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Genotypes variations in fluorescence parameters among closely related groundnut (*Arachis hypogaea* L.) lines and their potential for drought screening programs

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Abstract

Groundnut is the most important oilseed and cash crop in the Sub-Sahel regions. It is mainly cultivated under late-season drought that occurs during the pod-filling phase and regularly causes great reduction in pod yield. Two sets of extra-early closely related groundnut lines (80 days) were developed in Senegal through back-cross between two productive 90 days cultivars, 55-437 and 73-30, and a precocity donor of 75 days, Chico. However, reduced genotypic variability of breeding lines is one of the major drawbacks encountered by breeders when improving materials for physiological traits. The objective was to improve selection criteria by establishing relevance of breeding traits based on the extent of their genotypic variation (ability to discriminate among closely related genotypes) and their relationship with yield under both well-watered and late-season drought stress. Yield, earliness, drought response indices (SSI and STI), and physiological traits – relative water content (RWC), and parameters of in vivo chlorophyll fluorescence (SFI, ''structure-function-index'' and Fv/Fm) – were measured on the two sets of lines during two crop seasons (2001 and 2002). Results showed that yields and maturities of pods of selected lines were higher and more stable across watering condition that those of the parental lines confirming their high adaptive capacity for reallocation of assimilates to the grains. The stress susceptibility index (SSI) did not allow genotypic discrimination and correlated differentially with yield under well-watered and yield under stress whereas stress tolerance index (STI) correlated positively with yield under both treatments showing that STI is more efficient to characterize end-of-season drought resistance. Yields obtained in 2001 correlated with yields of 2002 but yields under well-watered were not correlated with yields under drought showing the robustness of varietal yielding across years but not across treatments in groundnut. Few measurements of RWC discriminated genotypes and the genotypic differentiation was more marked on the 73-30 derived lines which also showed morphologic dissimilarities. Fluorescence parameters gave greater discrimination among lines especially SFI, which is defined as the resultant between resistance and activity components. Correlations between these physiological traits and yields or drought response indices were highly variable according to lines, treatments and years. However, significant correlations were repeatedly established between SFI and pod yields across conditions indicating that the photochemical response is probably constitutive (independent of stress). The approach developed in this study provided SFI as potentially relevant selection criteria that can help screening lines because: (i) this trait was chosen on the basis of existing variability detected on appropriate genetic materials; (ii) it was correlated with yield performance in the field; (iii) and it is easy to measure on large collection of genotypes.

Keywords: Groundnut (*Arachis hypogaea* L.); Genetic variability; Closely related lines; Earliness; Drought adaptation; Fluorescence parameters; Yield correlations; Screening

1. Introduction

Groundnut (*Arachis hypogaea* L.) is the most important oilseed and cash crop in the Sub-Sahel regions (Ntare et al.,

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2001). It is cultivated during the rainy season, characterized by low rainfall ranging between 300 and 600 mm, and short growing season, starting in July and ending in October (Sivakumar, 1988). In these environmental conditions, groundnut generally undergoes late-season water deficit (Sivakumar, 1991; Ndunguru et al., 1995) causing reduction in pod yield when occurring during the pod-filling phase (Nageswara Rao et al., 1985; Wright et al., 1991). Stress occurring at this phase was founded to be detrimental to several physiological processes, such as a decrease on photosynthesis and stomatal conductance (Nautiyal et al., 1995). Therefore, development of early maturing groundnut cultivars that escape water deficit during pod-formation and pod-filling stages is a main breeding objective for this region (Annerose, 1991; Khalfaoui, 1991). Breeding programmes based on selection for earliness and grain yield have proven their efficacy in different drought-prone areas (Fukai et al., 1999; Reddy, 2003; Rosales-Serna et al., 2004) and specially on groundnut growing in Sub-Sahel zone where coarse textured soil are prevalent (Subbarao et al., 1995). But it is important that physiological differences accounting for drought resistance are also studied as potential selection criteria in particular to improve the genetic progress for yield stability (Fussell et al., 1991; Turner et al., 2001).

A certain number of physiological traits that contribute to drought resistance in groundnut have been identified (Nautiyal et al., 1995; Wright et al., 1991; Nageswara Rao et al., 1988) and recently reviewed (Turner et al., 2001; Reddy, 2003). However, these traits have not been fully exploited by breeders because physiological studies focused only on few contrasting genotypes while the extent of useful genetic variability remains unknown (Richards, 1996). For these reasons, this study proposes an approach using recently selected near-isogenic lines on earliness and yield to find out relevant physiological characters useful for screening breeding lines that generally possess relatively weak variability. The breeding program on which this study is based produces extra-early lines maturing in less than 90 days (Khalfaoui, 1991; Clavel and Annerose, 1997) destined for areas where rainy seasons were drastically shortened since about 20 years (Annerose and Diagne, 1990). This genetic material constituted by two series of near-isogenic lines possessing comparable genetic background and reduced variability. Since appropriate phenology and high yield under well-watered conditions are important characters for cultivars adapted to rainfed lowland conditions (Chapman et al., 1993; Fukai et al., 1999; Clavel et al., 2004), these lines were evaluated under both well-watered and end-of-cycle drought conditions.

This study aimed to compare genetically close lines thus reducing genotype by environment interaction. As proposed elsewhere for rice (Lazar et al., 1995; Jackson et al., 1996; Fukai et al., 1999), genetic and developmental similarities of lines will be used to determine specific physiological distinctions accounting for variation in susceptibility to drought. The extent of the variation of the physiological traits among genotypes and their relationship to grain yield will be analysed in order to identify relevant selection criteria in breeding cultivars with enhanced yield under drought.

