Pericarp structure and hullability in sunflower inbred lines and hybrids

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agronomie (1994) 14, 453-461

Plant breeding

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(Received 20 May 1994; accepted 10 October 1994)

Summary — The breeding of sunflower varieties whose seeds (achenes) can be easily hulled would help to optimise industrial hulling before oil extraction, and thus improve the protein content of sunflower seedmeal. Laboratory hulling tests require samples of about 10 g of seed, and so a search was made for characteristics that would permit indirect selection for improved hullability using smaller numbers of seed. Anatomical characteristics of the pericarps of 12 inbred sunflower lines and 18 hybrids were observed using light microscopy. Significant differences between genotypes in the frequency of parenchyma rays separating sclerenchyma zones, and in the proportion of wide and wedge-shaped sclerenchyma zones were observed. However, the phenotypic correlations between these characteristics and hullability measurements with a laboratory huller were too weak to be usable alone in hullability breeding programmes. Observations of hull structure however will probably serve most usefully to explain certain aspects of variation in hullability.

correlation / hull / parenchyma / sclerenchyma / sunflower

Résumé — Structure du péricarpe et aptitude au décorticage d'hybrides et de lignées de tournesol. *La sélection de variétés de tournesol dont les graines se décortiquent bien constitue l'un des facteurs déterminant pour l'optimisation du décorticage industriel effectué avant l'extraction de l'huile afin d'améliorer la teneur en protéines des tourteaux. La réalisation des tests de laboratoire nécessite environ 10 g d'akènes, ce qui constitue une quantité relativement importante ; c'est pourquoi une sélection indirecte basée sur des caractères mesurables sur un petit nombre de graines a été envisagée. Diverses caractéristiques anatomiques des coques des akènes de 12 lignées et 18 hybrides de tournesol ont été observées en microscopie photonique. Des différences significatives entre les génotypes ont été mises en évidence pour la fréquence des travées parenchymateuses et les nombres de massifs larges et de massifs en forme de coin par centimètre. Les liaisons des caractères étudiés avec le taux de décorticage sont trop faibles pour qu'une sélection indirecte puisse être envisagée. Néanmoins, l'observation de la structure des coques devrait contribuer à une meilleure compréhension de la variation de l'aptitude au décorticage.*

corrélations / coque / sclérenchyme / parenchyme / tournesol

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INTRODUCTION

A sunflower 'seed', or more strictly achene, is made up of a true seed (kernel) enclosed within a ligno-cellulosic pericarp (hull). The hulling process, carried out just before oil extraction, consists of separating as far as possible hull and kernel, in order to reduce the cellulose content, and thus increase the energy and protein content of the seedmeal obtained. This would improve its ability to compete commercially with other seedmeals, soyabean in particular (Evrard, 1984; Bourdon, 1985; Signoret and Evrard, 1987).

Many studies have been made to determine the best methods of hulling and separating sunflower kernels from hulls (Defromont, 1972; Ashes and Peck, 1978; Wan *et al*, 1978; Tranchino *et al*, 1984; Karlovic *et al*, 1992a,b; Simic *et al*, 1992). Environmental and cultural conditions (irrigation, nitrogen supply) are known to affect hullability (suitability for industrial hulling (Dedio and Dorrel, 1989)), but genotypic factors appear to be predominant (Beauguillaume and Cadeac, 1992b; Merrien *et al*, 1992; Denis, 1994), so that breeding programmes to improve hullability should be the most efficient method to provide material better adapted to industrial processes.

At present, laboratory hullers require samples of 5–10 g of seed. Thus, in early generations of breeding programmes, this destructive test uses a large proportion of the seed produced by individual plants, especially in the case of branched genotypes with small main capitula (inbred sunflower lines produce from 2 to 100 g of seed per plant, with an average of about 10 g). It therefore appeared useful to search for characters observable on a smaller number of seeds and closely correlated with hullability, which could be used for indirect selection on inbred lines or individual plants in segregating generations.

