On the basis of the scanning micrographs the genotypes may be classified as highly glossy (IS 1096, IS 2205, IS 2312, IS 2396, IS 4776, IS 5282, IS 5567; medium glossy (IS 3962, IS 4663, IS 5282, IS 5359, IS 5484, IS 5692, IS 18390 and less glossy (IS 4661, IS 5622, IS 5642).

The intensity of platelike wax crystals varied among genotypes viz., IS 4661, IS 18390, IS 5484, IS 5622, IS 5642; but was sparse and widely spaced as in IS 4663, IS 5484, IS 2312, IS 2396, IS 4776 and IS 1054 (Plate 9.2).

Silica cells present on epidermal cells are dumbell shaped, bi- or trilobed varying in size and intensity in different genotypes. These are covered partially or heavily with amorphous or threadlike filaments (Plate 9.2).

Epicuticular wax content

Maiti *et al.* (1991) reported that glossy sorghum lines show variability in the contents of epicuticular wax, total, a and b chlorophyll and hydrocyanic acid at the seedling and adult stages suggesting that this variation in chemical components among glossy sorghum lines may be related to resistance to drought and insects. Variability in epicuticular wax among sorghum lines were also reported by Ebercon *et al.* (1977).

Epicuticular wax (EW) was found in trace amounts in all genotypes at 7th day. At 14th and 28th days the EW was higher in nonglossy line, (CSH 1), compared to that in glossy lines and showed a tendency to decrease with an increase in glossiness intensity (Table 9.5).

Table 9.5 Epicuticular wax content (EW/cm² leaf area) of 4 sorghum genotypes at 7, 14, 21 and 28 days after emergence (Score 1= Highly glossy, 3= Intermediate, 5= Non-glossy).

		Days after emergence			
Genotype	Score	7	14	21	28
IS 18551	1	Trace	0.020	0.042	0.029
IS 1046	3	Trace	0.018	0.020	0.019
IS 1054	4	Trace	0.015	0.024	0.022
CSH1	5	Trace	0.025	0.032	0.032

The results show that the chlorophyll contents were slightly higher in nonglossy lines, but were significantly different among seedling stages for chlorophyll a, b and total. Therefore, the higher chlorophyll contents in nonglossy lines is responsible for imparting their dark green leaf color. This result coincides with the observation of García-Mendoza (1986) that nonglossy lines had higher chlorophyll content compared to glossy ones. The difference in chlorophyll content between glossy and nonglossy lines were reduced at advanced seedling stage.

Epicuticular wax structure

The reflectance of sorghum leaves at 500-2000 nm was found to vary with epicuticular wax content (Blum, 1975a), and their glossy appearance was found to be related to epidermal hairiness and degree of wax deposition (Traere *et al.*,

989). The glossy appearance of the leaf was estimated by visual scoring or by serving sprayed water droplets under bright light (Maiti *et al.*, 1984; Traere *et al.*, 1989). The nature of the structure present on the leaf surfaces of sorghum notypes varying in glossiness and their optical properties were studied. The notypes used in the study had different glossy intensity: IS 18551(1), IS 1046 (3), 1054 (4) and CSH 1 (5).

A comparison of micrographs before and after dewaxing revealed the presence epidermal structures in all genotypes with variation in density. The aggregation epidermal structures after dewaxing could be due to epidermal deformation as result of chloroform treatment. These observations clearly demonstrate the ssibility of the presence of epidermal structures which were not affected by ganic solvents that look like alveolar material with waxlike appearance. The ensity of these epidermal structures decreased with the intensity of glossiness. Plates 9.3 & 9.4).

It is possible to explain the difference in glossiness of various genotypes irrespective of the amount of the epicuticular wax content. When light falls on latively smooth leaf surface the wave length of the reflected light depends on e pigments in the leaf. However, if the leaf surface is rough it acts as a diffuser us resulting in an uniform white appearance of the leaf surface together with e reflected wave lengths characteristics to leaf pigments. Thus glossiness is versely related to the roughness of the leaf surface. Glossy leaves show higher flectance and transmittance of light compared to nonglossy ones.

No specific relationship has been observed in EW content between glossy and nglossy lines. Trace levels of EW were detected at the early seedling stages (7th ay), but EW was higher in nonglossy lines compared to glossy ones at 14th day. It seems that EW is deposited in thick layer on the cuticular surface of the nglossy lines and thin layers on glossy lines. Therfore, it is assumed that the presence of a thin film of EW on the cuticular surface in glossy lines contributes to hining appearance. More studies are needed for confirmation.

Differential resonance activity between glossy and nonglossy lines

Epicuticular substances, probably smooth waxy coating on glossy leaf surface, offer resistance to the penetration of light causing less resonance units without any change even at higher wave lengths. In the case of nonglossy lines, this substance is absent causing easy penetration of the wave length of light and higher excitation of cytoplasmic materials resulting in higher resonance units. These units decreased with an increase in wave length in nonglossy lines unlike that in glossy ones (Fig. 9.5; unpublished).

Variability in morphological, anatomical and biochemical characters

Epidermal trichomes: Many of the glossy lines possessed microscopic hairs (trichomes) on both sides of their leaf surfaces (Maiti and Bidinger, 1979). The trichomes are frequently pointed at the tip. The size and morphology of the trichomes differ from genotype to genotype (Maiti *et al.*, 1980). They are directed towards the base, with more of them on the upper than on the lower surface (Plates 9.5 & 9.6).