2. Materials and methods

2.1. Genetic material

Two series of experimental lines were created using back-crosses (BC) schemes from two initial crosses involving two 90 days (90-day) varieties used as female recurrent parent. The 90-day parental lines were: 55-437, a Spanish non-dormant Sub-Sahelian cv. referred by Khalfaoui (1990a) as drought-adapted and 73-30, a Spanish dormant Sub-Sahelian drought-adapted cultivar crossed with the 75-day line, Chico, as male parent and precocity donor. Four successive BC were realised on 55-437 and five on 73-30, leading about 200 lines per BC with a theoretical isogeny of 97% and 98.5%. The lines were then selected on the basis of precocity and yield traits and stabilised by selfing in F4. Twelve F4 lines from 55-437 and eight from 73-30 were evaluated and selected under field conditions during the 1999 and 2000 rainy seasons, accounting for a total amount of 505.9 and 613 mm of rainfall, respectively.

All experiments were carried out in the experimental station of Bambey (14.42°N and 16.28°W), located in the semi-arid zone of the 'Groundnut Basin' in Senegal. Soils of this station are sandy (91–94%) and ferruginous with low clay content (3–6%), typical of the Sub-Sahelian region. Lines were compared with three control lines, both parents and the commercial line, GC8-35 (Clavel and Annerose, 1997), which also has 55-435 and Chico as parents. The check lines were regularly inserted in the field between series of four successive experimental lines with two replicates. Lines that were superior or equivalent to the recurrent parent or commercial line on precocity and yield were chosen for further evaluations. This procedure resulted in eight F6 lines from 55-437, named BC55 (Table 1A), and four F7 lines from 73-30, named BC73 (Table 1B), that constituted the plant material used in the present study. Three lines (two from 55-437 and one from 73-30) evaluated from 1999 to 2001 were not re-evaluated in 2002 because of poor yields and/or high phenotypical with another line.

2.2. Experimental conditions

The study was carried out during 2001 and 2002 rainy seasons in the experimental station of Bambey. Two seeds pre-treated with Granox (Captafol 10%–Benomyl 10%–Carbofuran 20%) per hole were hand-sown. Inter-and intra-row spacing was 50 and 15 cm, respectively. The seedlings were thinned to one per hole 1 week after sowing resulting in a density of 133,300 plants ha^{-1}. Usual cultural practices for groundnut in the Sahel were applied. The crop was

Table 1
Yield-based drought stress tolerance index (STI) on BC55 lines (above) and on BC73 lines (below) compared to parental lines in 2001 and 2002

Genotype	2001		2002
	Indice[a]	STI	STI
(A) BC55 lines			
55-128		0.95	0.86
55-15a		0.69	–
55-26		0.78	0.95
55-314		0.58	–
55-315		0.79	0.85
55-319		0.96	0.83
55-36		1.06	0.82
GC8-35		1.00	0.91
55-437 ♀		0.65	0.81
Chico ♂		0.76	0.55
S.D.		0.206***	0.170***
(B) BC73 lines			
73-28-1		1.14	0.62
73-43		1.04	–
73-44		1.12	0.72
73-9-11		1.09	0.77
73-30 ♀		0.53	0.60
Chico ♂		1.11	0.49
S.D.		0.212***	0.204*

S.D. is the standard deviation value around the mean of the data and significance of the genotypic effects was represented by *, *** at the 0.05, 0.001 probability levels, respectively.

[a] STI (stress tolerance index according to Fernandez, 1992), data from stress susceptibility index (SSI, according to Fisher and Maurer, 1978) were not presented because they did not show statistical differences between genotypes.

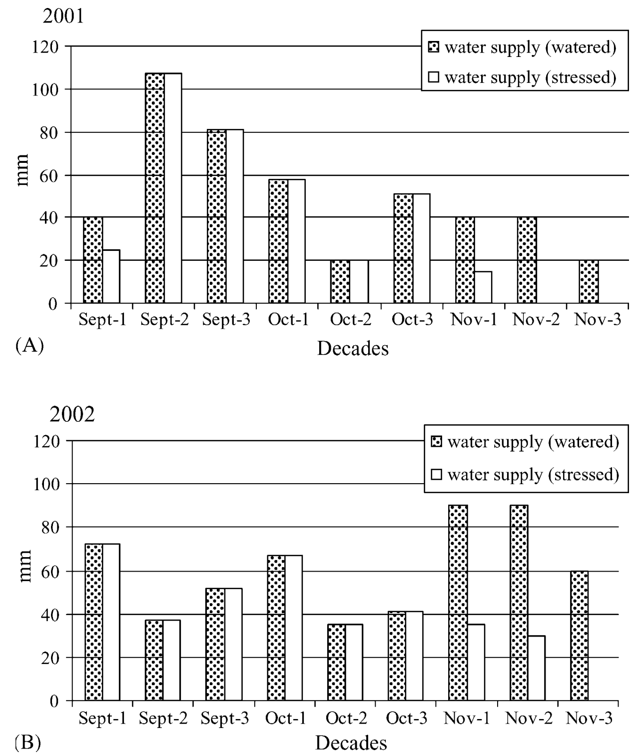

Fig. 1. Rainfall and irrigation supply at Bambey (Senegal) in 2001 (A) and 2002 (B) during the cycle of culture in decadals totals.

maintained manually weed-free and no fertilizer either pesticide was applied except a powdering with Granox on the drying pods after harvest.

2.3. Rainfall pattern and watering regime

The sowing was made in early September at a period prevailing soil water saturated conditions. This sowing date was 5–6 weeks later than optimal practices in order to submit the plants to terminal water deficit in the stressed treatments. In this geographic area, this delay has little effect on temperature and photoperiod experienced by the plants (Ndunguru et al., 1995). Mean temperatures were comprised between 28 ± 1.5° (night) and 36 ± 1.5° (day), which are the same order in rainy season with no significant variation from 1 year to another. In addition, groundnut is weakly sensitive to photoperiod, which is not considered as an environmental impact factor for a breeding program with limited geographical mandate (Williams and Boote, 1995).

