Evaluation of Peanut Genotypes for Membrane Thermostability[1,2]

D. L. Ketring[3]

ABSTRACT

Optimum mean ambient temperatures for vegetative growth of peanut (*Arachis hypogaea* L.) plants are in the range of 25 to 30 C, while those for reproductive growth may be somewhat lower (20 to 25 C). Under field conditions the peanut crop is frequently subjected to temperatures in the range of 35 to 40 C, which adversely affect growth and development. Differences in heat tolerance have been found among genotypes of other crops. This was determined by the extent of electrolyte leakage from leaf discs exposed to elevated temperature treatment *in vitro*. These investigations were undertaken to use the *in vitro* leaf disc method as a means to evaluate field-grown peanut genotypes for membrane thermostability. A preliminary test in 1981 with ten genotypes showed significant differences in membrane injury among genotypes (G) and a significant day after planting (DAP) effect. However, CV's were excessive (about 38%). Modification of the procedure and method of leaf sampling reduced CV's to an acceptable level for field data (15-20%). Significant G and DAP effects were found. Hovever, G X DAP interactions were significant at $P<O.05$ in only one of three years of the tests, and this was due to the response of just one cultivar. Genotype differences also varied between seasons. Thus, the *in vitro* leaf disc method of testing for membrane thermostability appears useful for selecting peanut genotypes for improved crop tolerance to temperatures that adversely affect presently grown cultivars.

Key Words: *Arachis hypogaea* L., groundnut, heat tolerance, rainfed, temperature, *in vitro* leaf disc method, leaf electrolyte leakage test.

Data from both controlled environment (1, 2, 3, 4, 7, 12) and field studies (11) indicate that optimum temperatures for vegetative growth of peanut (*Arachis hypogaea* L.) are in the range of 25 to 30 C, while for reproductive growth they may be similar or somewhat lower (20 to 25 C). Many of the peanut growing areas in the United States are subjected to temperatures above 35 C during July and August. In recent years peanut yields have been severely reduced by heat and/or drought. Although the effect of temperature *per se* is difficult to separate from the heat-load imposed by high temperature that results in moisture stress, studies in a controlled environment where moisture was not limiting (4) indicated that a temperature of 35 C reduced both vegetative and reproductive growth of peanuts. These studies also indicated that there were differences among genotypes in their response to a temperature of 35 C. Thus, selection of parental germplasm for improved heat tolerance of this crop seems possible if a suitable field technique could be developed.

Sorghum (*Sorghum bicolor* L. Moench) genotypes have been found to differ in membrane thermostability (9, 10) using the *in vitro* leaf disc method, which evaluates membrane thermostability at elevated temperatures by electrolyte leakage from damaged leaf cells. This was used as an estimate of heat tolerance of genotypes. Similarly, soybean (*Glycine max* L. Merr.) and cotton (*Gossypium hirsutum* L.) genotypes have been found that differ in membrane thermostability (5, 8).

The objectives of this study were to determine the feasibility of using the *in vitro* leaf disc method as a means to evaluate peanut genotypes for membrane thermostability and to determine whether differences in membrane thermostability existed among peanut genotypes.

Materials and Methods

The method for determining cellular membrane thermostability was described by Sullivan (9) and more recently by Martineau *et al.* (5) and by Quisenberry *et al.* (8). The procedure measures electrical conductivity of electrolyte leakage from heat-damaged leaf tissue cells after exposure to elevated temperatures. The extent of electrolyte leakage, expressed as a percentage after correction for control tissue, indicates the degree of membrane injury.

Peanut (*Arachis hypogaea* L.) genotypes used in these studies were planted in 1981, 1982, and 1983 in 0.91-m two-row plots 6.1 m in length. The field was located on the Oklahoma State University Agricultural Experiment Station at Perkins, Okla. The plants were grown under rainfed conditions each year.

