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Analysis of early responses to drought associated with field drought adaptation in four Sahelian groundnut (*Arachis hypogaea* L.) cultivars

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Abstract

Groundnut (*Arachis hypogaea* L.) is the most important oil and cash crop in the sub-Sahelian tropics. Plant adaptation to drought, i.e. cultivars (cvs) that can maintain yield when water is limited, is a complex phenomenon which is not yet fully understood. This study aimed to identify traits expressed at the early stages of the cycle that could reveal cv differences in drought adaptation in the field. The field productivity of four Sahelian groundnut cvs was assessed during three crop seasons in Bambey (Senegal). The same cvs grown in rhizotrons were subjected to early drought stress and to a desiccation test to assess cell membrane tolerance. Between-cv differences were found with respect to pod yield, biomass production, water use efficiency (WUE), stomatal regulation and cell membrane tolerance. Two strategies to cope with water deficit were identified. The first behaviour was characterised by high rapid water loss, late stomatal closure and low cell membrane damage during drought. These traits are all found in the semi-late Virginia cv 57-422 and, into a lesser extent, in the early Spanish cv Fleur 11. For both cvs, biomass production was boosted under favourable conditions in rhizotrons but the semi-late cv had poor pod yield under end-of-season water deficit conditions. The second strategy involved opposite characters, leading to the maintenance of a higher water status, resulting in lower photosynthesis and yield. This characterised the early Spanish cv 73-30, and also, to some extent,

Abbreviations: cv, cultivar; RLA, relative leaf area (cm^{-2}); TDM, total dry mass (g); SLW, specific leaf weight ($g\,cm^{-2}$); ADM, above ground dry mass ($g\,plant^{-1}$); RDM, root dry mass ($g\,plant^{-1}$); DAS, days after sowing

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the early Spanish cv 55-437. Earliness associated with high WUE, stomatal conductance and cell membrane tolerance, were the main traits of Fleur 11, a cv derived from a Virginia × Spanish cross, which was able to maintain acceptable yield under varying drought patterns in the field. These traits, as they were detectable at an early stage, could therefore be efficiently integrated in groundnut breeding programmes for drought adaptation.

Keywords: Groundnut (*Arachis hypogaea* L.); Cultivars; Sub-Sahelian Africa; Responses to early drought; Stomatal regulation; Protoplasmic tolerance; Breeding traits

1. Introduction

Groundnut, *Arachis hypogaea* L., is the most important oilseed and cash crop in the semi-arid tropics (Ntare et al., 2001). Over the last 20 years, a serious decline in rainfall levels has been observed in the Sahel and sub-Sahelian regions. In Senegal, one of the most drought prone areas is the groundnut growing area, usually called the 'groundnut basin'. The main focus of the groundnut improvement programme carried out in the central and northern regions of the Senegalese 'groundnut basin' was to create early groundnut varieties that could withstand severe drought spells thanks to their physiological adaptive capacities (Annerose, 1991; Khalfaoui, 1991).

Understanding the mechanisms of drought tolerance in leguminous species naturally adapted to drought, such as bean or groundnut, can help to improve their agronomic performance (Subbarao et al., 1995; Cruz de Carvalho et al., 1998; Costa França et al., 2000). Considerable research has been undertaken on the physiological and molecular mechanisms involved in drought adaptation (Bohnert et al., 1995). However, there is still no comprehensive standard system for measuring drought resistance (Blum, 1999), especially because the physiological model approach is not always adequate for selection because of negative correlations between physiological traits involved in drought adaptation (Turner et al., 2001). Breeding groundnut would seem possible by using substitute measurements of water use efficiency in the field (Wright et al., 2002) or using a crop growth model (Subbarao et al., 1995), but these field studies are costly, time-consuming and affected by environment variations. Data on early physiological or growth changes obtained under controlled conditions are more precise than field results, but a simple and direct link between a particular trait and the maintenance of yield under drought has never been proven. Consequently, plant improvement programmes have not been able to fully exploit existing physiological data (Richards, 1996; Turner et al., 2001). Nevertheless, the use of early physiological variations between genotypes could be of substantial help for plant breeding provided that these changes also reveal behavioural differences in the field.

The aim of this study was to look for variety differences at an early growth stage in groundnut, which could reflect variations in yield performance under drought in the field. This required the following two steps: (i) to characterise early physiological differences among cvs; (ii) to check if these differences are linked to field production under drought stress. This study involved four Sahelian groundnut cultivars (cvs) whose growth parameters and physiological traits were measured in rhizotrons under controlled watering conditions, while yield components were determined under varying drought patterns during three crop seasons.

2. Materials and methods

Two types of experiments were carried out on four cvs: in the field and in rhizotrons.