The density of trichomes on the leaf surface is highly variable, being maximum towards the tip, less at the base and intermediate at the mid portion. The trichome length varies from 20 to 55 μm (Maiti *et al.*, 1980).

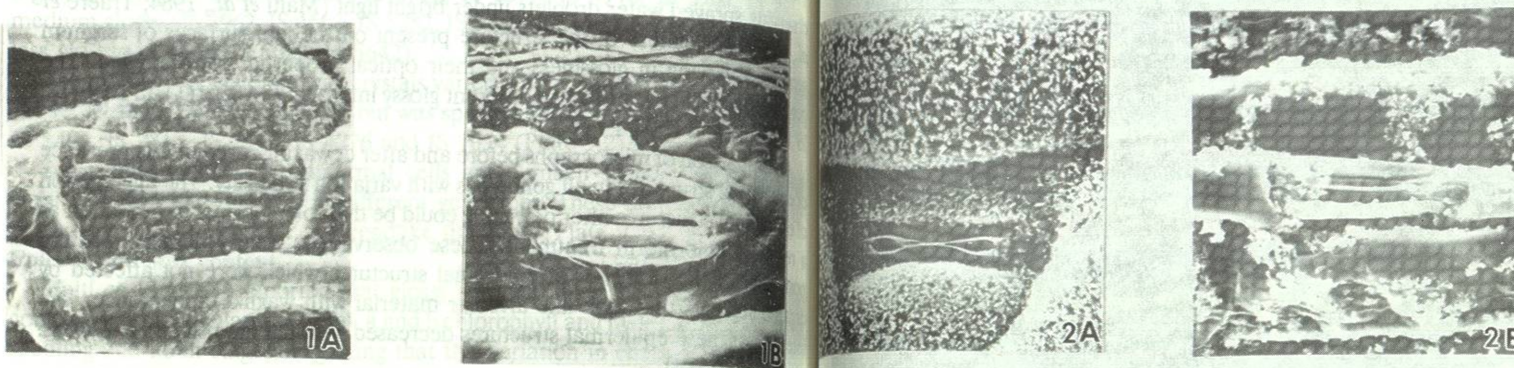


Plate 9.3 Scanning electron micrographs of adaxial surfaces of 4th leaves (suffixes A and B refer to After and Before dewaxing): 1A/B) IS18551 2A/B) IS 1046.

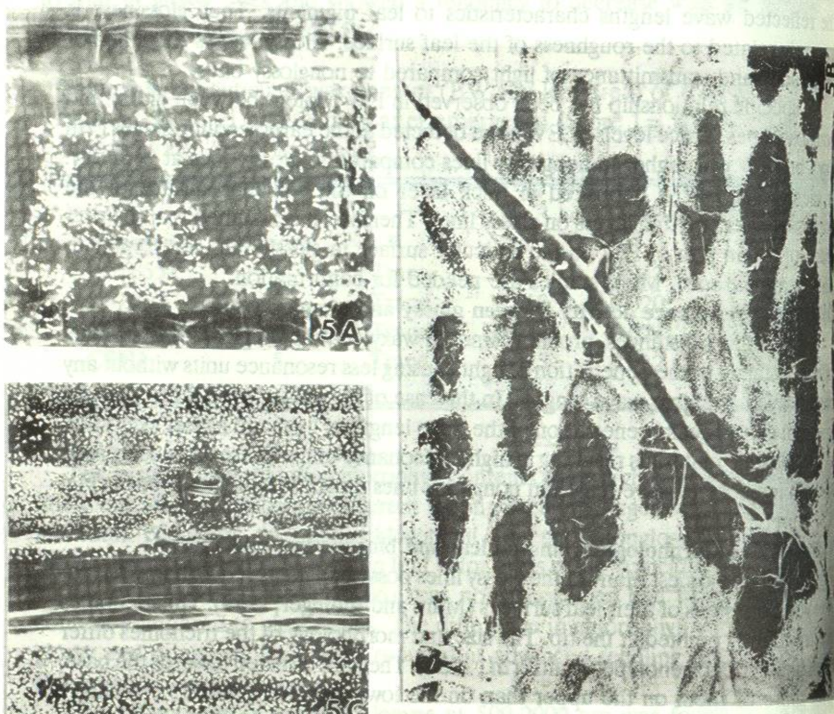


Plate 9.4 Sorghum CSH1: 5A) Sheath segment of 2 leaf (3rd leaf stage); 5B) Leaf lamina close to junction. 5C) Sheath segment after dewaxing.