The present study concerned anatomical characteristics of the sunflower hull, in particular those observed by Leprince-Bernard (1990) and Beauguillaume and Cadeac (1992a,b). The sunflower hull is composed mainly of sclerenchyma, divided into zones by parenchyma rays which become compressed at maturity. Beauguillaume and Cadeac (1992a,b) observed 2 types of sclerenchyma zone: narrow, wedge-shaped zones with vascular bundles at their base and wide non-vascularised zones. These authors reported differences between the proportions of the 2 types of sclerenchyma zone and the frequencies of separation by parenchyma rays in 6 commercial varieties. These differences appeared to be related to varietal differences in hullability. Leprince-Bernard (1990) applied compression tests on sunflower achenes and showed that breakage of the pericarp occurs along the length of the seed, preferentially along parenchyma rays which may be considered as the weak points of the hull.

If such characteristics are to be used in breeding programmes, they must be closely correlated with hullability for a wide range of genotypes. This paper reports studies to determine whether this was the case for 12 inbred lines and 18 F1 hybrids.

MATERIALS AND METHODS

Sunflower genotypes

The study was made on 12 inbred lines, 6 female (male-sterility maintainers), ZF, CAJ, RC, UD, CD, CAS, and 6 male (male sterility restorers), PBP4, 89HR2, PB3, PSC5, HA61, PSC6, whose origins are given in table I, and 18 hybrids obtained by crosses between these lines.

For both the inbred lines and hybrids, the plants were grown in 2 row plots at Clermont-Ferrand in 1992 at a density of 50 000 plants/ha. Ten plants per genotype were harvested, and their seed pooled for measurements of both mechanical hullability and anatomical observations. However, for 2 lines, PBP4 and 89HR2, seed from only 2 plants was available, so these were observed separately.

Measurements of mechanical hullability

The laboratory huller used to carry out hulling tests is based on a centrifuging process, with a disc turning at 3 800 rpm. Achenes are thrown lengthwise against the vertical wall of the huller. The product obtained is then sorted on a laboratory separator. This gives 3 fractions: 'fines' made up of small fragments of kernel; 'industrial kernels' which are a mixture of kernels and partially hulled achenes; and 'free hulls'. The 2 machines used were designed by Techmachine (Hydromécanique et Frottement group), Andrézieux-Bouthéon, France.

Hullability (*H*) was calculated from the ratio of the percentage of hulls extracted by a mechanical huller, measured on samples of 10 g of achenes (*MH*) compared with the total hull content expressed as a percentage of seed weight, determined by manual hulling of 50 achenes (*HL*)

$$H = (MH / HL) \times 100$$

For each genotype, total hull content and hullabilities were calculated from 3 measurements carried out on

Table I. Origin of the 12 inbred sunflower lines used for studies of hull anatomy.

Line	*Version*	*Origin*	*Breeder*
Female lines			
ZF	M	Recurrent selection seed yield	INRA
CAJ	M	CM303 from VNIIMK 8931	Agriculture Canada/INRA
RC	M	Recurrent selection seed yield	INRA
UD	M	USDA breeding population	INRA
CD (HA89)	M	Open pollinated Russian variety VNIIMK 8931	USDA
CAS (HA850)	M	Breeding population of Russian material	USDA
Male lines			
PBP4	R	Recurrent selection seed yield	INRA
89HR2	R	Breeding population of restorer	INRA
PB3	R	*H petiolaris* restorer x RHA299 (USDA)	INRA
PSC5	R	PAC1 x RHA299	INRA
HA61	R*	Russian open pollinated variety x wild *H annuus*	USDA
PSC6	R	RHA272 (USDA) x *H petiolaris* restorer	INRA

M: maintainers of French cytoplasmic male sterility; R: restorers of French cytoplasmic male sterility; R*: partial restorer.

mixtures of seed harvested. However, for some inbred lines 1 measurement was made for each plant individually.

Light microscopy observations

The achenes were soaked in water for 12 h at room temperature. Transverse sections 30 µm in thickness were cut on a freezing microtome equipped with a razor blade, halfway along the length of the achenes.

The sections were stained for 10 min in a mixture of alum carmine and iodine green, rinsed and mounted on a slide in a drop of water. The stains coloured lignin blue-green and cellulose pink.