2.1. Plant material

Four groundnut cultivars selected by the Institut Sénégalais de Recherches Agricoles (ISRA) and the Centre de Cooperation International en Recherche Agronomique pour le Développement (CIRAD), were used in this study: two commonly cultivated Spanish type drought-tolerant cvs, 55-437 and Fleur 11, with a 90-day cycle (Clavel and N'Doye, 1997), and two formerly released cvs, 57-422, a Virginia type with a 105-day cycle and 73-30, also a Spanish type with an

90-day cycle (Lauriano et al., 1997; Lauriano et al., 1997, 2000).

2.2. Field experiments

Comparative variety trials were conducted during the 1999, 2000 and 2002 rainy seasons at the experimental station of Bambey (14.42 N and 16.28 W), located in the semi-arid zone of the 'groundnut basin' in Senegal. Two seeds pre-treated with Granox (10% Captafol-10% Benomyl-20% Carbofuran) per hole were hand-sown. The seedlings were thinned to one per hole 1 week after sowing, resulting in a density of 133 300 plants/ha. No fertilizer was applied, in compliance with farmers' usual groundnut cropping practices in the Sahel. The crop was treated with pesticides and the field was maintained weed-free throughout the study.

2.3. Water supply and statistical design of the field trials

The trials were sown in mid-July after the first substantial rainfall and conducted during the rainy season without supplementary irrigation except in 2002 when two water treatments were applied. The first treatment was strictly rainfed, with 385 mm total rainfall, and the second was supplemented by irrigation at the end of cycle, for a total water input of 560 mm. Field trials were generally arranged in a randomised complete block design with four replicates, except in 2002 when a split-plot design was used, with cvs as the main factor and water treatment as the secondary factor.

2.4. Productivity measurements in the field

The plot size for the productivity measurements was 12.3 m^2. The plants were harvested 90 or 105 days after sowing (DAS) depending on the earliness of the cvs. The harvested plants were exposed to ambient temperatures of 30–35 °C so as to allow complete drying of pods and haulms to less than 5% pod moisture. Pod yield was determined as the dry mass of pods, and the harvest index (HI) was calculated as the ratio of the dry weight of pods and haulms.

2.5. Rhizotron experiment

The rhizotron experiment was carried out for 6 weeks (42 days) and conducted at the end of the 1997 dry season at the experimental station of Bambey. The rhizotrons were built with 100 cm high, 15 cm diameter PVC tubes with a plexiglas window. They were filled with sifted sandy soil, called 'dior', obtained from the Bambey station, and then placed at an angle of 30° on a metal chassis, so that the root system adhered tightly to the window and was thus easily measurable. A transparent tarpaulin shelter was installed to protect the experiment from rain. Minimum and maximum recorded air temperatures were 28 and 44 °C, respectively, with a mean temperature of 34 °C. Relative humidity averaged 75%. Seeds were first powdered with 'Granox' and pre-germinated 24 h before sowing. Two seeds per rhizotron were sown and the seedlings were later thinned to one per rhizotron. One litre of NPK fertilizer (6-18-27), at a dose of 0.3 $g\,l^{-1}$, was applied for each rhizotron.

2.6. Water supply and statistical design of the rhizotron trials

The initial water supply was calculated to simulate a 25 mm rainfall, which is the average level for the first rain of the crop season in the Sahel (Annerose and Diagne, 1990). This limited quantity of initial water supply allowed us to quickly induce a water deficit while not completely waterlogging the soil column. During the first 2 weeks of growth, the plants were watered regularly (2.5 mm/day) so as to maintain optimum growth conditions. Half of the rhizotrons was subjected to water deficit 14 DAS by withholding irrigation, while the other half was watered as before. The rhizotrons were arranged in a two-factorial design, replicated four times, with the cultivar (cv) and water regime as the two studied factors. The experimental unit consisted of four plants.

2.7. Growth measurements in rhizotrons

Rooting depths were measured at 14, 24 and 35 DAS directly on the transparent side of the rhizotron, and noted as RD14, RD24 and RD35 cm, respectively. At the end of the experiment at 42 DAS, plants were unpotted, the roots washed and the root dry mass (RDM) determined. Individual aboveground dry mass (ADM) was measured after 48 h drying at 80 ± 1.5 °C. The total dry mass (TDM) was the sum of ADM and RDM. Since between-genotype variations in stem biomass are generally limited (Turner et al., 2001), it was considered that the major genotypic differences in aerial parts with respect to the water regimes was due to leaf number and size. The relative leaf area (RLA) was therefore estimated by the following formula:

$$\mathrm{RLA} = \frac{1}{\mathrm{SLW}}\mathrm{ADM}$$

where, SLW ($\mathrm{g\,cm^{-2}}$) is the specific leaf weight of the third leaf of each plant, determined at 42 DAS, and ADM, the aboveground dry mass ($\mathrm{g\,plant^{-1}}$).

Water use efficiency (WUE) was given in $\mathrm{g\,mm^{-1}}$ (El Hafid et al., 1978). It was determined by weighing rhizotrons at the beginning and end of the experiment and was calculated as the amount of TDM produced per unit of water lost by evapotranspiration.