Peanut leaf samples were randomly collected over the entire plot length in the field between 0830 and 1130 hours on each sampling date given below. Leaves in the uppermost part of the canopy, which had been fully exposed to the environment, were selected. They were placed in plastic zip-loc bags, moistened with water, and placed in a cooler with a frozen ice substitute to maintain a cool environment. The leaves were kept from direct contact with the cooling material so that no freezing injury occurred. The samples were then transported to the laboratory. Each leaf sample was washed briefly with tap water, and ten distal leaflets (peanut leaves are tetrafoliate) were stacked. The stacked leaflets were punched twice with a No. 3 (or No. 2 for narrow leaves) cork borer to obtain a paired set (control and treatment) of ten leaf discs each. The ten discs were transferred to a 105 mm x 16 mm polycarbonate tube with one end covered by nylon mesh held in place with an elastic band. The tubes with leaf discs were placed in test-tube racks within pans containing tap water to wash the leaf discs. The nylon mesh retained the discs within individual sample tubes, yet allowed entry of water to wash the discs free of exogenous contaminants adhering to tissue surfaces and endogenous electrolytes released from cut cell surfaces. From the time that the last set of discs were cut, the discs were washed for 0.5 hr in tap water followed by two changes of distilled water for a minimum of 1.5 hr of washing. After the final wash, the tubes were shaken to remove excess water, but sufficient moisture was retained by the discs and tube interior to maintain moist conditions. The 16-mm sample tubes were then put into 50-mL graduated, conical polycarbonate test tubes held in racks. The racks of tubes were covered with aluminum foil to prevent moisture loss during temperature treatment. In the later experiments (1982 and 1983), 6 mL of demineralized water was added to float and separate the discs during heat treatment. The injury-response curve shown for Comet (Fig. 1) used this latter procedure at temperatures from 46 to 56 C with a 60-minute treatment time. After treatment the discs were immediately cooled by immersing the tubes in cold tap water. Control discs remained at room temperature. The leaf discs plus water were then transferred from the 16-mm tubes into 50-mL tubes and brought to 25 mL volume with demineralized water. Tubes were covered with aluminum foil, and both control and treat-

[1] Cooperative investigations of the Southern Region, Southern Plains Area, USDA-ARS and the Oklahoma Agric. Exp. Stn., Stillwater, Ok 74078.

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[3]Plant Physiologist, USDA-ARS, Plant Science Research Laboratory and Dept. of Agronomy, Oklahoma State Univ., Stillwater, Ok 74076.

ment leaf discs were then incubated in a refrigerator at 5 C overnight to allow diffusion of electrolytes from the discs. Conductivity of the solution was measured with a Markson ElectroMark analyzer (Markson Science Inc.) at a constant temperature of 27 C. After the initial conductivity measurement the tubes were re-covered with aluminum foil to prevent moisture loss, and both control and treatment discs were autoclaved at 100 C for 10 minutes to completely kill the leaf tissue. After the tubes were cooled and equilibrated at 27 C, a final conductance measurement was made. The percentage of injury to cellular membranes was calculated as described by Martineau *et al.* (5).

Experiment 1981. This test was planted 15 May 1981 and included the ten genotypes listed in Table 1. Leaf samples were randomly selected from the uppermost part of the canopy to obtain leaves fully exposed to the environment. Leaf samples were collected 14 July and 25 August (56 and 98 days after planting (DAP), respectively).

Experiment 1982. This test was planted 9 June 1982, about three weeks later than the 1981 test due to extensive spring rains. Leaf samples were collected 2 August, 23 August, and 13 September (54, 75, and 96 DAP, respectively). To prevent compaction of the leaf discs and provide more uniform heat treatment, about 6 mL of demineralized water was added to the 50-mL tube to float and separate the leaf discs during treatment. This water was included in the final total volume used for the conductivity measurement. In addition, to provide greater opportunity for uniform heating of all discs, the temperature was reduced from 51 to 50 C and treatment time increased to 1 hr.

Experiment 1983. This test was planted 26 May 1983 with five genotypes including three cultivars that were previously tested in 1981 and 1982 and two breeding lines (Table 3). The number of replications was reduced to four in order to determine the least number that will provide a satisfactory test while maximizing the number of genotypes that can be evaluated during future growing seasons. Leaf sampling and heat treatment of the discs were conducted as in 1982.

The experimental design was a randomized complete block with 10, 6, and 4 replications in 1981, 1982, and 1983, respectively. Ten, 6, and 5 genotypes were used in 1981, 1982, and 1983, respectively. Data were analyzed by standard analysis of variance each year, and Duncan's multiple range test was used to determine differences among genotype mean values. LSD values were used for DAP comparisons. Since the same procedure was used in 1982 and 1983, these data were analyzed for comparisons between seasons.

