

Genotypic Differences and Water-Deficit Induced Enhancement in Epicuticular Wax Load in Peanut

M. Y. Samdur, P. Manivel, V. K. Jain, B. M. Chikani, H. K. Gor, S. Desai, and J. B. Misra*

ABSTRACT

The epicuticular wax load (EWL) on leaves reduces surface transpiration and thus improves crop water use efficiency. The objectives of this study were to evaluate peanut (*Arachis hypogaea* L.) genotypes for their ELW and also to determine the influence of water deficit stress on EWL. Peanut genotypes were grown in fields in two dry seasons (2000 and 2001) and one rainy (2000) season. Withholding irrigation water resulted in a significant increase in water saturation deficit in the stressed crop. At 45 d after sowing (DAS), significant genotypic differences were observed in EWL of 12 genotypes grown in the rainy season (2000). The values of EWL ranged from 0.91 mg dm⁻² in Chico to 1.74 mg dm⁻² in PBS 11049, with a mean of 1.27 mg dm⁻². Among six genotypes, which were also sampled subsequently, the mean values were 1.10, 1.58, 2.05 mg dm⁻² at 45, 75, and 95 DAS, respectively. In both dry seasons, significant genotypic differences were found in the EWL. In the dry season of 2001, the effect of various moisture deficit treatments and their interactions with the genotypes were highly significant. The values ranged from 0.653 to 2.878 mg dm⁻². On an average, the highest EWL was found in PBS 11049 (2.24 mg dm⁻²). Even under irrigated conditions, in summer 2001, the EWL increased with increased age of the crop. However, there was a greater increase in the treatments that were subjected to moisture deficit stress. It was concluded that genotypic differences exist in EWL of peanut and also that EWL increases with increased crop age. This increase is more pronounced in plants that are subjected to protracted moisture deficit stress.

PEANUT is one of the most important oilseed crops of the world. Because of its drought tolerant nature, this crop is grown under rain-fed conditions. As such, this crop is quite popular among the marginal farmers of semiarid tropics, where because of low and erratic precipitation the crop is subjected to mild to severe water deficit stress. Several morphological and physiological adaptations are known to impart drought tolerance to crop plants. Root structure, accumulation of osmotica, leaf folding, reduction in leaf area, and regulation of transpiration are some of the mechanisms known to enhance drought tolerance (Joshi et al., 1988; Subbarao et al., 1995; Blum, 1998).

As a consequence of decreased water availability, the stomata close to minimize the loss of water though stomatal transpiration and in this condition loss of water occurs mostly through the general surface of leaves. It is now known that epicuticular wax helps leaves in retention of water (Jordan et al., 1984) by minimizing cuticular transpiration (Jefferson et al., 1989; Premchandra et al., 1992). Genotypes with low cuticular transpiration rates usually have a functional advantage during water deficit environments due to more efficient water

use (Walker and Miller, 1986; Paje et al., 1988). Higher levels of leaf epicuticular wax have been shown to be correlated with seedling drought tolerance in *Eragrostis lahmanniiana* Nees (Wright and Dobrenz, 1973), with relative drought tolerance in oat (*Avena sativa* L.) cultivars (Bengston et al., 1978), and with greater water use efficiency in wheat (*Triticum aestivum* L.) (Johnson et al., 1983). Recently, it has been shown that in cocoa (*Theobroma cacao* L.), the leaf epicuticular wax content increases with increased in soil moisture deficit (Antwi, 1999).

Compared with several other crops, peanut has some drought tolerance. Specific leaf area has been shown to be inversely related to drought tolerance potential of peanut genotypes (Nageswara Rao and Wright, 1994). However, a comprehensive understanding of the contributions of various factors to imparting drought tolerance in peanut is lacking. There are reports that indicate accumulation of sucrose, proline, and amino acids in the leaves of peanut plant as a consequence of water deficit stress (Misra et al., 1991; Yadav et al., 1993). Earlier work on peanut did not indicate any accumulation of wax on leaves either in response to drought or after relieving the stress (Vakharia et al., 1993). Subsequently, on the basis of studies conducted on a single cultivar, Vakharia et al. (1997) observed that after imposing drought, the epicuticular wax increased while leaf moisture and relative water content declined. The objectives of this study were to evaluate peanut genotypes for their ELW and also to determine the influence of water deficit stress on EWL.