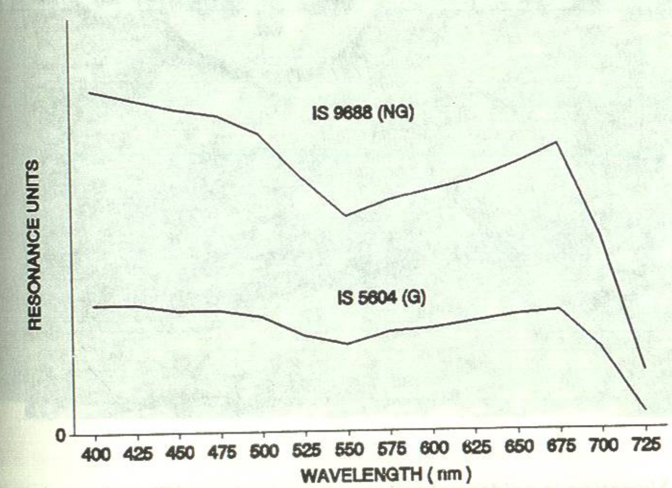
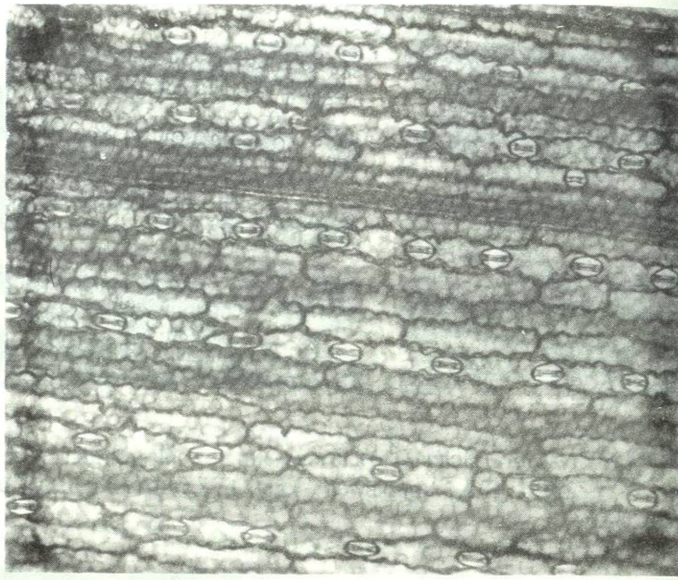


Figure 9.5 Photoacoustic response in glossy (G) and nonglossy (NG) sorghum leaves.

a)



b)

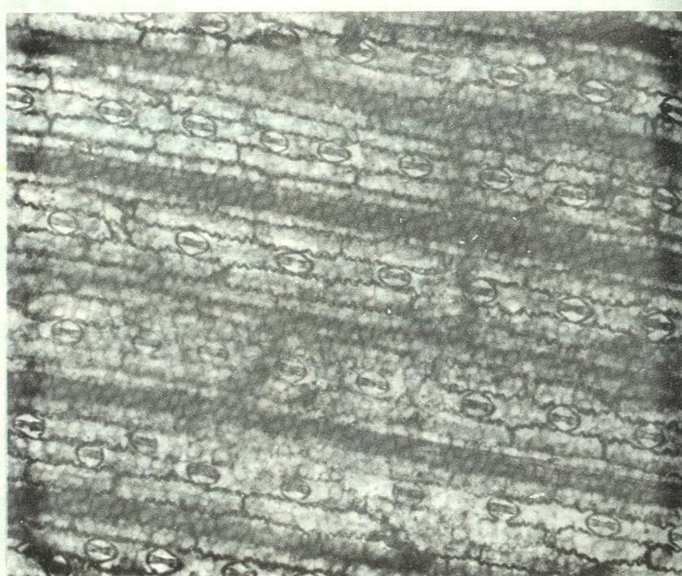
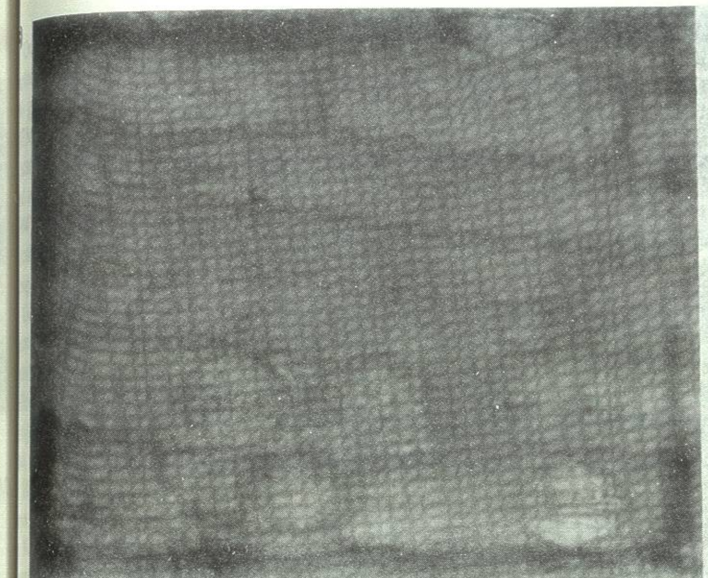
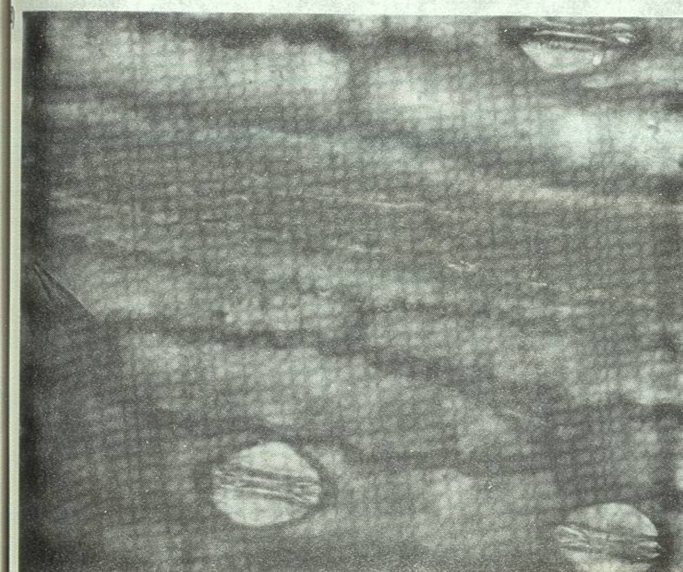