Results and Discussion

Experiment 1981. Figure 1 shows the relationship between percentage injury and heat-treatment temperature for the standard cultivar Comet. These data were obtained with the 1982 modified precedure, but a similar curve had been previously obtained in 1981, although with more variability in the data, which is explained below. Duration of heat treatment in 1981 was 45 minutes. This was 30 minutes longer than that used for soybeans (5) and the same as that used for cotton (8). The shape of the curve is similar to that for sorghum (9) and soybeans (5). The area around the 50% injury point is the most sensitive. A valid comparison of genotypes using this procedure assumes the shape of the response curve is nearly the same as for the standard genotype. This may not always be the case as shown for soybeans (5) so that some error in making comparisons between genotypes is introduced. However, this error is acceptable since in a screening procedure of this kind, large differences between genotypes are being sought, and it is not feasible to determine an injury response curve for every genotype to be tested. Thus, a temperature near the inflection point for 50% injury is usually chosen (5). For Comet, the temperature necessary to achieve about 50% membrane injury was 51 C (Fig 1).

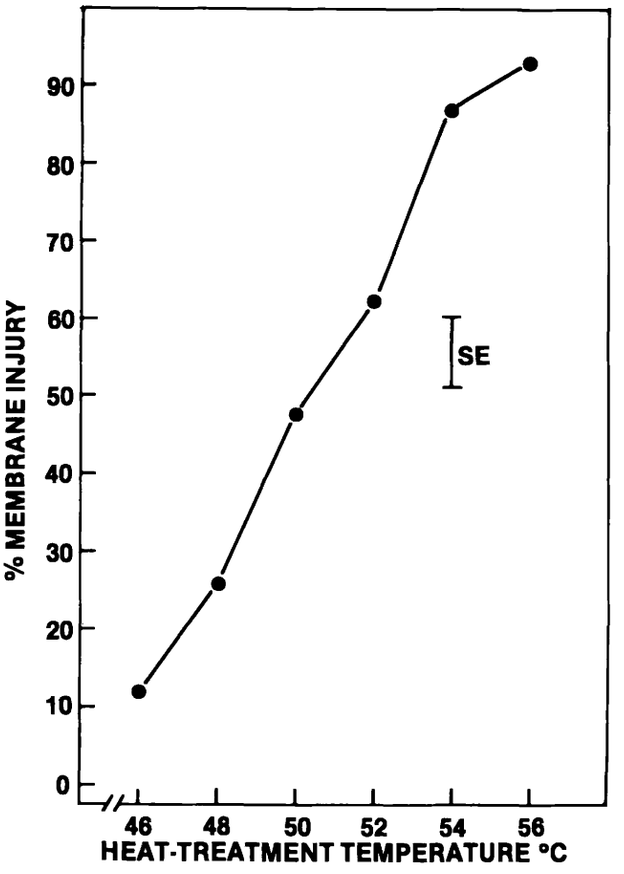

Fig. 1. Percentage membrane injury of the standard peanut cultivar Comet in relation to heat-treatment temperature. Data are the mean values for two experiments (1982 and 1983 procedure) with 5 replicate leaf samples per treatment. The data were obtained from leaf samples harvested one week before the first sampling date. Vertical bar indicates the SE of the mean value.

The genotypes listed in Table 1 were evaluated at 51 C for 45 minutes. The percentage membrane injury values shown in Table 1 were lower than expected based on the data in Fig. 1. The reason is unknown. But the data showed significant differences among the genotypes for both DAP. Also, there was a significant ($P<0.01$) DAP effect, with the later DAP showing greater injury (Table 1). A higher percentage value indicate more injury, i. e., less membrane thermostability. This occurred for all genotypes except Florunner, which explains a significant genotype X DAP interaction. Soybeans also showed differences in percentage membrane injury at different sampling dates (5). The coefficient of variation (CV) for the data in Table 1 was 38.3%, which is higher than acceptable if significant differences are to be found with limited replication. One reason for the large variation could be due to the method of leaf sampling. In this study, leaves were randomly sampled from those in the upper part of the canopy. For soybeans it was found that the node position from which the leaf was sampled influenced the extent of injury that occurred (5). Also, it was observed here that after shaking the excess moisture from the leaf discs before heat treatment, many of the discs remained compactly

stacked. This may have resulted in a lack of uniform heating of the leaf discs, which contributed to the variation of the data as indicated by the CV of 38.3%.