MATERIALS AND METHODS

The peanut genotypes were grown in the field of the National Research Centre for Groundnut, Junagadh, India (latitude 21°31'N, longitude 70°36'E). The soil was a Vertisol Ustochropt (pH7.5) with low organic matter, available nitrogen and phosphorus contents. A spacing of 450 mm (row to row) by 100 mm (plant to plant) was maintained, and recommended production practices for the region were used.

Field Exp. 1 was conducted in the dry season (February–June 2000) in a split plot design with two irrigation treatments (main plots), three replications, and six genotypes (subplots). The genotypes were breeding lines PBS 11023, Code 9, PBS 20055, PBS 11049, PBS 12067, and PBS 12115. The treatments were (i) crop irrigated to field capacity at regular 7-d intervals and leaves sampled for analysis at 80 d after sowing (DAS) and ii) crop irrigated at regular 7-d intervals only up to 47 DAS, subsequent irrigation discontinued until the sampling of leaves at 80 DAS, followed by resumption of regular irrigation. Three leaflets each from 3rd to 5th (top to bottom) leaves of plants were collected from 10 different plants of a genotype

National Research Centre for Groundnut, P.O. Box 5, Ivnagar Road, Junagadh 362 001, Gujarat, India. Received 15 Feb. 2002. *Corresponding author (misra@nrcg.guj.nic.in).

Published in Crop Sci. 43:1294–1299 (2003).

Abbreviations: DAS, days after sowing; EWL, epicuticular wax load; WSD, water saturation deficit; CD, critical difference.

from each treatment and used immediately for determination of water saturation deficit (WSD) and EWL.

Twelve genotypes were grown in field Exp. 2 during the wet rainy season (June–October 2000). Whenever rains were delayed, supplementary irrigation was provided to avoid moisture-deficit stress. The genotypes were CSMG 84-1, ICGV 86031, JL24, Chico, TAG 24, J11, PBS 12067, PBS 12115, PBS 11049, PBS 20055, PBS 11023, and Code 9. The trial was laid out in a RBD with five replications. Leaves of all the genotypes were sampled (as described for Exp. 1) at 45 DAS for studying the genotypic variability in EWL and subsequently leaves of only six genotypes (CSMG 84-1, ICGV 86031, JL24, Chico, TAG 24, and J11) were sampled at 75 and 95 DAS to study the changes in EWL with increased plant age.

Field Exp. 3 was conducted in the dry season (February–June 2001) in a split plot design. The main plot treatments were T1, regular irrigation and sampling at 45 DAS; T2, regular irrigation and sampling at 65 DAS; T3, regular irrigation and sampling at 80 DAS; T4, regular irrigation and sampling at 85 DAS; T5, no irrigation beyond 45 DAS and sampling at 65 DAS; T6, no irrigation beyond 45 DAS and sampling at 80 DAS; and T7, no irrigation beyond 45 DAS, resumption of irrigation at 80 and 84 DAS, which was followed by sampling at 85 DAS. The main plot treatments were arranged in a series with a gap of 3 m between the regularly irrigated plots (T1–T4) and the remaining plots (T5–T7). The genotypes (in subplots), in addition to the six breeding lines which were used in Exp. 1 (PBS 11023, Code 9, PBS 20055, PBS 11049, PBS 12067, and PBS 12115), three cultivars (J 11, GG2, and GAUG 1), and one germplasm accession, NCAc 17090, were evaluated. Three leaflets each from 3rd to 5th (top to bottom) leaves of plants were collected from 10 different plants of a genotype from each treatment and used immediately for determination of EWL. At the time of sampling of leaves, soil samples (representing 0- to 150-mm and 150- to 300-mm depth) were also taken from each treatment for determination of soil moisture content.