Concerning well-watered conditions (control plants), the total amount of rainfall plus irrigation accounting for the experiments were 456.8 mm in 2001 and 543.4 mm in 2002 while for stressed conditions (stressed plants) they were 356.8 mm in 2001 and 368.4 mm in 2002, under stressed conditions (Fig. 1). Irrigation was limited from 50 days after sowing (DAS) for stressed plants in both years. This water shortage period was chosen because previous experiments on comparable genetic material carried out in the same site showed that a water cumul of about 320 mm at 50 DAS conducted to an fraction of transpirable soil water (FTSW) that decreased progressively below 0.6 after this date on early groundnut (Clavel et al., 2004). This FTSW value represents a threshold below which plant generally begin experiences water deficit (Sadras and Milroy, 1996). For control plants, irrigation was added according to the rainfall pattern in order to provide optimal water supply. For groundnut in Sub-Sahel conditions, this optimal is below field capacity in particular at the beginning of cycle. As natural rainfall distribution resulted in a larger quantity of water during the first growing month in 2001 (228.4 mm in September) than in 2002 (115.4 mm in September), the water shortage for stressed plants at 50 DAS occurred after 340.6 mm in 2001 and 308.4 mm in 2002. In order to accentuate genotypic differences, which were weak in 2001, larger amount of irrigation was supplied the last month of the 2002 cycle (Fig. 1). Therefore, the differences in water supplies between stressed zplants and non-stressed plants were lower in 2001 (28%) than in 2002 (47%).

All plots were harvested 80 DAS which is the date of optimum maturity previously established on these lines under comparable water regimes.

2.4. Measurements

Pod and haulm yields were determined as the dry weights of pods and haulms after air-drying to constant weight done from the whole area of each given plots. For assessment of agronomic drought resistance, two yield-based drought responses indices were established from pod yield individual measurements. The stress susceptibility index (SSI) measures the relative yield reduction, normalised for the population and stress tolerance index (STI) integrates both stress induced yield reduction and yield potential of a genotype relative to the population yield potential, as following:

$$\mathrm{SSI} = \frac{1 - \left(Y_\mathrm{s}/Y_\mathrm{i}\right)}{1 - \left(\bar{Y}_\mathrm{s}/\bar{Y}_\mathrm{i}\right)} \quad \text{(Fisher and Maurer, 1978)}$$

$$\mathrm{STI} = \frac{Y_\mathrm{i} Y_\mathrm{s}}{(\bar{Y}_\mathrm{i})^2} \quad \text{(Fernandez, 1992)}$$

where Y_i is the pod yield of well-watered (irrigated) plots, Y_s the pod yield of stressed plots and $\bar{Y}_\mathrm{S}$ and $\bar{Y}_\mathrm{i}$, the same parameters averaged for the population.

Groundnut having indeterminate flowering, earliness is classically measured by the date of starting flowering and the percentage of mature pods at harvest. Flowering time was assessed as the date (in days after sowing) of appearance of at least one flower on at least 50% plants of each plot (F 50%). The maturity percentage of pods at harvest (Mat %) was determined on a 200 g sample of dried pods randomly taken from each plot and then hand-shelled. It was estimated based on visual classification of colour of the internal pericarp of the hull from opened pods (Williams and Drexler, 1981).

Relative water content (RWC) was measured around midday, weekly from the beginning of stress application until 2–3 days before harvest. The measurements were done during the last 5 weeks of growth. RWC was determined gravimetrically in sample of 10 foliar disks of 0.5 cm diameter sampled from the third leaf, counting from the top of the main shoot, as RWC = [(FM − DM)/TM − DM] × 100, where FM is fresh mass determined immediately after punching the discs, TM is turgid mass after 4 h rehydration of the discs in distilled water, under dark conditions, at room temperature (28 ± 1.5 °C) and DM is the dry mass after drying at 85 °C for 24 h.

Chlorophyll fluorescence parameters were quantified using a portable Plant Efficiency Analyser type MK2 (PEA, Hansatech Instruments Ltd., UK). The measurements were realised at 56, 63 and 70 DAS only in BC73 lines in 2001. In 2002, fluorescence was quantified weekly on both series of lines from 5 days after the application of the stress. Leaves were adapted to darkness for 30–45 min by attaching light exclusion clips to the surface on three topmost full-blown leaves in situ taken on three plants randomly chosen in each plot. The fluorescence responses were induced by flash exposure to saturated white light with a photon flux density of about 3000 μmol m^{-2} s^{-1}. Among the chlorophyll fluorescence parameters given by the equipment, only two of them, Fv/Fm, ratio of the maximal fluorescence in dark-adapted state, and SFI, were considered because these parameters showed significant differences according to treatments and/or lines in the analysis of variance. The Fv/Fm traduces the maximal quantum yield of photochemistry in dark-adapted state and SFI or Vitality Index expresses an ability of plant to avoid drought and maintains its physiological activity at a certain level. This Vitality Index combine criteria of structure and function: it reflects the fraction of non-photochemical phenomenon (fluorescence and heat dissipation) when the majority of the PSII reaction centres are open for maximal photon absorption (Strasser and Strasser, 1995; Strasser et al., 1999).

2.5. Experimental design and statistical analyses

The experimental designs of four experiments were in split-plot with three replications in 2001 and four in 2002. Watering treatment at two levels, well-watered and end-of-cycle water deficit constituted the main plots (secondary factor for analysis of variance) and the lines constituted the sub-plots (main factor for analysis of variance). Each plot consisted in five lines of 6.15 m long and spaced by 0.5 m of which the three central lines were used as given plot. The border plants at the beginning and at the end of each line were not considered for measurements and observations.