Table 1. Comparison of leaf membrane thermostability among peanut genotypes at 51 C for 45 minutes, 1981.

Genotype	% Membrane Injury	
	56 DAP†	98 DAP
Florunner	27.40a§	30.99 bc‡
UF 77318	26.39a	37.88 b
PI 404021	22.47ab	33.05 bc
PI 355993	21.45ab	50.57a
EM-9	15.94 bc	35.35 b
Spanco	15.84 bc	49.86a
Chico	12.93 c	34.88 bc
Pronto	12.33 c	37.75 b
PI 405915	12.08 c	23.85 c
Comet	7.85 c	27.66 bc

† DAP, Days after planting 15 May 1981.

‡ F value for DAP was significant at the 0.01 probability level except for Florunner. $LSD_{(0.05)}$=9.19 for DAP comparisons, CV=38.3%

§ Mean values within columns not followed by the same letter were different (P<0.05) among genotypes as determined by Duncan's multiple range test.

Experiment 1982. To overcome the possible effect of leaf node position on extent of membrane injury, leaves were selected only from the third node when counting the meristematic stem tip as the first node. This was the first fully expanded leaf. For soybeans, it appeared that the greatest genotypic differences in membrane injury were for newly developed leaves (5). Also, since the mainstem slows elongation earlier in the season than the lateral branches, leaves were collected only from lateral branches, which continue to produce new leaves for a longer time during the season. Six genotypes (three each from those least (high % injury) and most (low % injury) thermostable as indicated by the results from 1981) were tested with these modifications in procedure. Genotypes PI 355993 and PI 405915 retained their rankings as one of the least and most membrane thermostable, respectively (Table 2). The most notable difference in 1982 compared to 1981 was higher percent injury values for all genotypes at the earliest DAP (Tables 1 and 2). Comet showed higher percent injury at all DAP in 1982 compared to 1981 (Tables 1 and 2). Although in 1982, percent membrane injury was similar for most genotypes at all DAP, there was a significant (P<0.01) F value for DAP as occurred in 1981. The genotype X DAP interaction was significant only at P>0.1 in 1982 compared to P< 0.01 in 1981. However, the environmental conditions, as discussed in more detail below, were much different in 1982 than in 1981. Further, the changes in procedure provided a more acceptable method as shown by reduction in CV's from 38.3% in 1981 to 18.9% in 1982.

Table 2. Comparison of leaf membrane thermostability among peanut genotypes at 50 C for 60 minutes, 1982.

Genotype	% Membrane Injury		
	54 DAP†	75 DAP	96 DAP
PI 355993	55.55a§	51.62a	53.53a
Comet	53.48a	50.32a	51.21a
Florunner	44.46 b	34.86 b‡	44.84a
Pronto	43.02 b	47.43a	44.04a
UF 77318	41.78 b	23.42 c	32.89 b
PI 405915	25.83 c	22.87 c	25.44 b

† DAP, Days after planting 9 June 1982.

‡ F value for DAP was significant at the 0.01 probability level. $LSD_{(0.05)}$=9.04 for DAP comparisons, CV=18.9%

§ Mean values within columns not followed by the same letter were different (P<0.05) among genotypes as determined by Duncan's multiple range test.

Experiment 1983. There were no significant differences among the genotypes at 54 DAP. Comet had a larger percentage of membrane injury (lowest membrane thermostability) than all other genotypes at 96 DAP and all but Florunner at 75 DAP in 1983 (Table 3). The F value for DAP was significant (P<0.05), but the genotype X DAP interaction was significant only at P>0.1 as in 1982. The CV was 16.3%, with four replications in 1983. Since the CV's were less than 20% for two consecutive years, acceptable data can apparently be obtained with this procedure as adapted for peanuts.

When the data for the 1982 and 1983 seasons were compared for those cultivars grown in both years, there was clearly a seasonal effect on membrane thermostability (Table 4). Generally, membrane thermostability was less in 1983 than in 1982, particularly for 75 and 96 DAP (Table 4). Environmental data for the 3-year period 1981-1983 indicated that both the 1982 and 1983 seasons had higher temperatures (>35 C) for longer durations and had much less rainfall than the 1981 season (Table 5). The three cultivars (Florunner, Comet, and Pronto) that were tested all three years showed lower membrane thermostability in 1982 and 1983 compared to 1981 (Tables 1, 2, and 3). Average yields of these cultivars were reduced from about 2200 kg/ha in 1981 to about 600 kg/ha in 1982 and 1983. Both lack of rainfall and high temperatures probably contributed to reduced membrane thermostability and yields of the cultivars in 1982 and 1983 (Table 5). However, in studies under

Table 3. Comparison of leaf membrane thermostability among peanut genotypes at 50 C for 60 minutes, 1983.