From the lamina of each leaf (excluding the midrib), discs of 10-mm diameter were obtained with a leaf punch and then 30 such discs were used for determination of EWL by the colorimetric method as outlined by Ebercon et al. (1977). The moisture status of leaves was determined by the method outlined by Barrs and Weatherley (1962) expressed as water saturation deficit (WSD), which was calculated as follows:

$$\text{WSD} = \left[\frac{\text{Fully turgid weight} - \text{fresh weight}}{\text{Fully turgid weight} - \text{dry weight}} \right] \times 100,$$

where fresh leaf weight is the weight of leaf at the time of sampling in field and fully turgid weight is the weight of leaves recorded 6 h after immersion in water (so as to allow all the cells to acquire full turgidity). The soil moisture content was determined gravimetrically.

Statistical Analysis

Experiment 1 and Exp. 3 were both conducted in split plot design. In Exp. 1, main plot factor was two types of irrigation treatments and sub-plot factor was genotypes with three replications. In Exp. 3, main plot factor was seven types of irrigation treatments and sub plot factor was genotypes with three replications.

Experiment 2 was conducted in randomized complete block design (RCBD) in which genotypes was the sole factor with five replications. The statistical analysis for split plot and for RCBD were performed as outlined by Gomez and Gomez (1984).

To analyze the extent to which the results were reproducible

across the years the data pertaining to six genotypes that were common to Exp. 1 and Exp. 3 were analyzed in a combined manner by taking T3 and T6 treatments in Exp. 3 corresponding to irrigation and stress treatments of Exp. 1 as the two types of main plot factor and genotypes as subplot factor. The statistical analysis for split plot was performed as outlined by Gomez and Gomez (1984).

RESULTS

The range of values of WSD for six genotypes grown in Exp. 1 was 16.3 to 26.0% in the irrigated crop and 22.9 to 39.2% in the stressed crop (Fig. 1) and the genotypic differences were significant ($P < 0.05$). On an average, WSD of the stressed crop (28.9%) was significantly ($P < 0.05$) higher than that of the irrigated crop (20.9%). The differences due to genotype–treatment interactions were also significant ($P < 0.05$) and the change in WSD ranged from 3.5% (PBS 12067) to 16.6% (PBS 20055). The amount of change in the remaining genotypes was 10% in PBS 11023, 7.5% in Code 9, and 5.4% in both PBS 11049 and PBS 12115.

The range of EWL in six genotypes grown in Exp. 1 was 1.46 to 2.06 mg dm⁻² under irrigated conditions while it was 1.70 to 2.44 mg dm⁻² under stress conditions (Fig. 1). On an average, there was a significant ($P < 0.01$) and substantial increase (19.58%) in EWL because of imposition of stress. The individual genotypes, however, differed from the general trend since only three genotypes (PBS 11023, Code 9, and PBS 20055) registered a significant increase, while two genotypes (PBS 11049 and PBS 12067) showed a nonsignificant increase and one genotype (PBS 12115) did not show any change. The interaction between the genotypes and the treatments was significant ($P < 0.01$). The correlation between the values of WSD and EWL of genotypes or between the differences of values of stressed and irrigated plants was not significant. The significant genotype–treatment interaction explains the absence of a significant correlation between the values of WSD and EWL if worked out on the basis of combined data ($n = 12$), although the nature of response of WSD and EWL was similar in that both WSD and EWL values increased because of increased soil moisture deficit.

The 12 genotypes, grown in Exp. 2 and sampled at 45 DAS, differed significantly ($P < 0.05$) in their EWL. The values ranged from 0.91 mg dm⁻² in Chico to 1.74 mg dm⁻² in PBS 11049 with a value of 13.1% for coefficient of variation. The values for mean and critical difference ($P < 0.05$) were 1.27 mg dm⁻² and 0.21, respectively (Table 1).