2.8. Water status measurements in rhizotrons

The second pair of leaflets of the third leaf from the top of the main shoot was used to measure the leaf water potential (ψ_1) and relative water content (RWC). These measurements were obtained between 12 and 14 h, and weekly from 14 DAS. ψ_1 was determined with a Scholander pressure chamber (PWSC 3005, Soil Moisture Equipment Corp., CA, USA) and RWC was calculated from gravimetric measurements using the following formula (Catsky, 1960):

$$\mathrm{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}}$$

Samples of 10 leaf disks (0.5 cm diameter) were weighed immediately after they were punched out (fresh weight), rehydrated overnight at $5 \pm 1.5\,^{\circ}\mathrm{C}$ (turgid weight), and then oven dried for 24 h at $85 \pm 1.5\,^{\circ}\mathrm{C}$ (dry weight).

2.9. Stomatal regulation

Water loss from leaves when submitted to a dehydration treatment was linked to stomatal regulation, which may be variable according to cvs. The time decrease in RWC was monitored on excised leaves using the method of Hygen (1951), modified by Clavel and Annerose (1995). Leaves came from well-watered 28-day old plants grown in rhizotrons. The third leaf from the top of the main shoot was collected and immerged in distilled water for 4 h under natural full light at room temperature so as to maintain the complete turgor of cells and the stomatal aperture. The leaves were then quickly wiped and weighed to obtain their turgid weight. Eleven successive weighings of each leaf were made under ambient conditions during a dehydration period lasting 115 min. The first eight weighings of fresh leaves were carried out every 3 min, and the last three were conducted every 20 min. Dry weight was determined after oven-drying for 24 h at 85 °C. Each timely RWC was calculated using the formula given in the previous section. This procedure was repeated four times for each cv.

2.10. Electrolyte leakage measurements

Electrolyte leakage from leaf tissues in response to desiccation stress has been measured by different authors as a means of assessing membrane stability (Blum and Ebercon, 1981; Vasquez-Tello et al., 1990; Costa França et al., 2000). Desiccation stress was simulated using a polyethylene glycol (PEG) solution applied on 0.8 cm diameter leaf discs punched out of fully expanded leaves from 21 day-old well-watered plants. The discs were rinsed twice in distilled water for 1 h to remove solutes from damaged cells. A first set of 96 discs (24 per replicate and per cv.) were then incubated in distilled water (control discs) and another set was floated in PEG 4000 solution at 400 g/l concentration, with an osmotic potential of −2 MPa (treated discs). After at least 4 h incubation, all discs were rinsed three times in distilled water and floated overnight on distilled water in the dark at room temperature and a first conductivity reading was made. Leaf tissues of control and treated samples were then killed by 1 h autoclaving and a second conductivity reading was made after equilibration at room temperature. The conductivities of discs floating in solution were determined using a conductimeter (PROLABO, CD6-NG, Paris, France). The injury index (%) was measured according to Vasquez-Tello et al. (1990) using the following formula:

$$\text{injury}\,(\%) = \frac{1 - (1 - T_1/T_2)}{1 - C_1/C_2} \times 100$$

where T_1 and T_2 were the first and second conductivity readings for the PEG treatment and C_1 and C_2 the first and second conductivity readings for the control treatment.

2.11. Statistical analysis

Data were processed using the SAS/STAT software package (Version 6.22) for variance analyses, which were performed according to the statistical design used: randomised complete block or split-plot for field studies, and two-factorial design for the rhizotron experiment. Means were compared using the Student-Norman and Keuls (SNK) test for single effects at the 0.05 probability level. The significance of correlations between traits were determined using regression analysis at the $P = 0.05$ threshold.

3. Results

3.1. Field productivity according to the water regime

Although the rainfalls of 1999, 2000 and 2002 (irrigated treatment) were comparable (total: 498–566 mm), their distributions were different. In 1999, there was a water deficit at the end of the cycle, with only 60 mm for the last month of cultivation compared to 190 and 175 mm for the other two situations (Table 1). In 2002 (rainfed treatment), the total rainfall recorded was the lowest, totalling 385 mm, including only 55 mm for the last month of cultivation. Pod yield and harvest index (HI) are shown according to rainfall and watering conditions after day 60, corresponding to the pod filling and maturation phase (Table 1). In 2002, when rainfall was supplemented with irrigation (560 mm of rain, including 175 mm for the last month), no significant difference was recorded between cvs. On the contrary, when the water supply was very limited at the end of the cycle (2002-rainfed and 1999), cvs Fleur 11 and 55-437 gave the best yields and HIs, while 57-422 and 73-30 appeared to be the most sensitive to drought. Under mild terminal water stress conditions (2000), Fleur 11 showed the highest yield but the 105-day duration cv 57-422 yielded comparably. For the late cv, however, the end-of-season water deficit had a strong effect on pod yield, with HIs of 0.55 and 0.66 in 2000 and 2002-irrigated, respectively, compared to 0.14 and 0.24 in 1999 and 2002-rainfed, respectively. On average, Fleur 11 gave the highest pod-yields and HIs and 73-30 the poorest.