Observations were carried out at a magnification of 11 x 10 unless otherwise stated. Ten seeds per genotype were observed for the following 5 characteristics: average hull thickness in mm (T); external perimeter in mm (*E*), which was estimated from the 2 diameters *a* and *b* of the section considered to be an ellipse, observed at a magnification of 11 x 2.5, using the formula $(\pi / 4) \times [a + b + (2 \times a^2 + b^2)^{1/2}]$; number of wide sclerenchyma zones per cm (*W*); number of narrow, wedge-shaped sclerenchyma zones per cm (*N*); and number of parenchyma rays per cm (*P*). The last 3 characters were measured on a quarter of the perimeter of each seed observed.

RESULTS

Figure 1 shows the appearance of a transverse section of a sunflower hull. Externally, a melanised epidermis and a second layer of pigmented cells are visible. In agreement with the observations of Beauguillaume and Cadeac (1992a,b), the 2 types of sclerenchyma zone were observed: wide zones (W) alternating more or less regularly with narrow wedge-shaped zones (N) whose bases project into the internal space beneath the hull (I). However, this second type of zone was not observed in the achenes of the inbred line PSC6 and the hybrid UD x PSC5 (table II). In addition, of the 2 plants of 89HR2 observed, only one showed the narrow zones, indicating some variability within genotypes.

The sclerenchyma fibres in contact with the pigmented layer generally showed thicker, more lignified walls than the more internal cells. The internal parenchyma cells, with thin cellulose walls, were not usually individually distinguishable, being crushed between the kernel and the hull sclerenchyma.

Genotypic effects were highly significant for all the 5 anatomical characteristics observed (tables II, III) and for both inbred lines and hybrids. However, there was less variation between hybrids than between the inbred lines. In particular, the number of parenchyma rays per cm varied from 36.5 (CAJ) to 102.0 (PSC5) for inbreds, but from 45.7 (UD x PB3) to only 66.5 (CAS x PSC6) for hybrids. The coefficient of variation showed quite a wide intra-genotype variation for the numbers of wide and narrow sclerenchyma zones per cm.