With increased crop age, EWL also increased. On an average, there was a 43.6% increase between 45 and 75 DAS, 29.7% between 75 and 95 DAS, while the overall increase between 45 and 95 DAS was 86.4% (Fig. 2). The differences due to genotypes were nonsignificant. The differences due to interaction between the genotypes and the ages of crop were significant ($P < 0.05$). The extent of increase in EWL from 45 to 75 DAS was minimum in TAG 24 (12.3%) while the maximum was in Chico (76.9%) and during 75 to 95 DAS the minimum increase was in JL 24 (19.9%) and maximum in ICGV

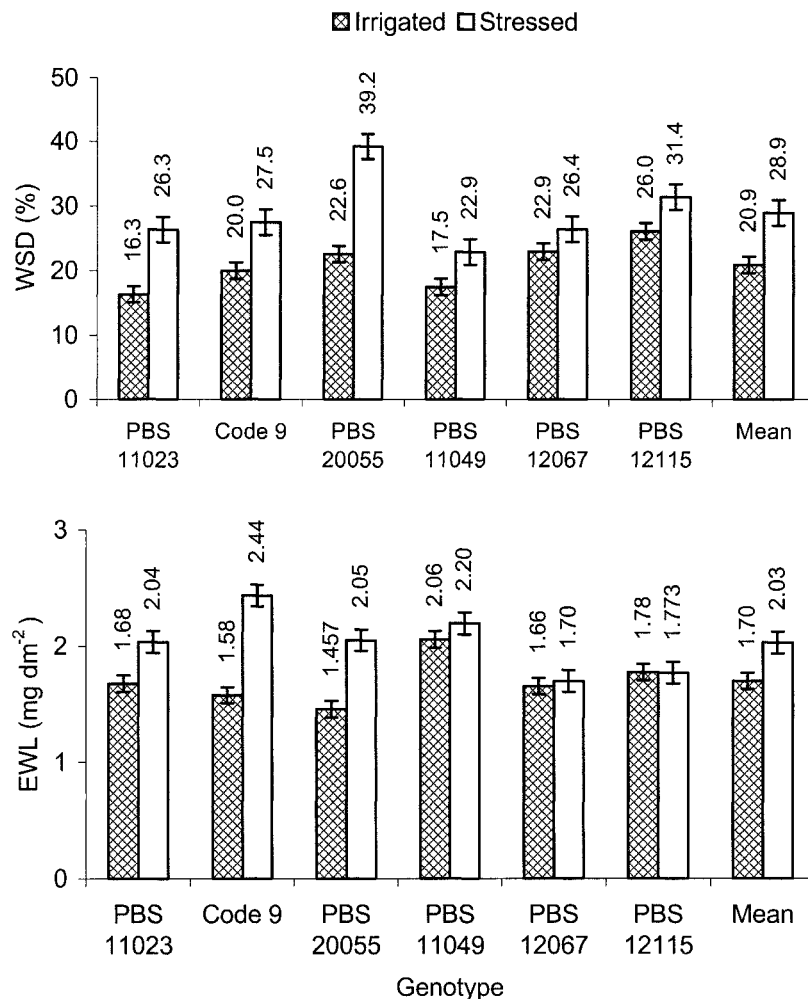


Fig. 1. Effect of soil moisture deficit stress on water saturation deficit (WSD) and epicuticular wax load (EWL) in some peanut genotypes in Exp. 1 (dry summer season 2000).

86031 (38.5%). The minimum overall increase (45–95 DAS) was observed in TAG 24 (51.5%), while the maximum was in Chico (124.2%).

The moisture contents in Exp. 3 of the top 150-mm and the subsequent 150-mm layer of soil was similar in various treatments, hence only mean data are given

Table 1. Leaf EWL at 45 DAS of peanut genotypes grown in Exp. 2 (wet rainy season 2000).