3.2. Leaf water status

Between 14 and 35 DAS, the three short cycle cvs showed a slow ψ_l decrease from −0.5 to −2 MPa

Table 1
Pod yield (kg ha^{-1}) and harvest index (HI) for the four groundnut cultivars (cvs) obtained in field conditions at Bambey (Senegal) during three crop seasons while varying the water supply

	Year				Mean
	1999 (rainfed)	2000 (rainfed)	2002 (rainfed)	Irrigated	
Annual rainfall	498	566	385	385	
After 60 DAS	60	190	45	175	
Total (mm)	498	566	385	560	502 ± 84
cvs					
57-422 (105-day)					
Pod	1362 ab	3080 ab	1510 ab	3897 a	2462 ± 66
HI	0.14 b	0.55 b	0.24 c	0.66 ab	0.40 ± 0.25
55-437 (90-day)					
Pod	1776 ab	2657 b	2908 a	3201 ab	2635 ± 188
HI	0.22 a	0.52 b	0.40 b	0.41 b	0.39 ± 0.12
73-30 (90-day)					
Pod	1232 ab	2418 b	1927 ab	2958 b	2133 ± 58
HI	0.13 b	0.62 b	0.33 b	0.36 b	0.36 ± 0.20
Fleur 11 (90-day)					
Pod	2624 a	3648 a	2899 a	3873 a	3261 ± 93
HI	0.24 a	0.79 a	0.72 a	0.75 a	0.63 ± 0.26

For each parameter, means followed by the same letter are not significantly different according to SNK mean comparison test at $P < 0.05$. Mean values across years are followed by a standard error value.

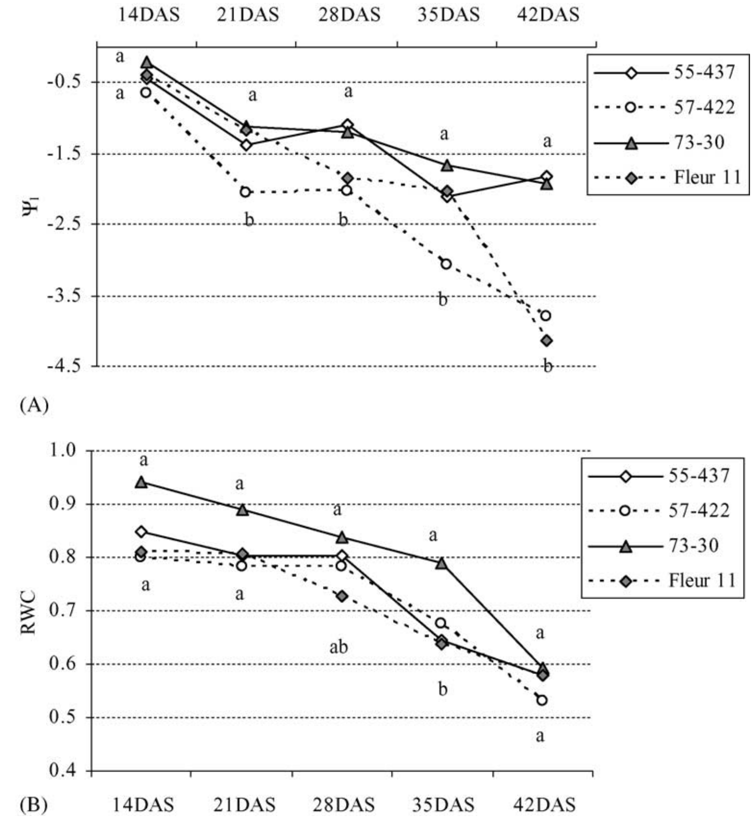

Fig. 1. Change in leaf water potential ψ_l (A) and RWC (B) with the water stress progress expressed in days after sowing (DAS). Water stress was applied by withholding irrigation at 14 DAS. For each cultivar and each time, means followed by the same letter are not significantly different according to SNK mean comparison test at $P < 0.05$.

(Fig. 1A). This behaviour corresponds to isohydric species, characterized by early stomatal closure, which prevents leaf dehydration. At the end of the experiment, 1 week later, Fleur 11 and 57-422 reached the same ψ_l, of about −4 MPa, which was significantly lower than that of 55-437 and 73-30 (Fig. 1A). Conversely, the regular ψ_l decrease observed on cv 57-422 illustrates the long opened stomata period typical of anisohydric species. The relative water content (RWC) data showed that the drought susceptible cv 73-30 always had higher water contents than the other cvs, particularly until 35 days of applied water deficit (Fig. 1B). In summary, the rapid and regular decrease in water status of 57-422 contrasts with the relative stability of the leaf water status of 73-30, at least until 35 days after stress application.