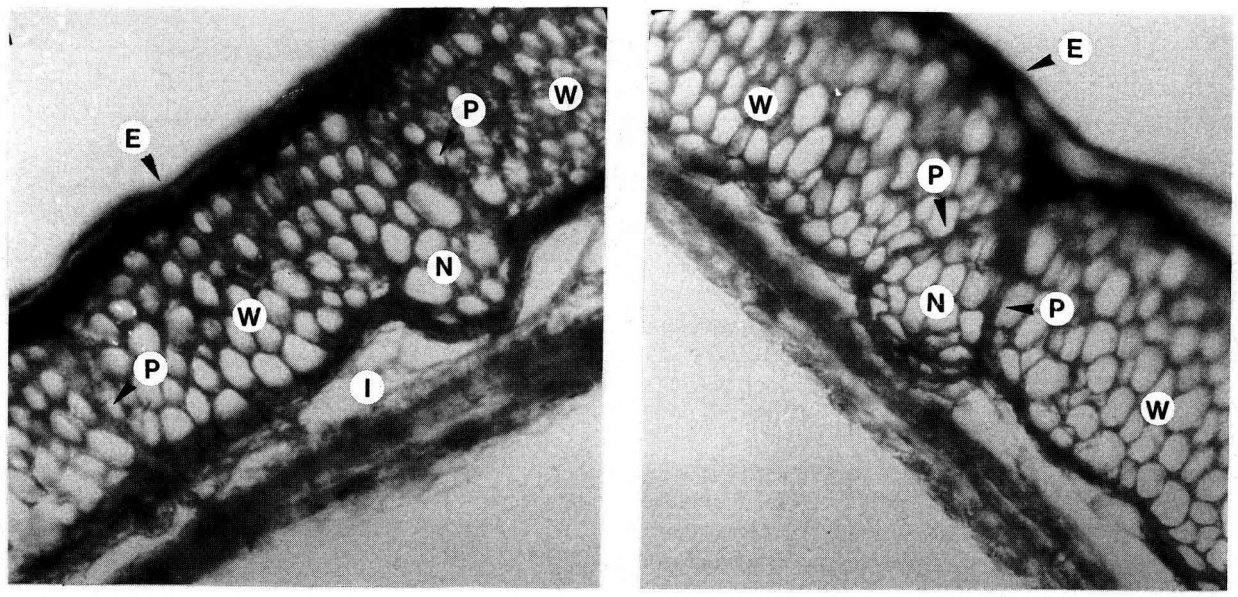

Fig 1. Transverse sections of a sunflower seed hull (pericarp) showing different aspects of anatomical structure. E: epidermis and layer of pigmented cells; W: wide sclerenchyma zone; N: narrow wedge-shaped sclerenchyma zone; P: parenchyma ray; I: internal parenchyma.

On average, the female lines generally had thicker hulls than the male lines (means respectively 0.21 and 0.15 mm) and fewer parenchyma rays than the latter (48.6 and 71.9 areas/cm) (table II).

The analyses of variance presented in table IV indicate that the effect of the male parent was much greater than the female effect for all the characteristics studied, with the exception of the number of wedge-shaped sclerenchyma zones per cm. The female effect was not significant for hull thickness, perimeter and number of parenchyma rays per cm.

The correlations between the mean parent values and those of hybrids are given in table V. The closest relationships were found for the numbers of parenchyma rays (r = 0.60**, $p < 0.01$) and wide sclerenchyma zones per cm (r = 0.58*, $p < 0.05$). However, the determination coefficients for these 2 correlations indicated that only about 35% of the value of a hybrid was explained by that of its parents.

Correlations between the different characteristics are presented in table VI. Concerning technical characters, it may be noted that hullability (*H*) and the quantity of mechanically extracted hulls (*MH*) were closely correlated (r = 0.96** (inbreds) and r = 0.98** (hybrids)).

Among the anatomical characters, the number of parenchyma rays and wide sclerenchyma zones per cm were negatively related to achene perimeter (r = –0.73** and –0.67** respectively) for inbred lines. These correlations also exist for hybrids, but to a lesser extent (r = –0.54* and –0.48*). The correlation between hull content and number of parenchyma rays per cm was positive for hybrids (r = 0.68**) but negative for inbred lines (r = –0.54*).

In this study, the achene character that most closely correlated with hullability and quantity of mechanical hulls was hull content (r = 0.56* to 0.76**). No close relation appeared between hullability and any of the anatomical characters observed. For inbred lines, perimeter and thickness showed certain relations with mechanically extracted hulls whereas in the case of hybrids this character was correlated significantly with the number of parenchyma rays per cm (r = 0.50*).

DISCUSSION

The different tissues in the sunflower hull described by Percie Du Sert and Durrieu (1988) were observed, with the exception of the hypodermis, which should be made up of several layers of regularly arranged cells. However, this tissue probably undergoes compression and progressive disorganisation during maturation (Beauguillaume and Cadeac, 1992a), which would explain why it is not observed in mature achenes. According to Perestova (1976), the formation of pericarp tissues and lignification of sclerenchyma fibres is complete 30 d after

Table II. Mean hull characteristics of 12 inbred lines and 18 hybrids of sunflower.