Genotype	EWL mg dm ⁻²
CSMG 84-1	1.02
ICGV 86031	1.14
JL 24	0.97
Chico	0.91
TAG 24	1.30
J 11	1.29
PBS 12067	1.12
PBS 12115	1.19
PBS 11049	1.74
PBS 20055	1.57
PBS 11023	1.58
CODE 9	1.38
Minimum	0.91
Maximum	1.74
Mean	1.27
CD (0.05)	0.21
CV (%)	13.1

(Fig. 3). The moisture content of T7 (no irrigation beyond 45 DAS for 40 d), recorded immediately after relieving the stress (at 85DAS) was 12.8%. This value was only marginally higher than that of T4 (regularly irrigated), which was also sampled the same day.

The differences in EWL due to genotypes and treatments as well as their interactions were highly significant ($P < 0.05$) and the values ranged from 0.65 to 2.88 mg dm⁻² (Table 2). On an average, the highest EWL was found in the genotype PBS 11049 (2.24 mg dm⁻²), which was not different from GG 2 and PBS 11023 but was higher than the remaining seven genotypes. The EWL of genotype PBS 11049 which ranked the highest in T2 and T4, was not different from the EWL of those genotypes which had the highest EWL in T1, T3, T5, T6, and T7 (Table 2). The highest average EWL value of 2.88 mg dm⁻² was found in genotype GG 2 in T7 while the lowest of 0.65 mg dm⁻² in PBS 20055 in T1. Among the treatments T1, T2, T3, and T4, which all received normal irrigation and differed only in age of the crop at the time of sampling, the mean EWL was lowest in T1 followed by T2, T4, and T3, thereby indicating that the EWL increased with increased crop age under irrigated conditions. On any given day of sam-

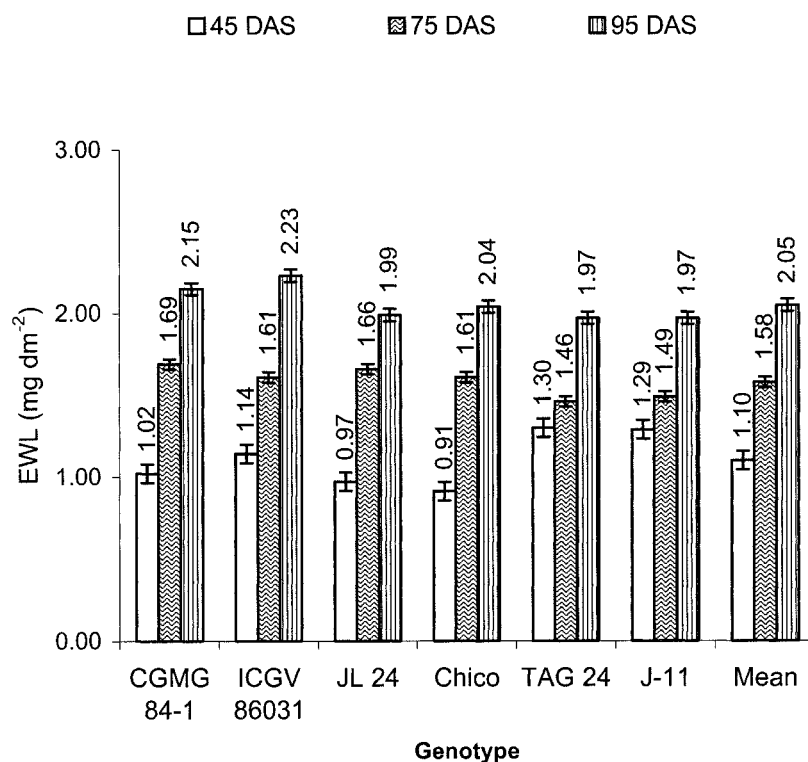


Fig. 2. Leaf epicuticular wax load (EWL) of six peanut genotypes at three stages of crop growth in Exp. 2 (wet rainy season, 2000).

pling, a greater increase in EWL was observed in the treatments that were subjected to moisture deficit stress than in those which were not. The differences were, however, significant only for 80- and 85-d old plants. Compared with its value in T1, the values of EWL in T2, T3, T4, T5, T6, and T7 were 2.33, 2.90, 2.80, 2.50, 3.10, and 3.24 fold, respectively.

