [mmol (H_2O) m^{-2} s^{-1}]
Efficient	*A. glabrata* 11,818	97	87	-0.7	-0.90	291	256	11.2	10.2
	A. prostrata 11,847	98	90	-0.7	-1.0	305	267	11.4	10.5
	A. marginata 17,206	97	91	-0.8	-1.0	291	267	10.9	10
Moderately-efficient	*A.pintoi* 12,990	96	87	-0.8	-1.2	291	267	10.5	9.4
	A. hagenbeckii 11,846	94	84	-0.7	-1.2	278	256	10	9.2
Inefficient	*A. appressipila* 12,035	96	85	-0.8	-1.1	278	246	11.7	9.4
SD (p=0.05)		NS	7.16	NS	0.70	9.9	8.4	1.5	1.2

Table 3. Fodder yield during 2008 and 2009 in fresh mass (FM) and leaf protein contents in selected wild *Arachis* species belonging to efficient, moderately–efficient and inefficient groups.

Species/Accession		Fodder yield [t ha^{-1}, FM] (2008)	Fodder yield [t ha^{-1}, FM] (2009)	Leaf protein [%]
Efficient	*A. glabrata*	3.8	3.7	16.9
	A. prostrata	3.6	3.8	14.2
	A. marginata	3.2	3	16.8
Moderately-Efficient	*A. pintoi*	2.7	2.6	12.1
Inefficient	*A. hagenbeckii*	2.3	2.4	11.1
	A. appressipila	2.5	2.3	14.8
	SD (p=0.05)	0.78	0.75	1.2