	T	*E*	*W*	*N*	*P*	*HL*	*MH*	*H*
Lines								
Female lines (maintainers of French cytoplasmic male sterility)								
ZF	0.178	12.1	24.5	14.6	40.8	19.1	3.90	20.8
CAJ	0.208	13.9	26.3	7.4	36.5	23.9	11.98	50.1
RC	0.183	12.2	30.5	16.5	51.0	21.4	10.24	47.8
UD	0.267	15.1	38.3	16.3	57.8	25.3	18.70	73.9
CD	0.218	13.8	33.0	13.9	49.4	21.0	9.40	43.5
CAS	0.175	12.0	43.1	9.9	55.9	15.2	3.37	22.2
Means	0.205	13.2	32.6	13.1	48.6	21.0	9.60	43.1
Males lines (restorers of French cytoplasmic male sterility)								
PBP4pl1	0.141	11.8	43.6	22.8	70.1	14.8	2.60	17.6
PBP4 pl2	0.125	11.1	41.7	34.1	79.6	14.9	2.10	13.8
89HR2 pl1	0.178	12.2	47.9	0.0	52.5	26.0	6.30	24.3
89HR2 pl2	0.181	12.8	43.0	8.2	53.0	21.5	3.40	16.0
PB3	0.143	11.3	44.2	24.6	71.6	17.7	4.90	27.7
PSC5	0.137	10.4	59.0	31.9	102.0	17.3	3.01	17.4
HA61	0.173	12.4	37.7	19.8	61.4	22.8	16.90	74.1
PSC6	0.135	10.0	81.1	0.0	84.8	18.2	9.38	51.6
Means	0.152	11.5	49.8	17.7	71.9	19.2	6.07	30.3
Means	0.174	12.2	42.4	15.7	61.9	19.9	7.58	35.8
Max–Min	0.142	5.0	56.6	34.1	65.4	11.2	16.60	60.3
Hybrids								
ZF x PSC6	0.219	13.3	35.5	27.3	65.9	19.1	7.96	41.7
CD x PSC6	0.201	12.9	37.0	22.7	62.9	21.9	9.35	42.7
CAS x PSC6	0.224	12.2	42.8	20.4	66.5	19.6	8.72	44.5
ZF x PSC5	0.225	14.4	27.8	25.8	56.4	19.4	7.49	38.6
UD x PSC5	0.201	13.2	62.1	0.0	65.2	20.2	8.49	42.0
CD x PSC5	0.203	13.8	38.1	23.8	64.8	22.2	9.23	41.6
UD x PB3	0.184	14.4	28.5	14.4	45.7	16.6	4.88	29.4
RC x PB3	0.205	15.2	23.1	22.0	47.7	16.8	6.17	36.7
CD x PB3	0.188	13.8	26.6	18.9	48.4	15.4	3.12	20.3
CAJ x PBP4	0.198	13.8	29.9	25.4	58.2	20.1	7.01	34.9
RC x PBP4	0.185	13.4	30.9	18.0	51.9	19.2	10.2	53.1
ZF x PBP4	0.187	13.7	36.0	22.2	61.1	17.1	5.50	32.2
RC x 89HR2	0.206	13.2	26.0	19.0	48.1	18.1	5.24	29.0
CAS x 89HR2	0.192	12.6	31.7	17.6	52.5	17.2	3.54	20.6
CAJ x 89HR2	0.208	14.5	23.8	19.0	45.6	17.9	4.23	23.6
UD x HA61	0.206	14.8	25.0	23.0	50.7	19.1	8.84	46.3
CAS x HA61	0.197	14.1	25.9	21.0	49.8	16.5	8.19	49.7
CAJ x HA61	0.183	12.4	27.7	24.7	55.7	20.9	11.68	55.9
Means	0.201	13.7	32.1	20.3	55.4	18.7	7.21	37.9
Max–Min	0.042	3.0	39.0	27.3	20.9	6.8	8.56	35.6

Max–Min: maximum value – minimum value. *T* = hull thickness in mm, *E* = perimeter in mm, *W* = number of wide sclerenchyma zones per cm, *N* = number of narrow wedge-shaped sclerenchyma zones per cm, *P* = number of parenchyma rays per cm, *HL* = hull content in %, *MH* = hull extracted by a mechanical huller in %, *H* = hullability in %.

Table III. Analysis of variance of measurements of hull characteristics for 12 inbred sunflower lines and 18 hybrids.

