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Evaluation of groundnut genotypes for heat tolerance[1]

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Summary

Heat tolerance of groundnut (*Arachis hypogaea* L.) genotypes was evaluated by solute leakage and chlorophyll fluorescence techniques in heat-hardened and non-hardened plants. To determine the appropriate hardening treatment, 1-month-old plants of two groundnut genotypes, ICGV 86707 and Chico were conditioned at five combinations of hardening (37°C) and non-hardening (30°C) air temperatures over a 5-day period. Heat injury, was assessed through measurements of electrolyte leakage after stressing leaf discs to 55°C for 15 min. The relative injury was significantly influenced by the conditioning temperatures and by the temperature during 24 h prior to measurement if those involved non-hardening conditions. Relative injury and chlorophyll fluorescence were measured after stressing leaves of six genotypes at a range of temperatures between 49°C and 55°C. Significant genotype × hardening treatment interactions were observed in relative injury and chlorophyll fluorescence. Chico was susceptible to heat stress, the relative injury test identified ICGV 86707 as tolerant, and the chlorophyll fluorescence test identified ICGV 86707 as tolerant under hardened conditions and ICGV 87358 as tolerant when non-hardened. When expressed as percentage of control values, the relative injury and chlorophyll fluorescence measurements over the 49–53°C stress temperature range were strongly correlated. Chlorophyll concentrations were increased by hardening in all genotypes except Chico. In Chico, chl_b concentration was decreased and the $chl_{a/b}$ ratio increased by hardening, and chlorophyll concentrations were correlated with chlorophyll fluorescence parameters. Chlorophyll concentration may therefore provide an alternative means of screening for heat tolerance.

Key words: *Arachis hypogaea L.*, peanut, chlorophyll concentration, chlorophyll fluorescence, electrolyte leakage, heat hardening

Introduction

Temperature is an important factor in all aspects of plant growth and development. The optimum temperature for growth of a plant or crop species is an important determinant of its geographical distribution and growing season. Crops are generally grown in areas to which

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they are naturally adapted. However, to meet the growing demand for food, especially in developing countries in the tropical and sub-tropical regions, crops are increasingly grown outside their traditional area of adaptation and outside their natural growing seasons. Under these conditions, daily or seasonal temperature often exceeds the optimum (30°C) for growth and reproductive development and high temperature becomes a major factor limiting crop production (McWilliam, 1980). Growing environment may deteriorate further due to global warming (Schneider, 1989). Breeding of heat-tolerant genotypes in crop species is therefore necessary. To do this requires sources of heat tolerance to be identified, an understanding of the mechanisms of heat tolerance to be developed and screening methods to rapidly measure tolerance to be produced. Little information is available on the responses of groundnut to high temperature (Ketring, 1984; Srinivasan, Takeda & Senboku, 1996).

Although several plant processes are more sensitive to heat, plant adaptation to high temperature essentially requires a cell membrane system that remains functional during heat stress (Raison, Barry, Armond & Pike, 1980). Schreiber & Berry (1977) also considered the thermostability of photosynthetic apparatus as being an important determinant of heat tolerance. Direct high temperature injury in crop plants has been evaluated by electrolyte leakage (Sullivan, 1972) and chlorophyll fluorescence tests (Aoki, 1989; Moffet, Sears & Paulsen, 1990) which measure thermostability of cell membrane and photosynthesis, respectively. In several crops, such as sorghum (Sullivan & Ross, 1979), soybean (Martineau, Specht, Williams & Sullivan 1979), and wheat (Blum & Ebercon, 1981), genotypic differences in heat tolerance have been determined by the electrolyte leakage test, and in some cases, a significant relationship found with performance in high temperature environments (Martineau *et al.*, 1979). A high correlation between heat and desiccation tolerance has been reported (Sullivan & Ross, 1979). It has also been shown that hardening treatments improve the heat tolerance of wheat, cucumber, and soybean, and that an appropriate hardening treatment is necessary for detecting genotypic differences in heat tolerance with this method (Chen, Shen & Li, 1982; Lester, 1985; Sadalla, Shanahan & Quick, 1990). No information is available on heat tolerance of groundnut using any of the above mentioned methods. The objectives of this study, therefore, were to: a) determine appropriate hardening requirements to detect genotypic differences in heat tolerance, b) evaluate a limited number of genotypes using electrolyte leakage tests and chlorophyll fluorescence, and c) examine the effects of hardening on chlorophyll concentration and its relationship with chlorophyll fluorescence.