Plate 9.5 Variation in epidermal cell structures in glossy (G) and nonglossy (NG) sorghum genotypes. a) CSH1 (NG) showing general surface morphology and absence of nonglandular trichomes; b) IS 4664 showing general surface





morphology and presence of nonglandular trichomes; c) IS 1062, d) IS 1082 depicting the presence of nonglandular trichomes.

a)

b)

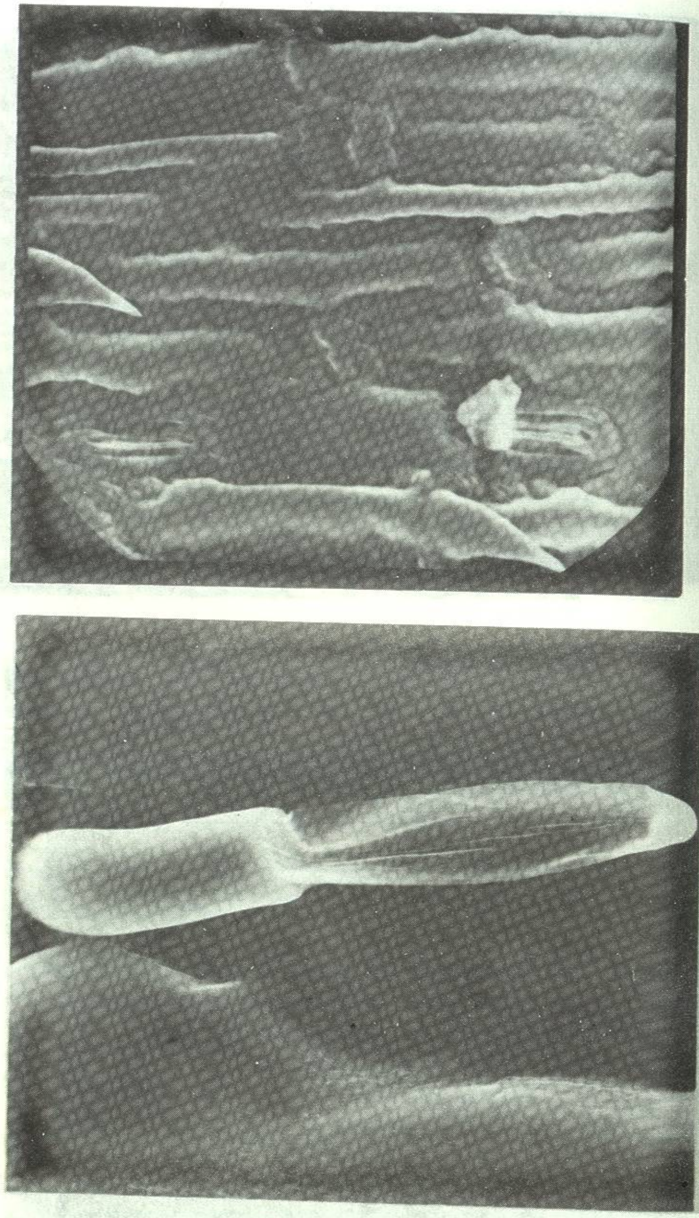


Plate 9.6 Variation in trichome morphology in glossy and nonglossy sorghum genotypes: a) M-35-1 (SEM) showing epidermal cells, stomata, silica crystals and pointed nonglandular trichomes; b) CSH1 showing bicellular trichomes, characteristic of nonglossy sorghum genotypes.

Out of 495 glossy lines, 272 lines had trichomes on both upper and lower faces and 169 lines had no trichomes. The leaf surface of only 50 sorghum lines glossy and 10 nonglossy) were studied with scanning electron microscopy EM). The glossy lines possessed amorphous smooth waxy lines associated with maller number of large irregular crystals in contrast with irregular leaf surface d a large number of needle shaped crystals in the nonglossy lines (Maiti *et al.*, 94). Nonglossy lines had a high density of needleshaped wax crystals unlike the shaped crystals reported by Tarumoto *et al.* (1981).

op age

Growth analysis indicated that leaf area, plant height and plant dry weight eased with age in both glossy and nonglossy lines. Glossy lines had larger and vier plants with lower leaf area (García-Mendoza, 1986). There was not much ference in epidermal cell, stomata and trichome numbers between glossy and nglossy lines. Glossy lines had predominantly nonglandular unicellular trichomes d the nonglossy lines bicellular glandular trichomes (Plates 9.5 & 9.6).

HCN content in glossy lines showed a slight decrease from seedling up to 45 ys but a sharp reduction at 60 days (Fig. 9.6). Nonglossy lines showed a decrease 30 days with an increase at 45 days and a drastic decrease at 60 days. Wax ntent increased at 30 days in both glossy and nonglossy lines, but decreased at vanced growth stages. Glossy lines showed higher wax content at 15 days mpared to nonglossy ones (Fig. 9.7). Chlorophyll content increased with age in th lines, remaining stable at 45 days in glossy lines, but increasing in nonglossy es. Chlorophyll content was always higher in nonglossy lines compared to glossy

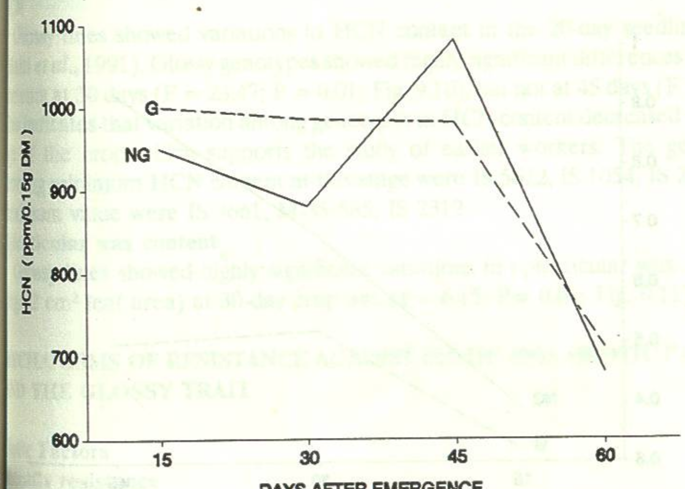


Figure 9.6 Average HCN content (ppm / 0.15 g DM) in glossy (G) and nonglossy (NG) sorghum at dirrerent crop age.