Variable (abbreviation used)	*F genotype*	*Coefficient of variation*	*lsd*
Lines			
Hull thickness (*T*)	26.2**	13.6	0.021
Perimeter (*E*)	13.4**	9.6	1.04
Number of wide sclerenchyma zones per cm (*W*)	50.9**	15.9	5.66
Number of narrow, wedge-shaped sclerencyma zones per cm (*N*)	34.9**	35.4	4.97
Number of parenchyma rays per cm (*P*)	42.7**	14.0	7.76
Hybrids			
Hull thickness (*T*)	3.2**	11.5	0.020
Perimeter (*E*)	4.7**	8.9	1.08
Number of wide sclerenchyma zones per cm (*W*)	19.4**	20.7	5.96
Number of narrow, wedge-shaped sclerenchyma zones per cm (*N*)	18.3**	22.0	3.99
Number of parenchyma rays per cm (*P*)	8.1**	15.0	7.38
Hull content (*HL*)	29.9**	3.2	1.01
Hull extracted by a mechanical huller (*MH*)	40.8**	8.9	1.08
Hullability (*H*)	28.1**	9.1	5.7

** Highly significant ($P < 0.01$).

flowering. The pigmented layer observed probably corresponds to the non-cellular phytomelanin 'armor layer' described by Perestova (1976) and Knowles (1978).

The studies described here indicate that the use of anatomical characteristics of sunflower achenes in breeding programmes would raise a number of technical problems. In particular, the

Table IV. Male and female parental effects on hull anatomical characteristics of 18 sunflower hybrids.

Variable (abbreviation used)	*Source of variation*	F *test*
Hull thickness (*T*)	Female	1.63ns
	Male	5.28**
Perimeter (*E*)	Female	1.37ns
	Male	3.67**
Number of wide sclerenchyma zones per cm (*W*)	Female	9.90**
	Male	23.1**
Number of narrow, wedge-shaped sclerenchyma zones per cm (*N*)	Female	18.97**
	Male	8.40**
Number of parenchyma rays per cm (*P*)	Female	0.49ns
	Male	14.71**

** Highly significant ($P < 0.01$); * significant ($P < 0.05$); ns: non-significant.

Table V. Correlations between *per se* values of inbred lines and those of their hybrids for 7 characteristics of sunflower hulls.

Characteristic	*Correlation*
T	−0.28ns
W	0.58*
N	−0.23ns
P	0.60**
HL	0.04ns
MH	0.38ns
H	0.50*

T = hull thickness, *W* = number of wide sclerenchyma zones per cm, *N* = number of narrow, wedge-shaped sclerenchyma zones per cm, *P* = number of parenchyma rays per cm, *HL* = hull content, *MH* = hull extracted by a mechanical huller, *H* = hullability.

Table VI. Correlations between different characteristics of sunflower hulls and achenes calculated for 18 hybrids (below the diagonal) and 12 inbreds (above the diagonal).

	T	E	W	N	P	HL	MH	H
T		0.67**	–0.14ns	–0.03ns	–0.14ns	0.47ns	0.63*	0.55*
E	0.07ns		–0.67**	–0.24ns	–0.73**	0.66**	0.60*	0.49ns
W	0.13ns	–0.48*		–0.15ns	0.77**	–0.28ns	–0.17ns	–0.08ns
N	0.24ns	0.11ns	–0.61**		0.52ns	–0.51ns	–0.24ns	–0.24ns
P	0.36ns	–0.54*	0.76**	0.04ns		–0.54*	–0.29ns	–0.22ns
HL	0.26ns	–0.39ns	0.44ns	0.14ns	0.68**		0.71**	0.60*
MH	0.09ns	–0.29ns	0.29ns	0.17ns	0.50*	0.76**		0.98**
H	0.06ns	–0.19ns	0.19ns	0.16ns	0.38ns	0.56*	0.96**	

** Highly significant ($P < 0.01$); * significant ($P < 0.05$); ns: non-significant. *T* = hull thickness, *E* = perimeter, *W* = number of wide sclerenchyma zones per cm, *N* = number of narrow, wedge-shaped sclerenchyma zones per cm, *P* = number of parenchyma rays per cm, *HL* = hull content, *MH* = hull extracted by a mechanical huller, *H* = hullability.

seeds would be observed individually and since they show some heterogeneity for the different anatomical characters, about 10 (as in the present study) would be necessary to obtain significant differences between genotypes. Perestova (1976) and Beauguillaume and Cadeac (1992a) have also reported that hull thickness and anatomical characteristics may vary according to the position of the achene on the capitulum.