The value for EWL content averaged over six genotypes (PBS 11023, Code 9, PBS 20055, PBS 11049, PBS 12067, and PBS 12115) which were common to Exp. 2 and 3 and both sampled at 45 DAS was 1.43 mg dm⁻² in Exp. 2 and 0.79 mg dm⁻² in Exp. 3. Thus at 45 DAS, the EWL was lower in dry season (Exp. 3) than that in wet rainy season (Exp. 2), thereby indicating that the build up of EWL in dry summer was rather slow in the initial stage of crop growth. However, with increased crop age, the values for EWL became similar in both seasons (Table 2 and Fig. 2). This difference in the pattern of build up of EWL could be to some extent attributed to the differing weather condition prevailing in the early growth phase of the crop in dry summer (Exp. 3) and wet rainy (Exp. 2) seasons.

DISCUSSION

The results of Exp. 1, which was conducted in the dry season, indicated that at 75 DAS, peanut genotypes differed significantly ($P < 0.01$) in their EWL under both irrigated and stress conditions (Fig. 1). The plants subjected to water deficit, however, accumulated a greater EWL than those grown under regularly irrigated conditions. The peanut genotypes also differed significantly ($P < 0.05$) in their WSD even under uniform

irrigation conditions. The magnitude of response had a significant genotype-treatment interaction as was evident from the ranges of change of WSD (3.45–16.65%) and EWL (–0.2–54.5%).

The results of Exp. 2 indicated that at 45 DAS, signifi-

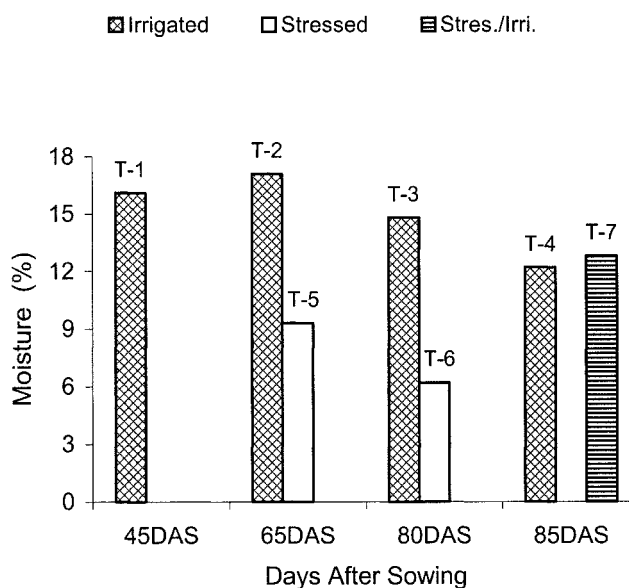


Fig. 3. Soil moisture content (%) in various treatments of Exp. 3 (T-1, regular irrigation and sampling at 45 DAS; T-2, regular irrigation and sampling at 65 DAS; T-3, regular irrigation and sampling at 80 DAS; T-4, regular irrigation and sampling at 85 DAS; T-5, no irrigation beyond 45 DAS and sampling at 65 DAS; T-6, no irrigation beyond 45 DAS and sampling at 80 DAS; and T-7, no irrigation beyond 45 DAS, resumption of irrigation at 80 and 84 DAS, which was followed by sampling at 85 DAS).

Table 2. Epicuticular wax load (mg dm^{-2}) of peanut genotypes at various stages of crop growth and moisture regimes in Exp. 3 (dry season 2001).