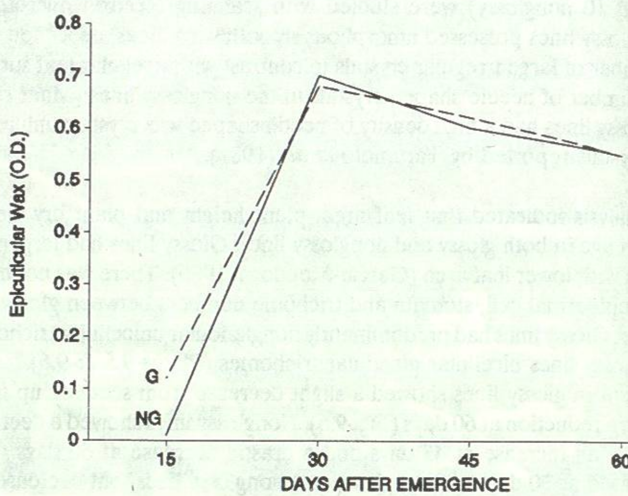


Figure 9.7 Average epicuticular wax content (OD) in glossy (G) and non-glossy (NG) sorghum at different crop ages.

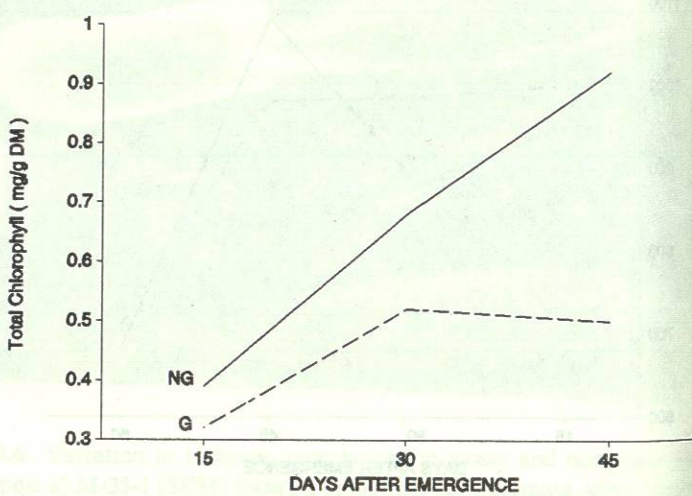


Figure 9.8 Average chlorophyll content (mg/g DM) in glossy (G) and non-glossy (NG) sorghum at dirrerent crop ages.

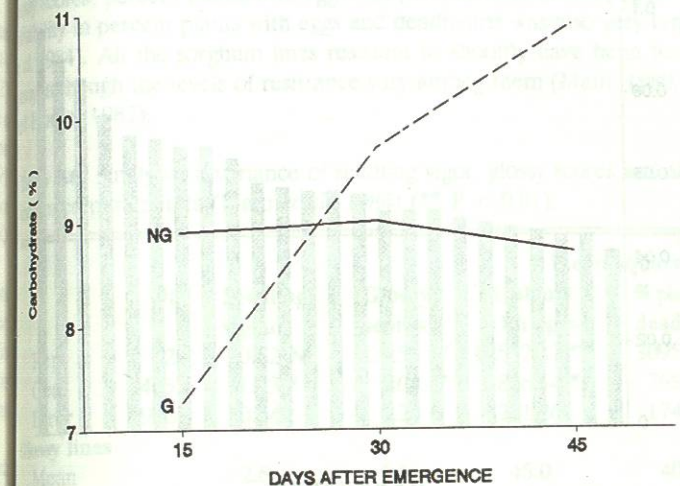


Figure 9.9 Average percentage carbohydrate content in glossy (G) and non-glossy (NG) sorghum at dirrerent crop ages.

es at all growth stages (Fig. 9.8). Carbohydrate content was higher in nonglossy es, but decreasing at subsequent growth stages, then it showed a sharp increase m 15 to 45 days in glossy ones (Fig. 9.9).

CN

Glossy lines showed variations in HCN content in the 20-day seedling stage Maiti *et al.*, 1991). Glossy genotypes showed highly significant differences in HCN ntents at 30 days (F = 23.47; P = 0.01; Fig. 9.10), but not at 45 days (F = 2.40). his indicates that variation among genotypes in HCN content decreased with the e of the crop which supports the study of earlier workers. The genotypes owing minimum HCN content at this stage were IS 5622, IS 1054, IS 2205 and aximum value were IS 4661, M-35-585, IS 2312.

picuticular wax content

Glossy lines showed highly significant variations in epicuticular wax contents mg/32 cm² leaf area) at 30-day crop age (F= 6.15; P= 0.01; Fig. 9.11).