3.3. Effect of drought on plant growth parameters

Under well-irrigated control conditions, in the rhizotron experiment, cv 57-422 (105-day) and Fleur 11 (90-day) had the highest TDM. In contrast, the poorest growth performances were recorded in cvs 55-437 and 73-30 (Table 2). The ADM and RLA were directly linked since the specific leaf weights did not vary according to varieties and watering conditions (Table 2b). ADM and RDM and RLA were reduced in all cvs under drought. Fleur 11 and 57-422 showed the highest WUE under well-watered conditions and their WUEs did not increase under drought conditions. However, the WUE of Fleur 11 was the highest in all conditions (Table 2). In contrast, the WUE for 55-437 and 73-30 was low under well-watered conditions but significantly

Table 2
Growth parameters of four groundnut cultivars grown in rhizotrons under well-irrigated (control) and water stress conditions (drought)

Cycle	cv	ADM (g plant^{-1})		RDM (g plant^{-1})		TDM (g plant^{-1})	
		Control	Drought	Control	Drought	Control	Drought
Aerial dry matter (ADM), root dry matter (RDM) and total dry matter (TDM)							
105-day	57-422	1.662 a	0.591 cd	1.373 ab	0.654 c	3.035 a	1.097 d
90-day	55-437	0.754 c	0.480 d	0.681 c	0.603 c	1.435 c	1.083 d
90-day	73-30	1.119 b	0.558 cd	1.014 b	0.605 c	2.133 b	1.163 d
90-day	Fleur 11	1.357 ab	0.570 cd	1.627 a	0.664 c	2.984 a	1.234 cd

Cycle	cv	RLA (cm^2)		WUE (g mm^{-1})	
		Control	Drought	Control	Drought
Relative leaf area (RLA) and water use efficiency (WUE)					
105-day	57-422	342.331 a	126.655 cd	0.0115 ab	0.0105 ab
90-day	55-437	164.029 c	100.397 d	0.0054 cd	0.0103 ab
90-day	73-30	208.556 b	130.414 cd	0.0080 c	0.0111 ab
90-day	Fleur 11	305.906 a	124.660 cd	0.0113 ab	0.0118 a

Cycle	cv	RD14 (cm)	RD24 (cm)		RD35 (cm)	
		Control	Control	Drought	Control	Drought
Rooting depth under control conditions at 14 DAS (RD 14) and under both control and water deficit conditions (drought) at 24 (RD 24) and 35 (RD 35) DAS						
105-day	57-422	23.6 b	45.5 b	46.0 b	57.0 a	47.9 b
90-day	55-437	19.5 c	48.5 ab	45.5 b	52.5 ab	53.2 ab
90-day	73-30	23.1 b	48.5 ab	49.2 ab	52.2 ab	45.5 b
90-day	Fleur 11	28.3 a	53.2 a	47.0 b	56.3 a	49.7 b

For each parameter, means followed by the same letter are not significantly different according to SNK mean comparison test at $P < 0.05$ probability; drought was applied by water shortage 14 days after sowing.

higher under water deficit conditions, as generally observed.

Rooting depth (RD) was measured every 10 days in order to monitor progress of the root front according to watering conditions. Fleur 11 showed the deepest root system whilst 55-437 showed the most superficial root system before water deficit application, as shown by the RD14 results (Table 2). Significant genotypic differences in root length thus existed prior to the application of water stress. Differences between cvs diminished with time under both control and water deficit conditions. Between 24 and 35 DAS, only weak progression of root growth was observed and root elongation was not boosted by drought. These phenomena were attributable to the partial waterlogging of the soil in the rhizotrons. Nevertheless, cv Fleur 11 still had the deepest roots under control conditions. It should also be noted that the rooting depth of 73-30 was slightly increased under drought conditions. However, since there was no difference as regards RDM, this increase probably concerned only thin roots.

3.4. Relationships between water status parameters

RWC and ψ_l were closely related, as expected: the coefficient of correlation from the logarithmic type regression curve (Fig. 2A) was 0.73 ($P < 0.001$). No direct relationship was found between ψ_l and any of the other growth parameters (not shown). On the contrary, RWCs at 35 and 42 DAS were closely correlated (r = 0.64, $P < 0.001$, and r = 0.59, $P < 0.001$, respectively) with TDM (Fig. 2B and C). It is, however, interesting to note that this relationship became negative at 35 DAS (r = −0.54, $P < 0.05$, Fig. 2D) or disappeared afterwards (results not shown) when only stressed plants were considered in the regression calculation. This means that although high leaf water status levels allow the plant to grow well, high water status under stressed conditions