Materials and Methods

Plant material

The experiments were conducted during June to August 1993. Seeds were sown in vinyl plastic pots (12 cm diameter) containing 800 g of powdered sandy soil (a Kaolinitic hyperthermic Ultisol). The pH of the soil used was 4.5 initially, but was raised to 5.5 by mixing it with 4 g of coral limestone kg^{-1} soil and incubating it for 3 wk at 12% soil moisture. In addition, a commercial fertiliser containing 14% each of N, P and K was mixed with the soil at 0.5 g kg^{-1} soil before filling the pots. Four seeds per pot were sown which, when emerged, were thinned to two per pot. The pots were kept in plastic trays and irrigated daily by adding water to the trays. The pots were kept in a naturally-lit greenhouse maintained at 30/25–28°C day/night temperatures. The two genotypes used for the hardening studies were ICGV 86707 and Chico and the six genotypes to determine differences in heat tolerance were ICGV 86635, ICGV 86707, ICGV 87358, Chico, TMV 2 and JL 24, which were selected as showing differences in drought tolerance.

Growing conditions during the hardening treatment

To study the conditions of hardening, 1-month-old plants of ICGV 86707 and Chico were subjected to different combinations of non-hardening (30°C) and hardening temperatures (37°C) in two growth chambers (Eyelatron, FL1 301N, Tokyo Rikakikai Co. Ltd, Japan) over a 5-day period. Both chambers were held at 12 h photoperiod and 400 μ mol m^{-2} s^{-1} photosynthetic photon flux. Five combinations of conditioning temperatures were used: a) 4 days at 30°C, 1 day at 37°C (NH4H1); b) 2 days at 30°C, 3 days at 37°C (NH2H3); c) 3 days at 30°C, 1 day at 37°C, then 1 day at 30°C(NH3H1NH1); d) 1 day at 30°C, 3 days at 37°C, then 1 day at 30°C (NH1H3NH1); e) a control treatment of 5 day at 30°C (NH5). For the electrolyte leakage and chlorophyll fluorescence tests, plants were conditioned at 30 days after sowing with the NH3H1NH1 and NH5 treatments, and with the NH2H3 and NH5 treatments for the determination of the effects of hardening on chlorophyll concentration.

Electrolyte leakage test to measure cell membrane thermostability

Cell membrane thermostability was measured using the procedure described by Martineau *et al.* (1979). Each assay sample consisted of two sets of 12 leaf discs cut with a 1.2 cm diameter punch from 12 leaflets from the fully expanded leaves at the uppermost two nodes of each plant. There were three replications.

Before each assay, the two paired sets of leaf discs were placed into two separate test tubes and washed thoroughly with at least four changes of distilled water to remove electrolytes released from cut cells at the periphery of the discs. The excess water was removed from the tubes, and the tubes covered with plastic film. One set of discs was then incubated for 15 min at 55°C in a temperature-controlled water bath whilst the other control set was maintained at 25°C. After the temperature treatment, the incubated tubes were quickly cooled to 25°C before adding 15 ml of distilled water to both sets of tubes. The tubes were then placed in an incubator for 18 h at 10°C to allow leakage of electrolytes from the discs. The tubes were then brought back to 25°C, inverted several times to mix the contents, and an initial measurement of solution conductance made using an electrical conductivity meter (CM-115, Kyoto Electronics, Japan), after which tubes were covered with aluminum foil and autoclaved at 120°C for 10 min to kill the leaf tissues. The autoclaved tubes were cooled to 25°C, the contents mixed thoroughly, and a second conductance measurement taken. The relative injury (*RI*) to cell membranes resulting from the hardening temperature treatments was calculated as:

$$RI(\%) = [1 - (1 - (T_i/T_f))/(1 - (C_i/C_f))] \times 100$$

where T and C refer to the conductances of the treatment and control solutions, respectively, and the subscripts i and f to initial and final conductance, respectively. The ratio of initial to final conductance (i.e. T_i/T_f) is a relative measure of electrolyte leakage caused by elevated temperature, and consequently a measure of the extent of damage to cellular membranes; it should reflect the injury to the cell membrane at the elevated temperature.