Genotype	% Membrane Injury		
	54 DAP†	75 DAP	96 DAP
Florunner	60.54a§	65.82ab	49.00 b‡
Breeding Line-14	58.37a	61.34 b	49.95 b
Breeding Line-13	57.59a	58.60 b	54.66 b
Comet	57.23a	79.02a	75.26a
Pronto	48.93a	63.63 b	60.65 b

† DAP, Days after planting 26 May 1983.

‡ F value for DAP was significant at the 0.05 probability level. $LSD_{(0.05)}$=13.98 for DAP comparisons, CV=16.3%

§ Mean values within columns not followed by the same letter were different (P<0.05) among genotypes as determined by Duncan's multiple range test.

controlled environment where moisture was not a limiting factor, a temperature of 35 C inhibited both vegetative and reproductive growth of peanut genotypes (4). Field planting dates may differ from those used (mid-May to early June) here, but present agronomic practices in the U. S. dictate that some critical reproductive phase (flowering, pegging, pod development, seed fill) will happen during July or August when conditions as given in Table 5 frequently occur. The coincidence of these events can be expected to reduce peanut yields unless more tolerant cultivars are developed. An approach may be through more thermostable membrane systems. Such adverse environmental conditions that injure cellular membranes can reduce photosynthesis, respiration, and other membrane-associated metabolism. Photosynthesis of sorghum is affected by direct high-temperature effects apart from stomatal closure (10).

Table 4. Comparison of leaf membrane thermostability between 1982 and 1983 seasons for three peanut cultivars grown under rainfed conditions.

Genotype and year	% Membrane Injury		
	54 DAP†	75 DAP	96 DAP
Florunner			
1982	44.46a‡	34.86a	44.84a
1983	60.54 b	65.82 b	49.00a
Comet			
1982	53.48a	50.32a	51.21a
1983	57.23a	79.02 b	75.26 b
Pronto			
1982	43.02a	47.43a	44.04a
1983	48.93a	63.63 b	60.65 b

† DAP, Days after planting.

‡ Mean values within columns and genotypes not followed by the same letter were different (P<0.05) except Florunner at 54 DAP (P<0.10) as determined by Duncan's multiple range test.

Table 5. Rainfall and number of days with high temperature for July, August, and September during three peanut-growing seasons at Perkins, Oklahoma.

	July			August			September			Totals	
	Temperature C		Monthly	Temperature C		Monthly	Temperature C		Monthly		
Year	35-38	> 38	Rainfall	35-38	> 38	Rainfall	35-38	> 38	Rainfall	Days	Rainfall
	No./days		mm	No./days		mm	No./days		mm	No.	mm
1981†	12	6	124.2	2	0	128.5	0	0	49.0	20	301.7
1982	5	0	94.2	9	7	8.4	6	2	22.4	29	125.0
1983	11	8	0.5	20	8	24.4	8	0	48.8	55	73.7

† The average maximum temperatures in July and August were 32.8 and 30.0 in 1981, 32.2 and 35.0 in 1982, and 35.6 and 37.2 in 1983, respectively.

Cellular membranes of soybeans tend to acclimate or harden in response to prevailing ambient temperatures (5). Most of the peanut genotypes tested in this study apparently did not acclimate to high temperatures, since membrane thermostability decreased with increased environmental severity across years (Table 4). Selection of peanut genotypes that show membrane stability across seasons, such as PI 405915 (Tables 1 and 2), or that acclimate to high temperatures may be a means to improve heat tolerance and hence yields of this crop.

Sufficient variability occurred in soybeans so that heritability estimates for membrane thermostability indicated that selection for this trait is feasible (6). Among breeding populations of sorghum there were those with similar yield, but one population showed less percentage membrane injury than the other (10). Thus, there appears to be a wide range of variability among crop genotypes for membrane thermostability. Such variability was found among the peanut genotypes tested in these studies and may be useful in attempts to adapt this crop to high field temperatures.

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