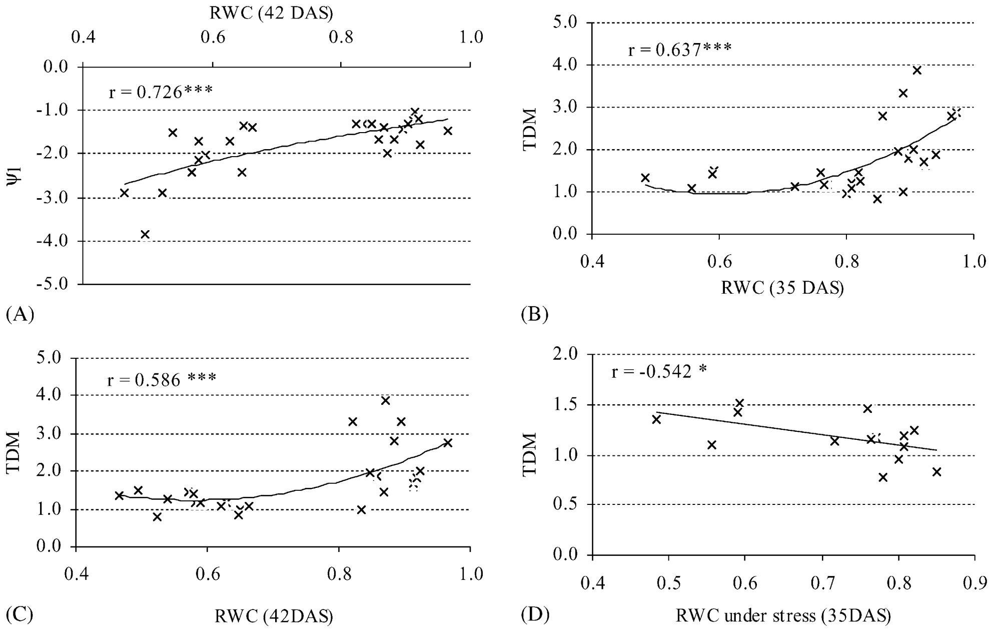

Fig. 2. Relationship between RWC at 42 DAS and ψ_l (A), RWC at 35DAS and TDM (B), RWC at 42 DAS and TDM (C) under both conditions and regression between RWC at 35 DAS and TDM (D) under water deficit conditions, for the four cultivars. (***) correlation is significant at the $P < 0.001$ level; (*) correlation is significant at the $P < 0.05$ level.

is not necessarily advantageous for biomass production, at least at the young stage.

3.5. Stomatal regulation

RWC was determined using young leaves excised from well-hydrated plants, which were left to dry up progressively under ambient conditions. The curves shown in Fig. 3 were established by calculation of RWCs after determination of leaf weight losses during a drying period of 115 min (11 weighings). The standard curves obtained fitted power regression curves with a determination coefficient higher than 0.95. The inflexion point indicates a rupture, corresponding to the RWC (and time) at which stomatal closure occurred. After this point, only cuticular transpiration was responsible for water loss. The time of stomatal closure was very similar irrespective of the cv, but there were differences between cvs for RWC at stomatal closure. The group of 90-day cvs showed early stomatal closure, which was obtained for RWC ranging between 83 ± 1 and 76 ± 2% (Fig. 3A–C). In this group, Fleur 11 closed its stomata the latest (Fig. 3C). The second type of stomatal behaviour was represented by the cv 57-422, which showed late stomatal closure when leaf RWC reached 68 ± 5% (Fig. 3D).

3.6. Cell membrane tolerance

Ion leakage, as a measurement of protoplasmic tolerance, allows evaluation of the ability of cell membranes to maintain their integrity at low water status (Costa França et al., 2000; Bajji et al., 2002). The suitable osmotic potential of the PEG solution was first established at −2 MPa to reveal maximum genotypic differences. The injury index of 73-30 was significantly ($P < 0.05$) higher than the indices obtained for Fleur 11 and 57-422, and that of 55-437 was intermediate (Fig. 4). This data confirms previous results, showing that 57-422 presented higher protoplasmic tolerance than 55-437 and 73-30 (Lauriano et al., 2000; Khalfaoui, Annerose, pers. comm.). Based on these results, the four cultivars could be ranked as follows: Fleur 11 and 57-422 were the most tolerant, 55-437 in an intermediate position and 73-30 was the most susceptible.