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured using a modulated fluorimeter (Hansatech Electronics Ltd, UK) on the abaxial surface of five leaflets taken from 8 fully-expanded young leaves, four from each plant, excised from heat-hardened (NH3H1NH1) and non-hardened (NH5) plants. There were five replicated pots per genotype for each hardening treatment. The leaflets were placed in 1 ml distilled water in test tubes and the tubes were then covered with plastic film, and either left at room temperature or heated for 5 min at four stress

temperatures of 49°C, 51°C, 53°C or 55°C in a water bath to induce heat injury to the photosynthetic apparatus. A set of non-heated tubes served as control. After the heat treatment, leaflets were placed in the dark for 30 min at room temperature, after which dark-adapted leaflets were placed into a leaf clip to which a modulated light probe and a detector probe were attached. The leaflets were exposed to actinic light and saturating light pulses through the fiber-optic cables connected to a Björkman Lamp (1800 μ mol m^{-2} s^{-1} photosynthetically photon flux; Hansatech Electronics Ltd, UK). The fluorescence signal at 700 nm, read directly to computer was used to calculate the initial fluorescence (F_0), the maximum fluorescence (F_m) and the variable fluorescence (F_v) derived by subtracting F_0 from F_m and the F_v/F_m ratio. This F_v/F_m ratio is a measure of efficiency with which light is utilised for photosynthesis. Upon heat treatment, F_v/F_m ratio declines indicating photo-inhibitory damage due to heat stress. There were five replications for each temperature treatment.

Chlorophyll determination

Chlorophyll was extracted from 12 leaf discs, each 1.2 cm diameter, in 10 ml of 80% acetone for 24 h in the dark at room temperature (25°C). The volume of the chlorophyll extract was made up to a 50 ml with 80% acetone, and the absorbance read at 645 and 663 nm on a spectrophotometer (U2000. Hitachi Ltd, Japan). The concentrations (mg cm^{-2} leaf area) of chl_a and chl_b were calculated using the following equations:

$$Chl_a = 12.7 \times A_{663} + 2.69 \times A_{645}$$
$$Chl_b = 22.9 \times A_{645} - 4.68 \times A_{663}$$

Three replicated measurements were made.

Data analysis

The results of electrolyte leakage tests and chlorophyll concentration data were analysed in a split plot design with genotypes as main plots and the hardening as sub-plots. The chlorophyll fluorescence data were analysed in a split-split-plot design taking genotypes as main plots, stress temperatures as sub-plots and hardening treatments as sub sub-plots. The association between the *RI* and chlorophyll fluorescence and chlorophyll fluorescence and chl_b concentration was determined by standard correlation analysis. The GENSTAT software (version 4.01) of the Rothamsted Experimental Station was used for statistical analysis.

Results

Effect of hardening

Preliminary experiments showed that the threshold stress temperature that caused a relative injury (*RI*) score of 50% was 55 ± 0.7°C. The score was significantly decreased ($P < 0.01$) by the hardening treatments (Table 1). The highest *RI* score was obtained for the non-hardened treatment (NH5) and the score was higher for plants in which the hardening treatment was followed by a non-hardening treatment (i.e. NH1H3NH1 and NH3H1NH1) than when the hardening treatment was not followed by a non-hardening treatment (i.e. NH4H1 and NH2H3). Three consecutive days of hardening (i.e. NH2H3) had a greater effect on the *RI* score of Chico than a single day's hardening (i.e. NH4H1) and a greater effect than in ICGV 86707. The largest differences between the two occurred when the plants received one day's

Table 1. *Effect of hardening treatment on the relative injury (%) to heat treatment at 55°C to two groundnut genotypes.* SE = *standard error of mean*

Hardening treatment			Genotype		
30°C	37°C	30°C	ICGV 86707	Chico	Mean
4	1	—	36.8	51.1	44.0
2	3	—	36.0	42.8	39.4
3	1	1	53.1	69.3	61.2
1	3	1	50.8	66.3	58.6
5	—	—	69.1	78.2	73.7
Mean			49.2	61.5	
SE					
Genotype (df = 2)			1.07		
Hardening (df = 16)			1.67		
Genotype × hardening (df = 16)			2.36		

hardening followed by one day's no-hardening (i.e. NH3H1NH1) with ICGV 86707 always having a lower *RI* score than Chico.

Genotypic differences in RI in relation to hardening

The genotype × hardening interaction was highly significant ($P < 0.01$) (Table 2). Although hardening the plants reduced *RI* score in all genotypes, the effect was more pronounced in some genotypes than others. The cv. Chico showed most injury in both the treatments, ICGV 86707 the least in the non-hardening treatment, and JL 24 the least in the hardening treatment.