Data for each trait were processed for analysis of variance (ANOVA) and means comparisons using the SAS/STAT software (version 6.22). The extent of the genotypic variation for each measured trait was evaluated by its ability to statistically discriminate among closely related genotypes under varying watering conditions. The standard deviations (S.D.) of the distributions were calculated as S.D. $= \sqrt{\sum d^2/n}$, with d is the deviation from the mean and n is the the number of observations. Genotypic means were compared using Student–Newman–Keuls (SNK) test for single effects at the 0.05 probability level.

The relationships between traits and pod yields and between drought response indices and pod yields were determined using simple linear regression analysis at the $P < 0.05$.

3. Results

3.1. Yield components and stress response indices

The amount of rainfall was higher and its distribution was better in 2002 than in 2001 for both conditions (Fig. 1): weak water deficit occurred at the beginning of cycle in 2002 but limited water supply before flowering is considered as favourable to pod and haulms production on groundnut (Nageswara Rao et al., 1985, 1988). High yields in 2002 relatively to 2001 (Figs. 2 and 3) were, therefore, principally due to the better 2002 rainfall. The end-of-cycle water deficit significantly decreased pod yields in 2001 and in 2002 on both series of lines (Figs. 2 and 3). Increased pod yield under stress were observed for all selected BC55 lines compared to their female and male parental line, 55-437 and Chico, excluding 55-314 and 55-15a in 2001 (Fig. 2A). These two lines were eliminated in 2002 and the selected lines showed equivalent or better performance than the female parent, 55-437, and better performance than the male parent, Chico (Fig. 2B). In 2001, all BC73 lines performed better than the recurrent parent, 73-30, on the average of yield but not better than Chico (Fig. 3A). In 2002, however, the lines performed better than Chico under water deficit (Fig. 3B). These results show that the precocity donor, Chico, has low yield potential. On the other hand, the BC73 lines were more severely affected by the water deficit (Fig. 2B) than the BC55 lines (Fig. 3B), as showed by the data obtained in 2002 when the water regimes were highly differentiated.

The comparison of STI values allowed a statistical differentiation of genotypes for both seasons and series of lines whereas SSI data (results not shown) did not in any case; it is the reason why the SSI data were not presented in Table 1. Statistical differences on STI appeared markedly in 2002 for the male parent Chico, which confirms its poor yield potential (Table 1). The parental recurrent line 55-437

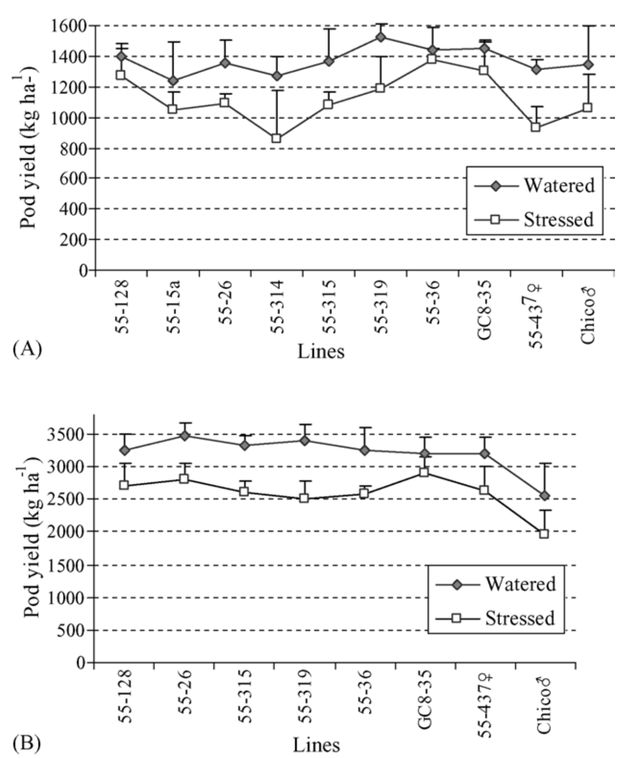

Fig. 2. Pod yields means of BC55 lines compared to parental lines according to water treatment in 2001 (A) and 2002 (B).

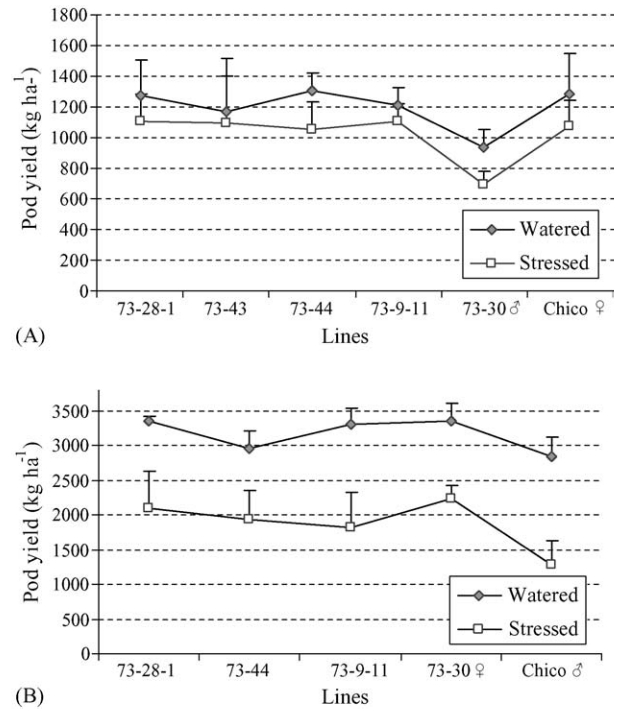

Fig. 3. Pod yields means of BC73 lines compared to parental lines according to water treatment in 2001 (A) and 2002 (B).

was classified as among the more susceptible lines, significantly in 2001 along with 55-15a and 55-314 but not in 2002 (Table 1A). The drought susceptibility of the recurrent parent 73-30 comparing to its progenies was significant in 2001 whereas the STI gave Chico as the more susceptible in 2002 (Table 1B). Thus, the drought susceptibilities of genotypes given by the STI values provided information in good agreement with the yield performance analyses contrary to SSI values.