ECHANISMS OF RESISTANCE AGAINST BIOTIC AND ABIOTIC FACTORS ND THE GLOSSY TRAIT

iotic Factors

hootfly resistance

Glossy sorghum genotypes (495) and 2 checks, CSH1 and Swarna were evaluat-d for resistance to shootfly during the postrainy season, 1981 in India at CRISAT. Several shootfly incidence indices were recorded.

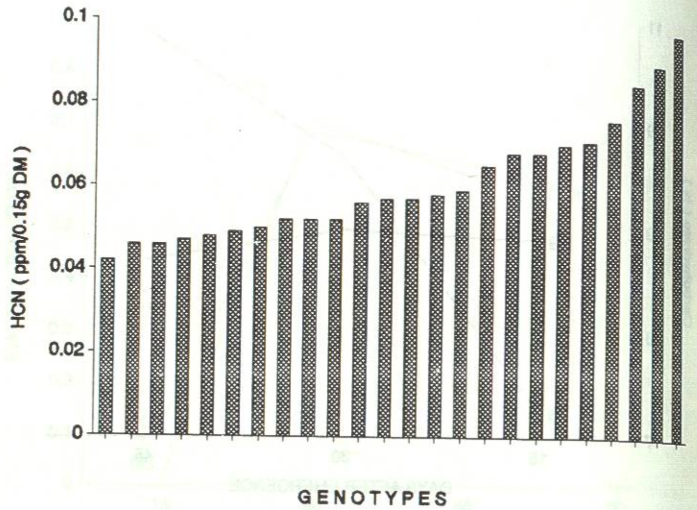


Figure 9.10 Average HCN content in glossy and nonglossy sorghum at 15 days after emergence (ordered according to increasing HCN contents).

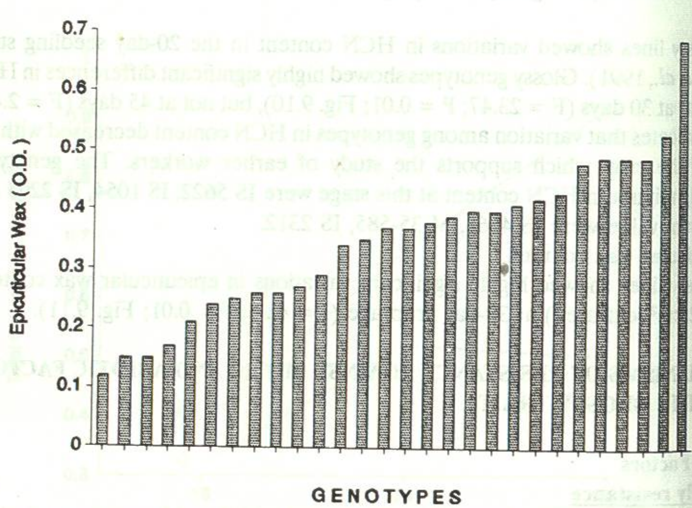


Figure 9.11 Average percentage epicuticular wax (EW) content in sorghum genotypes (ordered according to increasing EW contents).

ossy lines showed highly significant differences among them for seedling vigor, ossy scores, percent plants with eggs and percent dead hearts (Table 9.6). The riability in percent plants with eggs and deadhearts was also very large (Maiti al. 1984). All the sorghum lines resistant to shootfly have been found to be ossy, although the levels of resistance vary among them (Maiti, 1980; Agrawal d House, 1982).

Table 9.6 Analysis of variance of seedling vigor, glossy scores and shoot fly incidence parameters (Maiti *et al.*, 1984) (** P < 0.01).

		Mean square			
	df	Seedling vigour	Glossy scores	% plants with eggs	% plants with deadhearts
Rep.	2	0.82 NS	25.56 **	12572.37 **	3095.9 **
Gen.	495	1.73 **	1.04 **	826.54 **	768.34 **
Error	988	0.54	0.26	221.26	174.64
Glossy lines					
Mean	2.6		1.5	45.0	40.2
Range	1.0-4.7		1.0-4.7	12.6-94.7	7.9-90.1
C.V (%)	28.5		32.9	33.0	32.9
Nonglossy lines					
CSH 1		4.0	5.0	80.1	80.8
Swarna		4.0	5.0	74.8	74.8

echanism of resistance to shootfly - Trichome presence and density

Many sorghum lines having some field resistance had trichomes on their abaxial rface. In a wider range of materials (germplasm and breeding lines) under rying shootfly pressures, trichomed lines suffer comparatively less (fewer dead arts) than non trichomed lines (Maiti, 1980; Maiti and Bidinger, 1979; Agrawal d House, 1982; Maiti and Gibson, 1983; Gibson and Maiti, 1983.).

The presence of trichomes on the lower surface appears to confer the following dvantages: i- reduction in egg laying by the shootfly, and ii- reduction in the eadhearts with the presence of eggs.

Trichomes may be working as a mechanical barrier in the larval movement and causing death of the fly maggots. Therefore, their presence deters oviposition by hootflies. The correlation between trichomes and ovipositional nonpreference is early r=0.8 (Agrawal and House, 1982). Breeders have been able to reject usceptibles at all stages of testing of materials and finally increase the frequency f resistant genes (Agrawal and House, 1982).

Glossy trait

Glossiness is a monogenic recessive trait (Tarumato *et al.*, 1981). A set of enotypes with and without the glossy traits were tested 5 times for shootfly eaction under different fly pressures. Later, cluster analysis was done to categorize hem into different groups based on deadhearts incidence. The frequency of glossy lines in each group was calculated. There was a predominance of glossy lines in the group with the lower mean percentage of deadhearts (Maiti and Bidinger, 1979). With an increase in the level of susceptibility there was an increase in the number of nonglossy lines.