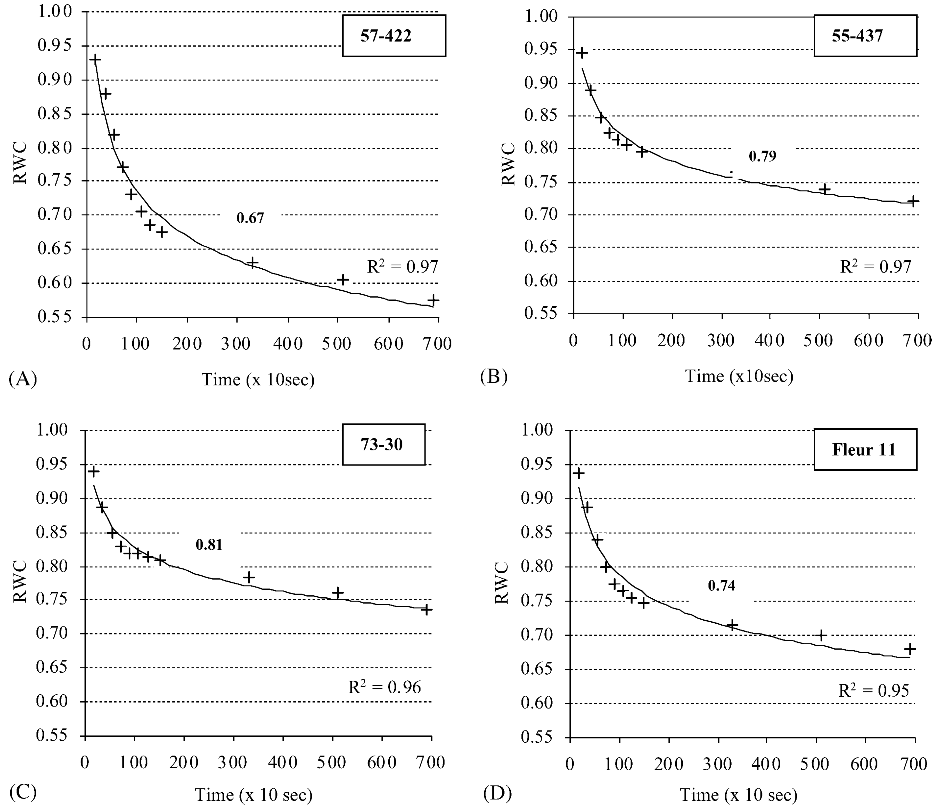

Fig. 3. Standard power regression curves of leaf relative water content (RWC) decreases during 6900 s (115 min) air-drying of excised leaves of the four well-hydrated groundnut cultivars (cvs). The inflexion point defined by the curves indicates the RWC at stomatal closure (RWCsc, indicated in bold in the figures) of each cv. Measurements were realised four times for each cv: means RWCsc were 0.68 ± 0.05 for 57-422, 0.81 ± 0.01 for 55-437, 0.83 ± 0.01 for 73-30 and 0.76 ± 0.02 for Fleur 11.

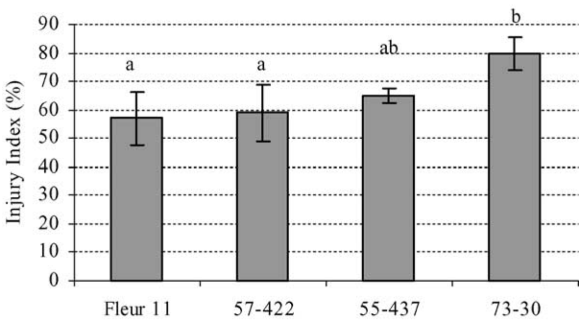

Fig. 4. Mean injury index of four groundnut cultivars subjected to PEG 4000 (osmotic potential: −2 MPa). Histograms represent means of four replicates of 24 leaf discs per cv. For each cultivar, means followed by the same letter are not significantly different according to SNK mean comparison test at $P < 0.05$ and bars represent the standard deviation.

4. Discussion

4.1. Physiological traits favourable to drought resistance in groundnut

Numerous physiological traits have been shown to potentially contribute to yield under stress, but the development of an efficient breeding method for drought resistance in groundnut is still a long-standing objective (Subbarao et al., 1995; Wright et al., 2002). Among these traits, stomatal control of water loss has been recognized as an early response for conditioning the leaf water status of plant in the field, but it severely limits carbon uptake and biomass production (Chaves, 1991; Chaves et al., 2002). Many experiments have shown that stomata close in response to hydraulic and chemical signals produced when soil moisture decreases (Davies and Zhang, 1991; Chaves et al., 2002). No evidence of involvement of a chemical signal has been demonstrated in groundnut, but a direct link between water status and stomatal closure has been proposed (Bennett et al., 1984). The fact that the cv with the lowest RWC at stomatal closure (57-422) presented a continuous reduction in RWC as the water stress increased whereas that with the highest RWC at stomatal closure (73-30) showed a more constant RWC agrees with this hypothesis. RWC is considered to be a more useful integrator of plant water balance than the leaf water potential (Sinclair and Ludlow, 1985; Wright and Nageswara Rao, 1994). The positive relationship found between RWC and TDM when all plants were considered demonstrated that biomass production is associated to the water status (Bennett et al., 1984; Sinclair and Ludlow, 1986; Wright and Nageswara Rao, 1994). However, in a certain number of cases, comparable TDM were associated with RWC varying substantially, suggesting differences in tolerance mechanisms according to cvs. The observed negative relationship between biomass production and RWC when only stressed plants were considered for regression calculation shows the detrimental effect of early stomatal closure for producing dry matter. This is probably due to the high degree of co-regulation of stomatal conductance and photosynthesis in groundnut (Farquhar, 2001). Since stomata influence the influx of CO_2 into leaves, the reduction in stomatal conductance to conserve water inevitably indicates a lower photosynthetic rate (Ludlow and Muchow, 1990; Medrano et al., 2002). It is thus quite possible that RWCs were negatively correlated with TDM in young groundnut plants subjected to drought.