3.2. Earliness

Except for the line 55-26, times to start flowering (F 50%) were generally significantly shortened for other breeding lines compared to the recurrent female parent 55-437 (Table 2A). Flowering earliness transfer was not so successful for the BC73 lines, which were, in the main, similar to 73-30 on this trait except for 73-43 and 73-44. This was observed both years (Table 2B). Similar differences between genotypes were observed for maturity percentages which were comparable to that of Chico, the precocity donor, for these lines (Table 2B). Well-watered conditions generally significantly increased the level of maturity of genotypes but variations between years, water regimes and genotypes were high on this trait. Under well-watered conditions, the maturity percentages obtained in 2002 were inferior to those in 2001 on both series of lines; it therefore seems that ample water resources at end-of-cycle (case of 2002) did not favour high maturity percentages.

Table 2
Flowering dates (F 50%) in days after sowing and maturity percentage of pods at harvest (Mat %) on BC55 and BC73 lines compared to parental lines according to water treatment in 2001 and 2002

Genotype	2001				2002			
	F 50%		Mat %		F 50%		Mat %	
	Watered	Stressed	Watered	Stressed	Watered	Stressed	Watered	Stressed
(A) BC55								
55-128	24.3	24.3	74.4	69.0	23.3	23.0	62.5	62.9
55-15a	24.3	24.0	70.7	61.6	–	–	–	–
55-26	24.7	24.3	62.8	60.2	24.5	24.3	49.6	47.8
55-314	24.0	24.0	64.1	48.9	–	–	–	–
55-315	24.0	24.0	67.5	47.4	23.3	23.8	56.3	40.2
55-319	24.0	24.0	70.7	60.0	23.5	23.8	42.2	40.9
55-36	24.0	24.0	71.5	65.7	24.0	24.0	58.2	52.6
GC8-35	24.0	24.0	73.1	62.7	23.5	23.0	62.5	57.2
55-437 ♀	24.7	26.0	60.4	45.2	25.0	24.3	38.0	36.9
Chico ♂	23.0	23.0	77.1	72.5	22.8	23.3	74.3	62.0
Genotype	***		***		***		***	
Treatment	n.s.		***		n.s.		*	
S.D.	0.68		1.61		0.83		12.61	
(B) BC73								
73-28-1	25.3	25.0	62.5	59.6	24.5	24.5	47.4	38.1
73-43	24.3	23.7	71.2	69.5	–	–	–	–
73-44	24.0	23.0	70.1	70.3	22.0	22.3	69.1	52.3
73-9-11	25.3	25.3	60.5	52.5	24.8	25.0	41.9	29.3
73-30 ♀	26.0	25.3	48.3	47.9	25.3	25.0	26.9	26.2
Chico ♂	24.7	23.3	72.5	74.1	23.0	24.5	75.4	50.9
Genotype	***		***		***		***	
Treatment	n.s.		n.s.		n.s.		*	
S.D.	1.05		11.48		1.54		18.27	

S.D. is the standard deviation value around the mean for each character. Significance of the genotype and treatment differences was represented by *, *** at the 0.05, 0.001 probability levels, respectively. Genotype × treatment effects were generally non-significant with the exception of Mat % in 2002 on BC73 lines where it was *.

3.3. Water status

No genotypic effect was observed on BC55 lines either in 2001 and 2002 whereas, highly significant genotypic effects were observed in 2001 on RWC responses of BC73 lines (Fig. 4A). For BC73 lines, the water treatment effect was not significant in 2001 except for 73-44 and Chico of which RWCs at 62 DAS decreased more rapidly under water shortage than that of other lines (Fig. 4A). This result (not shown) was confirmed on 73-44 in 2002. The absence of water treatment effect in 2001 on this trait was may be masked by high genotypic effect considered as main factor in the split-plot design. For BC55 lines, on the contrary, the RWC began to decrease from 70 DAS in 2001 (results not shown), and 7 days sooner (63 DAS) in 2002 (Fig. 4B).

In conclusion, the genotypic discrimination using RWC trait depends on the water regime and genetic background of compared lines. This discrimination was generally absent for the BC55 lines and high and early for BC73 lines. So, this trait has no value as selection criteria but serves to characterize finely the water status of plants.

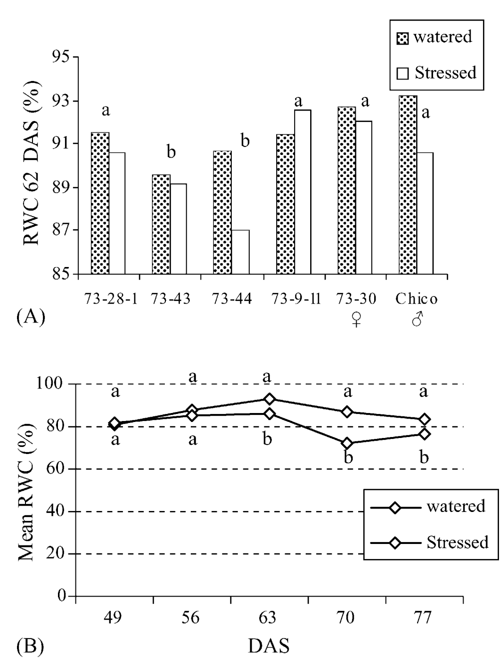

Fig. 4. Relative water content (RWC) of BC73 lines in 2001 (A) and BC55 lines in 2002 (B).