Differences in shootfly damage between glossy and nonglossy as well as trichomed and trichome free genotypes were always significant. At all fly pressures, glossy and trichomed lines overlap in the extent of shootfly damage indicating close association of these traits. Similarly, the differences between nonglossy and trichomefree lines were not significant. The correlations of trichome traits with shootfly indices were rather poor. This shows that the presence of trichomes imparts resistance to shootfly attack, but the increase in the number of trichomes was not seen to increase the level of resistance.

A principal component analysis on contribution of major factors to shootfly resistance, to trichome intensity, glossy intensity, eggs per plant and percentage dead hearts indicate that all traits are closely associated (Omori *et al.*, 1983). Both genotypic and phenotypic correlations made among these variables indicate a high degree of associations among them. Trichome intensity, glossy intensity and dead heart percentage are shown to be highly heritable traits (Agrawal and House, 1982). It is a monogenic recessive trait (Tarumato *et al.*, 1980). Shootfly incidence showed negative correlation with glossy and trichome intensity. These correlations were partitioned to show the contribution of individual trait by path coefficient analysis which indicated that high correlation is the result of exogenous traits and hence the glossy appearance may be an indicator to some other trait that contributes to resistance (Agrawal and House, 1982; Omori *et al.*, 1988). The genotypes showing a greater degree of intensity in glossiness were more resistant to shootfly. They were less affected by both shootfly egg laying and dead hearts (Maiti *et al.*, 1984). Omori *et al.* (1988) reported that trichome density contributed mainly towards genetic divergence in shootfly resistance, followed by glossiness. Heterosis for shootfly resistance was found to be associated with genetic divergence but not with geographic divergence.

There are several factors that contribute shootfly resistance, such as nonpreference for oviposition associated with the presence of trichomes on the leaf surface (Blum, 1968, 1972) and the glossy trait (Maiti and Bidinger, 1979). Similarly, the lignin and silica deposits may contribute towards the mechanical resistance of seedlings to penetration by larvae (Blum, 1968). Low leaf surface wetness (LSW) of the leaf whorl is an important factor in resistance to shootfly (Nwanze *et al.*, 1990). The susceptible lines show high LSW compared to the resistant lines. Glossy lines in general show less LSW depending of course on the intensity of glossiness (unpublished).

Quantitative relationship of morphophysiological traits with shootfly resistance

Some morphophysiological and anatomical traits of shootfly resistant and susceptible lines showed significant difference among them such as trichome number in the upper and lower surface. A correlation analysis has made among parameters determining resistance to shootfly deadheart in a set of lines. The parameters showing significant positive correlation with shootfly resistance (deadhearts) were: high glossy score, high seedling vigor, low leaf surface wetness, less

f width and lower stomata number in the upper surface in sorghum genotypes. e trichome number in the upper surface showed significant negative correlation th shootfly deadhearts (unpublished).

Another study with 520 sorghum germplasm determined that intensity of ssiness and trichomes were directly related with shootfly resistance, and not dling vigor (unpublished).

The distribution of traits for shootfly resistance such as glossy score (GS; 1 hly- to 5 non- glossy), seedling vigor (SV; high or low) and trichomes (T; none, per leaf surface, upper and lower leaf surfaces) were studied among sorghum otypes (1992, unpublished). The results indicated that intense glossiness (GS 2) persisted in the resistant (against shootfly and deadhearts) and moderately istant categories, whereas 80 to 95 % of the genotypes of the susceptible classes wed lower glossiness (GS 3-5). This clearly establishes that the intensity of ssiness is positively associated with the level of resistance against shootfly. milarly, the genotypes with highly vigorous seedlings resulted in plants resistant d moderately resistant to shootfly and deadhearts, whereas those genotypes with s vigorous seedlings resulted in susceptible or moderately resistant plants. The istance of the genotypes was also directly related to the presence and density f trichomes. In the resistant classes, the proportion of genotypes with trichomes n both the upper and lower surfaces was greater than in the susceptible classes th for oviposition and deadhearts.

A multiple regression equation was computed to determine the effect of the orphophysiological traits on oviposition and damage by shootfly (deadhearts). p to 40% of the variability could be explained by these traits in the case of iposition and about 47% in the case of deadhearts. The significant and negative gression coefficients of individual traits indicate that these traits substantially ntributed in reducing shootfly damage.

Shootfly infestation was direcly related to glossiness intensity score and was west in genotypes with high glossy score. Similarly, shootfly infestation increased n less vigorous genotypes and the lowest shootfly infestation was recorded when chomes were present on both leaf surfaces followed by the presence of trichoms only on the upper surface. The highest infestation was recorded in the genopes when the trichomes were absent on both leaf surfaces. Therefore, the resence of high glossy intensity and of trichomes on both surfaces of the leaf can e considered as reliable selection criteria in breeding for shootfly resistance.

Glossy characteristics and its role in genetic improvement in shootfly resistance

About 493 sorghum lines with varying glossy intensity were selected for their ossible utilization in breeding for shootfly resistance. These genotypes were istributed in groups depending on their resistance (shootfly oviposition and eadhearts) and their characteristics of glossy intensity (GS 1-5), geographical rigin and taxonomic group. None of the genotypes was highly resistant (< 10% nfestation) to shootfly for any of the resistance parameters. The genotypes in the esistant and moderately resistant categories (which made up 63-73% of the enotypes) had GS 1 or 2, with just a few having GS 3-5 in the moderately esistant group. Almost all of the genotypes with GS from 3-5 belonged to the usceptible group (27-37% of the genotypes). Shootfly infestation increased as the glossy intensity decreased. Genotypes with glossy score 1 had 40% oviposition and 35% deadhearts as against 74% oviposition and 69% deadhearts with glossy score 5. This establishes that the intensity of glossiness is positively associated with the level of shootfly resistance.