Observation of 10 or more seeds of each individual would be unrealistic in a breeding programme, since preparation of transverse sections takes about 25 min and observations 10 min. Sometimes, it is possible to cut satisfactory sections by hand, which reduces total manipulation time to 15 min, but this is still very long. In addition, cutting with a razor-blade may lead to loss of the epidermis or internal parenchyma, the latter tissue becoming more fragile especially when the achene is dry (Leprince-Bernard, 1990). It is also difficult in certain cases to obtain thin sections as the hull breaks up (for example, HA61).

For some of the genotypes very few parenchyma rays separating sclerenchyma zones were observed. Either they were absent or they may have been completely crushed. This observation was also reported by Beauguillaume and Cadeac (1992a,b), who observed in addition, that the degree of fragmentation of sclerenchyma by parenchyma varies according to pedoclimatic conditions and that different hybrids react differently to stress.

A larger male parent effect in the expression of the characters hull thickness, seed perimeter, number of wide sclerenchyma zones and parenchyma rays compared with the female effect was also observed for hullability of the same genotypes (Denis *et al,* 1994). This result may have been caused by the greater variability among male lines for numbers of wide sclerenchyma zones and parenchyma rays.

The close correlation between hullability (*H*) and mechanically extracted hulls (*MH*) is probably related to the fact that *H* is a function of *MH* and percentage hull content and that the latter shows less variation than the former. Practically it should make it possible to eliminate measurements of total hull content, which would be an advantage since this is a long, manual process. Hullability would be estimated directly from the quantities of hulls extracted mechanically. However, this method would have the disadvantage that genotypes with low hull contents but good hullabilities such as CAS x HA61, would not be retained since the amount of mechanical hulls would be small. As there is a positive correlation between hull content and hullability, these genotypes will be rare. Since the few that occur are likely to have also good oil contents, they should be identified.

In contrast with the quite close relation between hull content and mechanically extracted hulls, the anatomical characters observed do not show any close linkage with the last characteristic. The absence of any significant correlation between hull content and thickness agrees with the results of Leprince-Bernard (1990) who obtained similar values. The correlation coefficient between mechanically extracted hulls and number of parenchyma rays was significant only for hybrids, and lower than the figure obtained by Beauguillaume and Cadeac (1992b). Since the other anatomical characteristics in our study showed no significant cor-

relations with hullability, it was concluded that they cannot be used as indirect selection criteria. As a result, it does not appear justified to continue studies on heritability and genotypic correlations with hullability, or to compute a selection index involving anatomical characteristics.

Anatomical characteristics appear of more interest in understanding what happens when sunflower achenes are hulled and why there are differences in hullability. Hull structure does appear to be determined genetically; some genotypes showing much greater tissue differentiation than others, with different frequencies of narrow vascularised sclerenchyma zones and parenchyma rays. It would be interesting to know what effect the absence of vascular bundles has on both kernel and hull development. The frequency of parenchyma rays may depend both on their original production and on the extent of their lignification or compression by the surrounding sclerenchyma.

Leprince *et al* (1988) observed that hull thickness and the volume occupied by the internal parenchyma influence hullability. Beauguillaume and Cadeac (1992b) found that achene specific weight also affected hullability under certain pedoclimatic conditions. For partially filled achenes, the space between the hull and kernel may be predominant in determining hullability. However, when the kernel completely fills the hull, the frequency of parenchyma rays, the weak points, may determine how easily the pericarp can be split during the hulling process. In addition, the chemical composition of lignins and lignin / cellulose linkages might also affect hullability. It would be interesting to develop studies involving a wide range of factors including patterns of sclerenchyma fragmentation, seed specific weight and volume of space between kernel and hull, in order to obtain a better understanding of why some sunflower achenes can be more easily hulled than others. Further, Denis (1994) showed considerable environmental effects on hullability and it would be usefull to examine pericarp development according to environmental conditions.

ACKNOWLEDGMENTS

This study was financed by ECLAIR (European Community Linkage Agriculture and Industry through Research) contract number AGR2.0029, carried out in collaboration with the CETIOM (Centre Interprofessionnel des Oléagineux Métropolitains). We would also like to thank Promosol for general support of this programme.

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