3.4. Photosynthesis efficiency

Among the variables given by the equipment used, Fv/Fm and SFI presented the highest variation according to water treatment and lines. In 2001, the measurements were only made on the BC73 lines. In 2001, the water treatment had no significant effect for this trait, as for all other traits except pod yield. However, significantly higher SFI values were observed for the line 73-9-11 than for other lines while the lowest values were observed for 73-43 and 73-44 lines throughout the measurement period (result not shown). SFI, although repeatedly correlated with Fv/Fm (r between 0.89 and 0.94), gave better genotypic discrimination than Fv/Fm. In 2002, on the contrary to 2001, the water treatment effect occurred early, less than 1 week after stress application, for both series of lines. A significant genotypic effect appeared at the end of stress period for both experiments (Fig. 5). On BC55 lines, Fv/Fm and SFI values of the precocity donor, Chico, were the weakest under both conditions at 77 DAS (Fig. 3A). On BC73 lines, no genotypic effect was observed on Fv/Fm at any date but a significant water treatment and genotypic effects were observed since 55 DAS for SFI. The significant interaction genotype × treatment ($P < 0.01$) observed at 69 DAS on SFI showed that this parameter was more stable for the line 73-9-11 than for the other lines (Fig. 3B). These results were confirmed at the end-of-cycle where SFI value of 73-9-11 was significantly higher than those of 73-44 and Chico (Fig. 3C). In summary, data obtained on these fluorescence parameters showed strong sensitivity to drought and good genotypic discrimination particularly in the case of SFI. High photochemical efficiency was showed on 73-9-11 compared to other BC73 lines whereas the BC55 lines seemed very similar on this parameter.

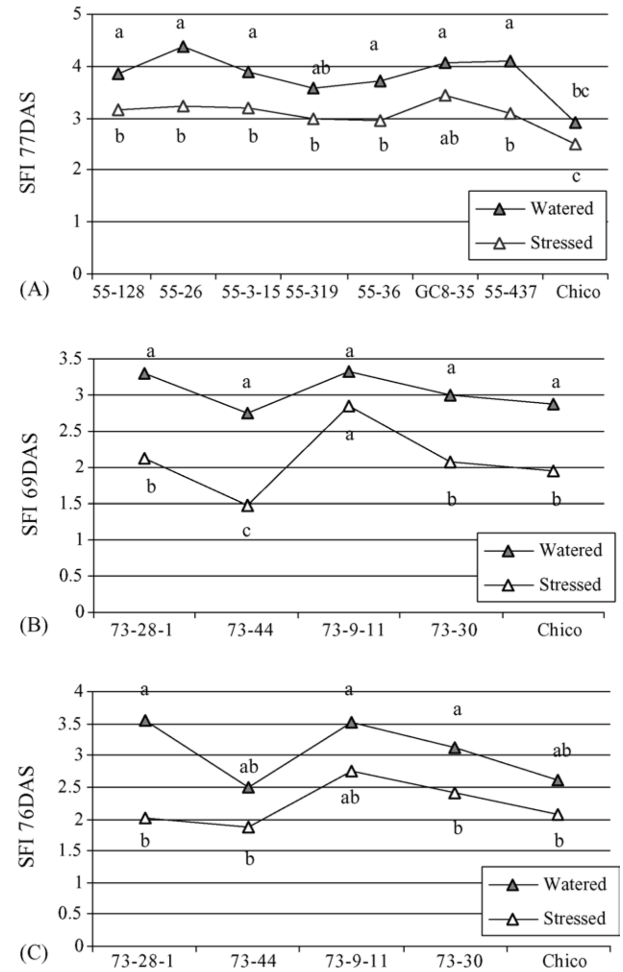

Fig. 5. Stucture-function-index (SFI) calculated from fluorimeter measurements on BC55 lines at 77 DAS (A) and on BC73 lines at 69 DAS (B) and 76 DAS (C) in 2002.

Table 3
Correlations coefficient (r) for regressions between physiological traits and pod yield under stressed and across conditions on BC55 and BC73 lines in 2001 and 2002

Traits[a]	2001		2002			
	Correlations (r) BC73 lines		BC55 lines		BC73 lines	
	Stressed	All plots	Stressed	All plots	Stressed	All plots
RWC 48DAS	−0.54*	−0.49*	−0.40*	n.s.	n.s.	n.s.
RWC 55DAS	n.s.	n.s.	−0.79***	n.s.	0.55**	0.66***
RWC 76DAS	−0.58**	n.s.	−0.52**	−0.47**	0.65***	0.80***
LAI 48DAS	−0.62**	n.s.	−0.40*	n.s.	0.70***	0.88***
LAI 69DAS	n.s.	n.s.	n.s.	n.s.	0.61***	0.45*
LAI 76DAS	n.s.	n.s.	0.37*	0.50**	0.69***	0.62**
Fv/Fm 56DAS	−0.59*	n.s.	0.30*	0.47**	n.s.	0.59**
Fv/Fm 63DAS	n.s.	n.s.	−0.55***	0.37**	n.s.	n.s.
Fv/Fm 70DAS	−0.45*	n.s.	n.s.	0.52***	n.s.	n.s.
SFI 56DAS	−0.66**	n.s.	−0.59***	0.41***	n.s.	0.44*
SFI 63DAS	−0.46*	n.s.	−0.66***	0.55***	n.s.	0.50*
SFI 70DAS	−0.51*	n.s.	−0.47*	0.63***	n.s.	0.48*

r values followed by *, **, *** indicate significance of regression at 0.05, 0.01, 0.001 probability levels, respectively. Fluorimeter measurements were not realised on BC55 lines in 2001and no correlation was significant in 2001 on these lines.