Of the Indian genotypes (83% of the genotypes), the majority were moderately resistant (47%) to shootfly, and moderately resistant to deadhearts (49%). Of the Indian genotypes, 24-36% were susceptible to shootflies and to deadheart damage. Half of the genotypes of African origin (12% of the genotypes) were susceptible for oviposition and deadhearts. Greater proportions of the genotypes of USA origin (5% of the genotypes) were either in the resistant or moderately resistant groups. The majority of genotypes with Indian and USA origin may be sources of higher resistance levels. The basis for the evolution of shootfly resistance is understandable, because shootfly has been a pest of sorghum in India since immemorial times. However, the sorghums genotypes of USA origin might not have exposed to insect infestations, since shootfly is absent in USA.

Considering glossy score, geographic origin and taxonomic race, minimum shootfly infestation was recorded in genotypes of USA origin and Bicolor race in glossy score 1. However in glossy score 2, Durra sorghums with Indian origin had the minimum shootfly infestation (Table 9.7). Maximum infestation was observed in Guinea sorghums of Africa, even with high glossy intensity, though the number of genotypes in this category were few (5). The variance ratio between genotypes and environment for shootfly resistant parameters *viz.* oviposition deadhearts among the glossy scores are shown in Table 9.7. The genotypes were significantly different for shootfly resistance parameters within each glossy score. Furthermore, genotype variation was higher for deadhearts compared to oviposition among the lines with high glossy intensity.

Table 9.7 Ratios of genotype to error mean squares (F ratio) for shootfly resistance parameters in different glossy score classes (** P=0.01; * P=0.05).

Glossy score	Resistance parameters	Geographic origin: Africa	India	USA	Taxonomic races: Bicolor	Caudatum	Durra
1	Oviposition	8.71**	3.26**	3.17**	3.56**	2.84	3.66**
	Deadhearts	9.54**	3.36**	5.32**	4.97**	3.36*	3.77**
2	Oviposition	2.48**	2.23**	-	1.41	5.84**	2.35**
	Deadhearts	3.56**	2.62**	-	2.29	8.34**	2.76**
3&4	Oviposition	1.57	2.76*	-	-	-	3.56*
	Deadhearts	2.05	3.66**	-	-	-	5.50**
5	Oviposition	-	2.84*	-	-	-	2.23
	Deadhearts	-	2.11	-	-	-	2.48

Correlation studies between oviposition and deadhearts in different geographic origin, taxonomic races and glossy scores showed that in all cases the coefficients were highly significant indicating close relationships between these shootfly resistance parameters (Table 9.8). Only in one case, no significant correlation was

d between oviposition and deadhearts in genotypes of African origin with sy scores 3-4.

ble 9.8 Correlations between oviposition and deadhearts in different geo-aphic origin, taxonomic races and glossy scores (** P=0.01).

Glossy score	Geographic origin: Africa	India	USA	Taxonomic race: Bicolor	Caudatum	Durra
1	0.95**	0.85**	0.88**	0.85**	0.96**	0.86**
2	0.91**	0.84**	-	0.83**	0.96**	0.85**
3 & 4	0.71	0.91**	-	-	-	0.90**
5	-	0.93**	-	-	-	0.84**

In order to assess the advantage of glossy trait for breeding of shootfly resis-ce, heritability percentage was calculated under geographical and taxonomic sification of glossy scores. Among the geographical groups, the heritability (%) sitively related with the level of glossiness in the genotypes both of Indian and ican origins. Glossy characteristics play an important role in improving the itability component in shootfly resistance parameters. This suggests that otypes of African origin with high glossy score are better for breeding programs improving shootfly resistance. Similar trends between glossiness and shootfly ameters were observed for taxonomic groups. High glossiness contributes orably for heritability improvement for both the resistance parameters in udatum and Bicolor races compared to Durra. But this needs further confirma-n with a larger number of genotypes of the Caudatum and Bicolor races. Glossy udatum sorghum of African origin can contribute well in the breeding program r shootfly resistance.

icuticular wax (EW) structure (scanning electron microscopy, SEM) in relation shootfly resistance

Sorghum genotypes with waxy bloom are reported to be drought and insect istant (Blum, 1975b; Chaterton *et al.*, 1975), improving their productivity in id and semiarid climates by reducing transpiration and increasing water use effi-ncy. Wax filaments types reported in *Sorghum bicolor* include tubular (Sánchez-las *et al.*, 1972), and filament- and ribbonlike (Atkins and Hamilton, 1982). *p*-Hydroxybenzaldehyde, a chemical contained in the EW of sorghum seedling aves is considered as major factor in reducing locust feeding, and related to the sistance to stem borer (Woodhead and Taneja, 1987). EW causes disorientation f stem borer larval movement (Taneja and Woodhead, 1989). Waxes with correct ysical characteristics and chemical composition are effective against insects. ummy waxes may stick the insect claws and feet to the leaf surface thus provid-g grip necessary to the insect to move around effectively (Atkin and Hamilton, 82; Mauseth, 1988).

It was mentioned earlier that glossy genotypes vary widely in epicuticular wax ructure. The smooth epicuticular waxy surface associated with trichome density