Genotypic differences in chlorophyll fluorescence

The genotypic differences were highly significant ($P < 0.01$) for F_v/F_m (Table 3) and there were also significant ($P < 0.01$) responses to temperature stress (S) and hardening (H) as shown by significant G × H and G × S interactions. The effects of temperature stress varied with hardening treatment. The G × H × S interaction was highly significant for F_v/F_m ratio

Table 2. *Differences in relative injury (%) at 55°C, as measured by solute leakage from leaf tissues, between six groundnut genotypes subjected to hardening (37°C) and non-hardening (30°C) temperature treatments.* SE = *standard error of means*

	Temperature conditioning treatment		
Genotype	Non-hardened	Hardened	Mean
ICGV 86635	68.6	54.2	61.4
ICGV 86707	65.5	62.2	63.9
ICGV 87358	68.2	57.5	62.9
Chico	76.6	71.5	74.1
TMV 2	75.6	61.5	68.6
JL 24	72.0	50.7	61.4
Mean	71.1	59.6	
SE			
Genotype (df = 10)	1.58		
Hardening (df = 12)	1.07		
Genotype × hardening (df = 12)	2.44 (2.63 for the same level of genotype)		

Table 3. *F_v/F_m ratio of chlorophyll fluorescence parameters of six groundnut genotypes subjected to hardening (37°C) and non-hardening (30°C) followed by five stress temperature treatments. SE = standard error of means*

Genotype	Control	Stress temperature °C 48	51	53	55
			Non-hardened		
ICGV 86635	0.79	0.52	0.39	0.25	0.20
ICGV 86707	0.80	0.65	0.39	0.31	0.24
ICGV 87358	0.82	0.59	0.41	0.34	0.35
Chico	0.81	0.43	0.30	0.22	0.13
TMV 2	0.80	0.58	0.50	0.38	0.16
JL 24	0.81	0.62	0.37	0.22	0.17
			Hardened		
ICGV 86635	0.80	0.71	0.67	0.50	0.17
ICGV 86707	0.79	0.71	0.59	0.52	0.25
ICGV 87358	0.80	0.75	0.57	0.35	0.20
Chico	0.80	0.61	0.51	0.22	0.14
TMV 2	0.79	0.67	0.46	0.40	0.10
JL 24	0.79	0.71	0.53	0.43	0.21
SE					
Genotype (df = 20)				0.010	
Genotype × stress temperature (df = 96)				0.024	
Genotype × hardening (df = 120)				0.016	
Stress temperature × hardening (df = 120)				0.014	
Genotype × stress temperature × hardening (df = 120)				0.035	

($P < 0.01$). Temperature stress followed by hardening accounted for the maximum proportion of the variance.

The F_v/F_m ratio decreased with increasing temperature stress (Table 3). Differences between genotypes in the ratio were greater in the non-hardened plants than in hardened ones. ICGV 86707, ICGV 86635 and JL 24 had greater F_v/F_m ratios than the other genotypes when hardened whereas the ratio was largest in ICGV 87358 when plants were not hardened. The cv. TMV 2 had a high F_v/F_m ratio when non-hardened, especially up to stress temperatures of 53°C. The cv. Chico generally had the lowest F_v/F_m ratios at all stress temperatures.

The F_v/F_m ratio, at 49°C, 51°C, 53°C and 55°C as per cent of control values decreased linearly with increase in the *RI* score; the correlation was highly significant at 49°C ($R^2 = 70\%$, n = 12), but less so at higher temperatures ($R^2 = 49\%$; n = 12) and was not significant at 55°C.

Chlorophyll concentration

Chl_a, chl_b and $chl_{(a+b)}$ concentrations and $chl_{a/b}$ ratio differed significantly ($P < 0.01$) in genotypes that were heat hardened (Table 4). Chlorophyll concentrations were increased by the hardening treatment in all genotypes except Chico, in which the concentrations were slightly decreased. The $chl_{a/b}$ ratio of Chico was increased by the hardening because of the decrease in chl_b concentration in response to hardening. The correlation between the chlorophyll fluorescence and chlorophyll concentration, measured after temperature stressing leaves at 49°C, was significant and positive ($R^2 = 56\%$, n = 12).

Table 4. *Effect of hardening on the chlorophyll concentration (mg cm^{-2} leaf area) of six groundnut genotypes subjected to hardening (37°C) and non-hardening (30°C). SE = standard error of means*

Genotype	Chl_a		Chl_b		$Chl_{(a+b)}$		$Chl_{a/b}$	
	NH[a]	H[b]	NH	H	NH	H	NH	H
ICGV 86635	30.4	48.1	9.5	10.5	39.9	58.7	3.2	4.6
ICGV 86607	50.2	68.0	14.4	16.4	66.6	84.4	3.7	4.1
ICGV 87358	33.0	49.4	9.7	11.2	42.7	60.1	3.4	4.4
Chico	27.1	24.7	7.6	2.1	34.6	26.8	3.6	11.9
TMV 2	40.4	60.2	11.2	11.4	51.6	71.6	3.6	5.4
JL 24	33.4	60.1	11.6	12.9	45.0	73.5	3.0	4.8
Mean	36.0	51.8	10.7	10.7	46.7	62.6	3.4	5.9
SE								
Genotype (G), (df = 10)	1.49		0.77		2.09		0.21	
Hardening (H), (df = 10)	1.03		0.37		1.28		0.13	
G × H, 12 (df = 12)	2.31		1.00		3.04		0.30	
for same level of G	2.51		0.91		3.13		0.31	