[a] Physiological traits were considered and times of measurements were indicated in day after sowing (DAS).

Table 4
Correlations coefficient (r) for regressions between traits under watered conditions and yields under drought stress (Y_s)

	Correlation (r) BC55 lines	Correlation (r) BC73 lines
Correlation between traits under watered and Y_s		
2001	n.s. for any trait	SFI 56DAS, $r = -0.58^{**}$ SFI 70DAS, $r = -0.47^{*}$
2002	RWC 70DAS, $r = -0.49^{**}$ SFI 70DAS, $r = -0.75^{***}$	LAI 62DAS, $r = 0.48^{*}$

r values followed by *, **, *** indicate significance of regression at 0.05, 0.01, 0.001 probability levels, respectively. Fluorimeter measurements were not realised on BC55 lines in 2001.

3.5. Yield or yield-related traits correlations

In 2001, the fluorescence parameters were not measured and no significant correlation was established on BC55 lines. On BC73 lines, by contrast, two measurements of RWC, and all fluorimeter measurements realised (Fv/Fm and SFI) were correlated to yield (Table 3). For RWC and fluorimeter measurements significant negative correlations with yield under stress were found (Table 3). Most of the negative correlations on RWC, Fv/Fm and SFI established in 2001 on BC73 lines under drought were also significant in 2002 but on BC55 lines instead of BC73 lines where there were positive or not significant (Table 3).

Correlations between traits measured under well-watered conditions and yields under stress (Ys) could reveal constitutive traits of genotypes. They were found (negative) on BC73 lines for SFI 56DAS ($P > 0.01$) and SFI 70DAS ($P > 0.05$) in 2001 (Table 4). These negative correlations were also observed for RWC ($P < 0.01$) and SFI 70DAS ($P < 0.001$) in 2002 on BC55 lines.

Yields observed under drought were not correlated with yields under well-watered conditions but yields obtained in 2001 were correlated with yields of 2002 for both series of lines (Table 5). On the other hand, STI, which integrates yield reduction and yield potential, showed high correlations with yields under both treatments (Table 5) whereas SSIs were weakly or not correlated with Yi (results not shown) confirming its poor value in identifying lines with high potential yields.

Table 5
Correlations coefficient (r) for regressions between pod yields (Y_i for well-watered and Y_s for stressed conditions) across treatments and crop seasons and between pod yields and for stress tolerance index (STI) in both series of lines in 2001 and 2002

	Correlation (r) BC55 lines	Correlation (r) BC73 lines
Across treatments		
Y_i and Y_s in 2001	n.s.	n.s.
Y_i and Y_s in 2002	n.s.	n.s.
Across crop seasons		
All plots in 2001 and 2002	0.43^{**}	0.50^{**}
Drought-responses indices		
Crop season 2001		
STI – Y_i	0.86^{***}	0.45^{*}
STI – Y_s	0.72^{***}	0.85^{***}
Crop season 2002		
STI – Y_i	0.78^{***}	0.58^{**}
STI – Y_s	0.87^{***}	0.95^{***}

r values followed by *, **, *** indicate significance of regression at 0.05, 0.01, 0.001 probability levels, respectively.

4. Discussion

For a selection trait to be potentially relevant for breeding, the basic requirements would be that this trait was chosen on the basis of existing variability detected on appropriate genetic materials, that it was correlated with yield performance in the field and that it was easy to measure on large number of genotypes.

4.1. Association of precocity traits and high yield

The earliness of flowering and high pod maturity at harvest are the primary attributes to consider for improving groundnut under end-of-season water deficit because only the first flowers emitted produced mature pods especially when the crop season is brief (Khalfaoui, 1990a; Wright and Nageswara Rao, 1994). The BC55 lines were superior to the female parent for both traits in 2002, which means that these traits appeared to be efficiently transferred. The precocity transfer was less efficient for the BC73 breeding lines probably due to the genetic background of the female line because the methodology of transfer by selection was the same. High susceptibility of maturity of pod at harvest to end-of-cycle drought (Chapman et al., 1993; Khalfaoui, 1990b) was confirmed.

Yield and earliness measurements confirmed that short cycle duration is not the only one factor that explains yield performances under end-of-cycle stress since the earliest line, Chico, showed, among the poorest values for yield and STI responses. These results show that the back-cross selection practised for both precocity and high yield under water stress, have been efficient for improving yield and precocity together.

