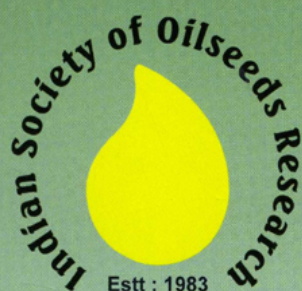


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Hull anatomy and hullability in safflower (*Carthamus tinctorius* L.)

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ABSTRACT

High fiber content makes safflower (*Carthamus tinctorius* L.) flour difficult to use as poultry and pork dietary supplement. During oil extraction, seed hulls are removed to improve flour quality, thus increasing its protein content and reducing, at the same time, its raw fiber content. Hulling of safflower in Argentina has technical difficulties resulting from the hull hardness and its adherence to the seed. Seed morphology and hull anatomy are related on how easily the hull is set apart from the seed. This is defined as hullability (H). In this paper, the genotype variability of safflower seeds was studied to identify which morphological characteristics are associated to changes in its H. The following parameters were determined in mature seeds of 9 safflower genotypes: H, hull dry weight (HDW), HDW/seed dry weight ratio and hull anatomy. H ranged between a minimum of 49% and a maximum of 60%, and only showed a positive correlation with the hull dry weight not being related to the other morpho-anatomical parameters of the seeds studied in this work.

Keywords: Hullability, Hull anatomy, Safflower

Safflower (*Carthamus tinctorius* L.) seed milling yields an average of 34% oil and a 61% of cake. The residual oil in the cake decreases to 6-8% when oil extraction is made by pressing and to 0.5 to 2% when solvent is used. In general, the cake is pelletized into fortified cubes or milled to be added into animal feed mixtures. When the cake is obtained after processing the seed without previous hulling, the protein and the fiber content is 22% and 40%, respectively and it is useful for ruminant feed (Lewis y Southern, 2000). However, its high fiber content becomes a problem if the cake is aimed at poultry and pork dietary supplement (Lewis and Southern, 2000; Smith, 1996).

Dehulling of seeds prior to oil extraction decreases the cake fiber content between 15 to 16% and increases protein content between 42 to 45%. This cake is then a good dietary supplement for monogastric animals (Smith, 1996).

The easiness with which the hull (botanically the seed's pericarp) separates from the kernel, known as hullability (H) of the seeds, is stated as the ratio between the percentage of hull removed mechanically, with small size hullers that simulate an industrial process, and the total amount of hull in seed weight, determined by manual separation (Denis *et al.*, 1994).

Modern safflower and sunflower genotypes show high oil percentage and a lower hull proportion in seeds (Leprince and Bernard, 1990; Smith, 1996). As the hull is more tightly attached to the kernel, its removal is harder. This is a compromise situation for breeders, as oil percentage and H are inversely proportional parameters.

In sunflower, there are genotypes with good H among oil producing hybrids, and with enough variability to select both parameters independently (Denis and Vear, 1996; Lindström *et al.*, 2000). Lindström *et al.* (2007) show that the H of sunflower seeds could be modified by environmental conditions or crop management (light stress). However, the morpho-anatomical parameters of the seed, which characterized each hybrid and determined the H difference among them, remained constant throughout crop sites, years and treatments.

The purpose of this work was to study, in nine safflower genotypes the effect of genotype on H and its correlation with seed's morpho-anatomical characteristics.

MATERIALS AND METHODS

Seed samples of the following safflower genotypes were used: Agrosearch L-7-3, AG Phoenix, CW 99 OL, Arizona L-7-5, Knowles 93079, L-8, Seedtech S-345, Seedtech S-3125 and Agrosearch T-21. All genotypes are of American origin and were cultivated in the network of Yield Comparative Tests (CYT for its acronym in Spanish) and supplied by INTA H. Ascasubi (Province of Buenos Aires. 39°23'S; 62°37'W). Sowing was made on September 29, 2009. Plots were of 1.4 x 6 m. The distance between rows was 20 cm and plant density was managed between 35 to 40 plants/m². The experimental design was completely randomized with 3 repetitions by genotype.

A laboratory huller as described by Bäumler *et al.* (2002), was used to carry out hulling tests. Hullability was calculated from the percentage of hulls extracted with the laboratory huller, measured on samples of 10 g of seeds (MH), compared to the total hull content expressed as a percentage of seed weight, determined by manual hulling of 50 seeds (HL; Denis *et al.*, 1994) as:

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

Hull dry weight (HDW), seed dry weight (SDW) and the relationship HDW/SDW were determined after manually hulling 50 seeds of each genotype. The samples were dried at 60 °C during 72 h.

Cross sections of the hull of each genotype were fixed in FAA (formaldehyde, acetic acid and absolute ethanol fixative solution) and processed according to conventional histological techniques of embedding, cutting (10 µm thick) and staining (Johansen, 1940). In addition, fresh cross sections were made of seeds previously hydrated in water at room temperature for 12 h. These were stained with acid phloroglucinol, a specific stain for lignin determination (Ruzin, 1999). Observation, measurements and photographic recordings were made with a Nikon Labophot-2 microscope with a digital photographic camera and ocular micrometer.

The following hull parameters were evaluated: number, thickness and sclerification of outer or inner parenchymatous strata. The differences between genotypes for each variable were analyzed by analysis of variance and the means were compared to the LSD test. In order to develop an explanatory model of H, simple and multiple linear regression analyses were made using the statistical package InfoStat (Di Rienzo *et al.*, 2009).