Genotype	Treatments†							Mean	Change (%)		
	T1	T2	T3	T4	T5	T6	T7		T2–T5	T3–T6	T4–T7
J 11	0.87	1.92	2.34	2.05	1.78	2.38	2.55	1.98	-7.2	1.4	24.2
PBS 12067	0.74	1.81	2.19	2.24	1.83	2.54	2.32	1.95	0.8	15.7	3.6
NcAC 17090	0.76	1.76	2.09	1.99	2.02	2.22	2.35	1.88	15.1	6.1	17.8
Code 9	0.79	1.80	2.11	2.00	2.17	2.56	2.65	2.01	20.5	21.6	32.4
PBS 20055	0.65	1.81	2.49	1.98	1.89	2.42	2.57	1.97	4.7	-2.8	29.6
PBS 11023	0.71	1.88	2.59	2.41	1.96	2.83	2.78	2.17	4.0	8.9	15.1
GG 2	0.73	2.00	2.45	2.46	1.96	2.76	2.88	2.18	-2.1	12.7	17.1
PBS 11049	0.87	2.02	2.35	2.64	2.15	2.82	2.86	2.24	6.1	20.1	8.5
GAUG 1	0.92	1.82	2.37	2.33	2.05	2.40	2.48	2.05	12.7	1.1	6.3
PBS 12115	0.96	1.86	2.31	2.32	2.23	2.46	2.54	2.10	20.4	6.4	9.4
Minimum	0.65	1.76	2.09	1.98	1.78	2.22	2.32	1.88	-7.2	-2.8	3.6
Maximum	0.96	2.02	2.59	2.64	2.23	2.83	2.88	2.24	20.5	21.6	32.4
Mean	0.80	1.87	2.33	2.24	2.00	2.54	2.60	2.05	7.5	9.1	16.4
CD (0.05)											
G‡	0.10										
T	0.20										
I	0.25										

† T1, regular irrigation and sampling at 45 DAS; T2, regular irrigation and sampling at 65 DAS; T3, regular irrigation and sampling at 80 DAS; T4, regular irrigation and sampling at 85 DAS; T5, no irrigation beyond 45 DAS and sampling at 65 DAS; T6, no irrigation beyond 45 DAS and sampling at 80 DAS; and T7, no irrigation beyond 45 DAS, resumption of irrigation at 80 and 84 DAS which was followed by sampling at 85 DAS.

‡ G, T, and I mean genotype, treatment and interaction, respectively.

cant genotypic differences exist in EWL in rainy season too. Incidentally, the genotype (PBS 11049) which recorded the highest EWL (1.74 mg dm^{-2}) among 12 genotypes in wet rainy season had also recorded the highest (2.06 mg dm^{-2}) EWL among six genotypes in dry season under irrigated condition (Table 1). The data on six genotypes of Exp. 2 conducted in wet season also showed that EWL increases with increasing age of the crop (Fig. 2).

In Exp. 3, the data on soil moisture content (Fig. 3) indicated that the plants in T5 and T6, with a moisture content of 93 and 62 g kg^{-1} , respectively, were suffering water deficit compared with the plants in T1, T2, T3, and T4 with moisture contents of 161, 171, 148, and 122 g kg^{-1} , respectively.

There were major differences in types of treatments in Exp. 1 and 3 but the treatments T3 and T6 of Exp. 3 corresponded to treatments (i) and (ii) of Exp. 1. Accordingly, when a combined analysis of the data of corresponding treatments of Exp. 1 (dry season 2000) and Exp. 3 (dry season 2001) was performed, it was revealed that there were significant differences due to genotypes as well as treatments (Table 3). Thus the pattern of change in EWL due to imposition stress re-

mained the same irrespective of whether data were analyzed separately for the individual years or by combining the data of both years. The genotypic differences were not consistent across the year so far as their ranking for EWL was concerned. This variation was perhaps due to a very strong (significant at 1% level) interaction among the genotype, year, and soil moisture status. The mean wax content of six genotypes for the year 2000 was 1.70 mg dm^{-2} for irrigated crop, 2.03 mg dm^{-2} for stressed crop, and 1.87 mg dm^{-2} for the overall mean. The corresponding values were 2.34, 2.60 and 2.47 mg dm^{-2} for the year 2001. Thus values of EWL for the year 2001 were higher than those for the year 2000. The differences could not be attributed to any clearly identifiable factor. The differences in the maximum temperature and relative humidity prevailing during early phase of crop may be one of the factors. Thus, the results suggest that there are factors other than soil moisture and WSD which also influence EWL of peanut leaves.