4.2. Responses on physiological parameters

RWC is considered as a more useful integrator of plant water balance than leaf water potential (Sinclair and Ludlow, 1985; Wright and Nageswara Rao, 1994). Moreover, our previous results have shown that it was more stable and water sensitive than water potential on groundnut (Clavel et al., 2005). It was therefore chosen instead of leaf water potential. Significant decreases on RWC seemed in close accordance with soil water availability which was related to rain distribution before the application of water deficit. Moreover, an interaction genotype by treatment was observed on the BC73 lines that showed that the parental line, 73-30, conserved high RWC under stress longer than the other lines. Consequently, RWC can be used to monitor differential plant responses to water supply. Genotypic discrimination for RWC occurred only for BC73 lines and it at different periods of the stress according to experiments. On the other hand, its measurement is time-consuming; these traits cannot, therefore, constitute valuable selection criteria. Fluorescence parameters, Fv/Fm and SFI, followed comparable patterns to RWC for treatment effect but contrary to the preceding trait, presented significant variability among lines. Chlorophyll fluorescence gives an indirect measurement of the efficiency of photochemistry processes involved in photosynthesis. The energy dissipated by fluorescence is inversely proportional to the conversion in chemical energy for photosynthetic carbon assimilation (Maxwell and Johnson, 2000). The fluorescence emitted is then closely related to the efficiency of photochemical apparatus, essentially the photosystem II (PSII), because it is more susceptible to drought and then, plays a major role on photosynthetic activity under water shortage. In fact, water deficit inhibits photosynthesis through stomatal (reduction of CO_2 uptake) as referred by Ludlow and Muchow (1990) and non-stomatal effects, which includes a direct damage of PSII (Glynn and Colin, 2002; Colom and Vazzana, 2003). The SFI is defined as ''vitality'' resultant of activity and resistance (Strasser et al., 1995). When plants close their stomata under water stress their photosynthetic activity decrease. However, a withered plant can posses a high photosynthetic activity and yield potential as shown by 73-9-11 compared to 73-30. The SFI is then a good indicator of this plant ability at chloroplast scale.

4.3. Associations of traits with yield

When contrasted materials are compared the relationship high RWC ⇔ high yield is habitually observed (Nautiyal et al., 1995). The negative correlations between RWC and yield under stress established for both series of lines (Table 3) could have been observed thanks to the genetically proximity of compared lines. This observation supports the hypothesis that a strategy of drought avoidance by early stomatal closure favouring high water status can be associated with poor yield potential on groundnut (Gautreau, 1977; Annerose, 1988; Clavel et al., 2004, 2005).

According to Fussell et al. (1991), traits that correlates with yield measured under stress and yield measured in the absence of stress were assumed to be constitutive (independent of the effects of the stress) whereas traits that correlate with yield only when measured under stress were assumed to be adaptive (reflecting the responses of genotypes to stress). Regarding data from Table 3, the majority of trait-responses studied in 2001 and 2002 would be adaptive except, maybe, the fluorescence parameters. Correlations between traits measured under well-watered conditions and yields under stress can also be used to identify traits that confer constitutively drought tolerance (Dingkuhn, personal communication). As correlations were found for BC73 and BC55 lines in 2001 and 2002 on SFI and RWC (Table 3), this confirmed that photochemical efficiency is probably constitutive. Moreover, the SFI values showed great genetic variation (Fig. 5). Thus, the integrity of PSII is likely a key character in the drought adaptation of groundnut. As PSII centre is situated on chloroplast thylakoïde membranes, decrease in membranes tolerance and restricted CO_2 availability under drought stress could possibly lead to increased susceptibility to photodamage (Colom and Vazzana, 2003). Observations that drought tolerant plants at the cellular level are able to keep their stomata open under severe water deficits (stomatal closure at low RWC) was documented in different studies on diverse plant species (Costa França et al., 2000; Bajji et al., 2002; Dhanda and Sethi, 2002) including groundnut (Clavel et al., 2005). The measurement of chlorophyll fluorescence in situ is then a useful tool to evaluate the tolerance of the photosynthetic apparatus to environmental stresses (Rizza et al., 2001; Maxwell and Johnson, 2000). However, opposite yield correlations with some RWC, and fluorimeter measurements were observed: negative correlations in 2001 under stress for BC73 lines and in 2002 for BC55 but not significant or positive for other situations (Table 3). A possible explanation of the negative correlations between physiological measurements and yields under stress is that under particular conditions, plants with weak stomatal conductance (thus with high RWC and weak CO_2 uptake) may have high photoinhibition but high yield under stress. It is not clear therefore if the value for SFI must be high or low to favour high yield under stress and what conditions allows expressing the largest genotypic variability and highest correlation with yield. Potential applications of chlorophyll fluorescence were recently reviewed (Baker and Rosenqvist, 2004). This review indicates that they can provide very rapid and efficient assessment of plant performances in a wide range of situations especially in the complex case of screening for tolerance to environmental stress. Once a comprehensive understanding of how changes in plant fluorescence relates with yield under stress of a particular species will be clarified, technical developments in fluorescence measurements can be imagined to allow rapid screening of numerous groundnut lines. From this complex pattern of correlations, it can be deduced that constitutive and adaptive traits probably influence interactively the phenotypic responses making it difficult to establish generally valid physiological selection criteria. The main reason for this is that plants, and especially a drought-adapted species as groundnut, possess several drought-adaptive strategies depending on their genetic background and environmental pressure. Nevertheless, it is likely that cell-level tolerance mechanisms (Wright and Nageswara Rao, 1994; Nautiyal et al., 1995; Clavel et al., 2005) and tolerance of photosynthetic apparatus are important constitutive component of drought adaptation in groundnut and need to be taken into account in variety selection.

5. Conclusion

The study demonstrated that the new lines selected using yield and earliness as selection criteria were superior to their parents and that they conserved genetic variation. In addition, the different agronomical significance of the two drought response indices used (SSI and STI) showed that it is very critical for progress in classical breeding to interpret these indices suitably in order to characterize properly the drought resistance variety in the field.

Genotypic responses were obtained whatever lines and treatments on some of the fluorescence parameters, in particular the structure-function-index values that traduce the status of photochemical apparatus. The technique of chlorophyll fluorescence, as it is rapid, sensitive and non-destructive, could therefore become a useful method for determining variations in tolerance of the photosynthetic apparatus in breeding for resistance to drought. Nevertheless, the role and value of the fluorimetric responses for maintaining yield under drought needs to be clarify due to difference existing in groundnut drought adaptation strategies. Future selection schemes for improvement of drought adaptation in the cultivated groundnut species are therefore possible by using fluorescence parameters in association with yield-based studies in the field.

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