[a]NH = non-hardened
[b]H = hardened

Discussion

Relative injury

The electrolyte leakage test is one of the more convenient methods for screening for heat tolerance in some crops (Sullivan, 1972; Martineau *et al.*, 1979; Lester, 1985). However, the method has some shortcomings. For instance, differences in leaf anatomy (MacRae, Hardacre & Ferguson, 1986) and hardening (Chen *et al.*, 1982; Lester, 1985) may induce differences in *RI* score. Therefore an attempt was made to standardise hardening in the present study. Hardening decreased the *RI* score, but the effects varied with the conditions under which hardening was induced. Differences between the two genotypes were greater if hardened plants were allowed to deharden for 24 h before measurement. The observed genotype differences could therefore conceivably be due to genotypic differences in the rate of dehardening. Similar results have been reported for musk melon (*Cucumis melo*) (Lester, 1985) and cabbage (Chauhan & Senboku, 1996). Chen *et al.* (1982), however, reported the greatest genotype differences when the *RI* score measured soon after hardening. The present results and those reported for other crops, suggest species-specific differences and similarities in the heat hardening of crops. The present study also suggests that the electrolyte leakage test, which has been developed mainly for screening purposes, can provide useful information on the mechanisms of heat tolerance when used in conjunction with different hardening treatments.

Whilst different hardening treatments may be useful in maximising detection of differences in heat tolerance among genotypes, they also simulate different environments for which heat tolerance is being sought. For example, where groundnuts are grown, temperatures may suddenly rise (e.g. in spring) or more slowly increase to injurious levels (e.g. in summer). The former is a situation in which plants are not likely to harden and the latter a situation that could enable hardening to occur. The significant interaction between genotype and hardening treatment in the present study suggests that screening at two levels of hardening may not be wholly satisfactory. Interestingly, genotypes showing the highest sensitivity to high

temperature showed similar responses to stress temperature when hardened but genotypes having least sensitivity differed more. The cv. Chico was most sensitive to high temperatures.

Chlorophyll fluorescence

Photosynthesis is one of the most heat labile of plant processes. Heat damage to the photosynthetic apparatus alters chlorophyll fluorescence (Kraus & Santarius, 1975; Schreiber & Berry, 1977) and its effects have been used to distinguish differences in heat tolerance between species. In the present study on groundnuts, the fluorescence parameters were changed significantly by the hardening and stress temperatures, and differed to a lesser extent between genotypes. The low F_v/F_m ratio of the cv. Chico indicated that this genotype is heat sensitive, in line with the results of the electrolyte leakage test. However, the genotypes that exhibited the higher fluorescence characteristics were not those that had the lowest *RI* score when non-hardened. ICGV 86707 (when hardened) and ICGV 87358 (when non-hardened) were more tolerant to stress temperatures. The fluorescence method allows many samples to be evaluated in a short time and is ideal for large scale screening, but because of strong genotype interactions, there may be a need to standardise the hardening and stress temperatures before measurement.

Chlorophyll concentration

The chlorophyll concentrations of all groundnut genotypes, except cv. Chico increased when plants were hardened. Chlorophyll concentration in cv. Chico decreased, especially that of Chl_b which resulted in an increase in $chl_{a/b}$ ratio. It is not known whether these changes are an adaptive response. In cabbage, chlorophyll concentrations were increased by hardening in heat-tolerant genotypes and decreased or unchanged in heat susceptible types (Chauhan & Senboku, 1996). In the present study, there was a significant positive linear correlation between chlorophyll fluorescence and the chlorophyll concentration and observed after subjecting leaves to a stress temperature of 49°C. The basis of this correlation and the reasons why chlorophyll concentrations increase upon hardening need to be understood if chlorophyll concentrations are to be used as a visual indicator of heat tolerance.

This study suggests that differences in heat tolerance exist between groundnut genotypes which are detectable by chlorophyll fluorescence or possibly chlorophyll concentration measurements. However, the relative injury test and chlorophyll fluorescence technique identified different heat-tolerant cultivars under non-hardened conditions and so may be responses to different mechanisms. Further studies are required to clarify this and to relate the performance of heat-tolerant genotypes identified by these *in-vitro* methods to field performance in hot environments.

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