RESULTS AND DISCUSSION

The H showed a gradient among genotypes that fluctuated between a minimum of 49% in L-8 and a maximum of 60% in AG Phoenix. H of L-8, Seedtech S-3125 and Agrosearch L 7-3 was lower than in the remaining genotypes that showed no difference among them (Table 1). The ethereal extract percentage of all the genotypes studied exceeded 48% except for L-8 (19.7%; Fernandez *et al.*, 2011), showing that there would be safflower genotypes with a combination of good H and high oil content.

The H measured in the different genotypes fluctuated between 41 to 55% and were similar to those obtained by Denis *et al.* (1994) for sunflower hybrids. There is only a reference of H for safflower in Argentina which reports an average H for high-linoleic genotypes of 7.13% (Baümler, 2002), significantly lower than those determined in this work.

The hull's cross section in the genotypes studied here showed that this structure is comprised by an outer epidermis followed by 8 to 11 outer parenchymatous cell strata, then a phytomelanin layer (Pandey and Dhakal, 2001) and finally an inner parenchyma formed by cells with thick, lignified secondary walls (Fig. 1).

The hull thickness varied from a minimum of 179 µm in L 7-3 and a maximum of 220 µm in T-21. These values were much lower than those observed by Baümler, (2002).

Hull sclerification comprised between 2 to 6 cell strata of outer parenchyma and the entire inner parenchyma (Table 1). Although significant differences were detected between genotypes regarding the hull sclerification level, it was not related to changes in their H (Table 1).

Within the set of variables studied, HDW was the only one that correlated with H ($r = 0.41^*$) (Table 2).

We have reported that in sunflower hybrids, 54% of H can be explained by the number of parenchymatous rays/mm and the thickness of the cell walls in the first stratum of the middle layer (Lindström *et al.*, 2006) in the hull. On the other hand Leprince-Bernard *et al.* (1990) observed that H increased in sunflower upon increase of the hull sclerified strata and decrease of inner parenchyma volume. As we show here, the arrangement of parenchymatous and sclerenchymatous tissues in the safflower's hull is inverted to that of sunflower. Probably this is the reason H only shows a positive correlation with the hull dry weight not being related to the other morpho-anatomical parameters of the seeds studied in this work.

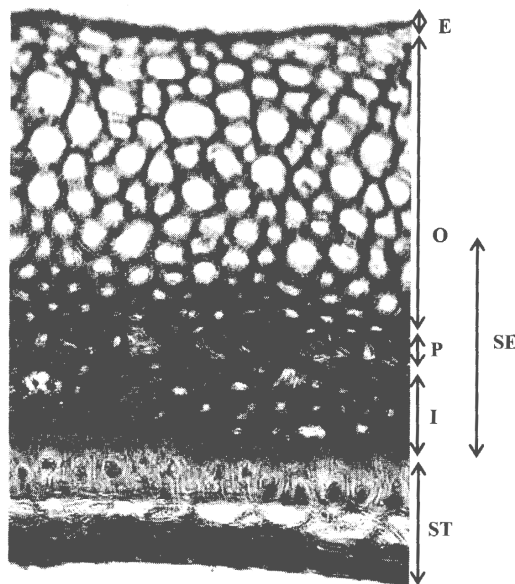


Fig. 1. Hull's cross section from AG Phoenix genotype seeds. E: outer epidermis; SE: sclerified strata; PL: phytomelanin layer; OP: outer parenchyma; IP: inner parenchyma; ST: seed tegument.

Table 1. Hullability, seed morphological parameters and hull's anatomical characters of nine safflower genotypes

	H	HDW	HDW/SDW	OPSN	OPSSN	IPSN
L-8	48.8 a	11.0 a	0.50 b	10.0 ab	2.7 a	4.0 a
Seedtech S- 3125	49.9 a	12.4 bc	0.52 c	9.3 ab	3.0 a	4.3 a
Agrosearch L7- 3	51.9 a	12.7 c	0.50 b	9.0 ab	2.3 a	4.0 a
Seedtech S- 345	56.6 b	13.6 d	0.50 b	8.3 a	2.0 a	4.0 a
Arizona L 7-5	58.4 b	11.8 b	0.46 a	10.7 b	3.0 a	4.3 ab
CW 99 OL	59.0 b	14.5 e	0.52 bc	9.3 ab	2.3 a	5.3 b
Knowles 93079	59.4 b	11.7 ab	0.52 c	9.7 ab	6.0 c	4.0 a
Agrosearch T-21	60.0 b	13.5 d	0.51 bc	10.0 ab	4.7 b	4.3 a
AG Phoenix	60.3 b	13.7 d	0.53 c	9.0 ab	2.7 a	4.7 ab

Values followed by different letters for each variable indicate significant differences ($p < 0.05$)

H: hullability; HDW: hull dry weight; HDW/SDW: hull dry weight/seed dry weight ratio; OPSN and IPSN: number of outer and inner parenchymal strata respectively, and OPSSN: number of sclerified strata in the outer parenchyma.

Table 2. Pearson correlation coefficients between hullability and morphological parameters and hull's anatomical characters of nine safflower genotypes

	H
HDW	0.41 *
HDW/SDW	0.02 ns
TT	-0.16 ns
OPSN	0.14 ns
OPSSN	0.24 ns
IPSN	0.37 ns

H: hullability; HDW: hull dry weight; HDW/SDW: hull dry weight/seed dry weight ratio; TT: total thickness; OPSN and IPSN: number of outer and inner parenchymal strata respectively, and OPSSN: number of sclerified strata in the outer parenchyma.

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