The rooting depth measurements obtained from plants cultivated in rhizotrons did not reveal any significant differences between cvs during stress because of the contradictory requirements to obtain a rapid response to stress and irrigate rhizotrons at field capacity. These conditions did not allow us to obtain specific information on genotypic differences concerning root length. For future studies on the root system it will be worth repeating this experiment with modified moisture conditions in rhizotrons.

The membrane injury index evaluated by electrolyte leakage measurements could be related to several physiological and biochemical parameters conditioning plant responses under water stress (Blum, 1988; Dhanda and Sethi, 2002; Bajji et al., 2002). In particular, photosystem II, the main reaction centre of the photochemical efficiency of photosynthesis, which is located on cell membranes, is very sensitive to water stress (Glynn and Colin, 2002). Conversely, restricted CO_2 availability under drought stress could lead to increased susceptibility to photodamage (Baroli and Melis, 1998; Colom and Vazzana, 2003). The hypothesis that drought tolerant plants at the cellular level are often able to keep their stomata open under severe water deficits (low RWC at stomatal closure) was established in a study involving four cvs of another leguminous plant, i.e. *Phaseolus vulgaris* (Costa França et al., 2000). In durum wheat, this index is not only correlated with the growth of seedlings of various cultivars but also with their field performances (Bajji et al., 2002) and the same relationship was observed in wheat (Dhanda and Sethi, 2002). In our study, the ranking obtained for the membrane injury index classified 73-30 as the most susceptible and Fleur 11 and 57-422 as the most resistant. The ranking of the four cvs based on their RWC at stomatal closure was the same as that of membrane injury.

4.2. Groundnut drought adaptation strategies

The first strategy developed by groundnut to cope with drought stress is earliness, which is a key factor under severe end-of-season water deficit conditions. In addition, we observed that Fleur 11 was the high yielding cv and 73-30 the poor yielding one, even though the latter developed avoidance mechanisms. Therefore, groundnut is able to develop at least two strategies to deal with water deficit. The first represented by the 105-day cv 57-422, is characterised by late stomatal closure and regular RWC decrease during drought that reflect a drought tolerant behaviour. This cv had also the highest biomass in well-watered conditions. The second feature is illustrated by 73-30 and 55-437 which can be considered as nearly isohydric, maintaining high RWC by early stomatal closure. These two opposite behaviours can lead to quite similar yields under mild drought, depending on the ability of the crop to store dry matter, which can be mobilised under drought so as to feed the pods. The cv Fleur 11 presents an intermediate behaviour with a high harvest index, water use efficiency and yield, an intermediate stomatal closure and high protoplasmic tolerance. This combination of traits probably characterizes a broad adaptation under a range of water limited environments (Wright et al., 1998). It can be thought that the cv optimises its biomass production before pod growth, due to its higher WUE, so as to mobilize stored assimilates when the stomata are closed. These results agree with those of Gautreau (1977), who concluded that among varieties with appropriate cycle lengths a high relative transpiration level, generally associated with a low water potential, is often observed in the most productive groundnut varieties under drought. Blum (1988) also stated that if selection were possible using this trait for stressed environments, plants with high transpiration levels should be promoted. The suitability of stomatal regulation as a comparison parameter for photosynthetic capacity was recently demonstrated for C3 plants (Medrano et al., 2002). Associating a high capacity to keep stomata open during stress and cellular membrane tolerance to dehydration thus seems relevant for breeding for drought resistance in groundnut. This objective could be achieved by combining the dry matter production efficiency from the Virginia types, such as 57-422, to the earliness and high WUE under drought of the Spanish type. To corroborate this hypothesis, it should be noted that cv Fleur 11 comes from a single cross combining Virginia and Spanish groundnut types (Mayeux, pers. commun.)

5. Conclusion

The results of this research highlighted the need for using different traits when evaluating drought adaptation of plants. In the case of the four cvs studied, the high productivity of Fleur 11 in the field under both water stress and non-stress conditions was probably associated with its early and efficient biomass production, high WUE and protoplasmic tolerance. All of these attributes could be measured in pots under controlled water supply conditions in a time-period of less than 42 days. Cell membrane tolerance has proven to be an important trait regardless of the type of water stress (Vasquez-Tello et al., 1990; Bajji et al., 2002), and could be associated with other favourable attributes for drought adaptation in groundnut. These results were obtained within the framework of a breeding programme geared mainly towards developing groundnut genotypes with physiological attributes of drought adaptation and they are now being used to this end. However, as the common drought pattern in the sub-Sahelian region is mainly an end-of-season water deficit (Annerose, 1991; Ndunguru et al., 1995), and since the maintenance of acceptable yield under water deficit is the first requirement, the identification of traits associated with yield variation in field conditions remains essential (Clavel et al., 2004).

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