Results show that peanut plants show an adaptive response to water deficit conditions by increasing EWL. This kind of adaptive response (Premchandra et al., 1992; McWhorter, 1993) and the associated genotypic differences (Castro-Nava and Huerta, 1994) have been reported to occur in *Sorghum* species also. A similar response has been observed in wheat (Blum and Johnson, 1992) and in cocoa (Antwi, 1999). Earlier, experiments conducted on peanut, however, remained inconclusive possibly because of the inclusion of only two genotypes (GG2 and J 11) in one study (Vakharia et al., 1993) and only one (GG 2) in another (Vakharia et al., 1997). These authors have reported EWL in a range of 2.67 to 4.15 mg g^{-1} dry weight in peanut in the former report and 0.63 to 0.92 mg g^{-1} fresh weight in the latter report. In this study, the EWL has been expressed in widely accepted units (mg dm^{-2}), hence a valid comparison of EWL of peanut with earlier reports is not possible. However, a recalculation made on the basis of some other data provided in one of these reports (Vakharia

Table 3. Analysis of variance for the combined data of Exp. 1 and 3 for common genotypes and corresponding treatments.

Source	Df	Mean squares
Replications	2	0.198
Years (A)	1	6.608*
Error	2	0.143
Irrigation treatments (B)	1	1.601**
AB	1	0.022
Error	4	0.031
Genotypes (C)	5	0.197**
AC	5	0.106**
BC	5	0.116**
ABC	5	0.124**
Error	40	0.027
Total	71	

* Significant at $P = 0.05$.

** Significant at $P = 0.01$.

et al., 1997), shows these values to be in the range 1.70 to 2.42 mg dm⁻², which are comparable to the values obtained in the current investigation.

Although at present the role of EWL in imparting drought tolerance to peanut genotypes cannot be defined with any degree of certainty, it can be said that EWL may contribute to drought tolerance of peanut plants in two ways. First, because of high initial levels (as has been observed for the genotype PBS11049, which has shown the highest level of EWL in both wet and dry seasons) the loss of water because of epicuticular transpiration remains low even under low soil water availability; and second, plants with otherwise low EWL may enhance their EWL in response to soil moisture deficit (as has been seen for the genotypes, PBS 11023, Code 9, and PBS 2005) and thus acquire tolerance by minimizing surface transpiration. Such reduction in transpiration rate because of epicuticular wax has also been reported by Denna (1970) in *Brassica* and O'Toole et al. (1979) in rice (*Oryza sativa* L.). Enhancement of harvest index and consequent improvement in pod yield because of imposition of transient water deficit stress has been reported in some genotypes of peanut (Nautiyal et al., 1999). Transient stress often increases the number of flowers. Conversion of flowers into pegs and subsequently conversion into pods are some other factors that may contribute to increase in pod yield (Chapman et al., 1993; Nautiyal et al., 1999). Whether these changes in the yield or harvest index are accompanied by a change in EWL is yet to be demonstrated.

Thorough studies are required to quantify the contribution of EWL in imparting drought tolerance to the peanut plant and its influence on reducing the loss of pod yield. Once systematic information on this aspect is available, suitable parents can be identified for introducing the trait of either high initial EWL or ability to enhance EWL as an adaptive response, in cultivated peanut.

ACKNOWLEDGMENTS

The authors acknowledge the physical facilities and the encouragement given by Dr. A. Bandyopadhyay, Director, NRCG, Junagadh, for conducting the studies.

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