Preliminary results on biomass heterosis in *Brassica carinata* A. Braun.

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Introduction

The cultivation of species of the genus *Brassica* dates back to ancient times, with reports of their uses as vegetables in the Neolithic. According to PRAKASH (1961), the use of oil from the seeds of these species is documented in Indian texts of 1500 to 2.000 A.C. In Medieval Europe, oil extracted from *Brassica* species was used as an illuminant, and in the industrial ages, as a lubricant. Thus, despite their present main uses as foodstuffs, in the past, rape seed and similar oils have had several industrial applications.

The present wide use of oil from *Brassica* for human consumption is attributable to the success of genetic improvement, resulting in an oil that is free of erucic acid with better nutritional properties than the natural oil (STEFANSON *et al.*, 1961; DOWNEY, 1964). In contrast, a high erucic acid (C22:1) content is required for its use as a lubricant since this fatty acid is able to lubricate metal surfaces subjected to high temperatures.

Species that have been widely cultivated worldwide include *Brassica oleracea* L., *B. rapa* L. and *B. napus* L., while other genetically related species such as *B. carinata* A. Braun and *B. juncea* (L.) Czern. are grown on a more local scale and presently show incipient domestication. Recently, rape is being cultivated, again on a reduced scale, for non-food use such as the manufacture of biodiesel obtained by the transesterification of the original oil. The use of this plant as a biomass producer to generate electrical energy is currently a subject of investigation. Both these applications of *Brassica* species could be substantially potentiated in the near future. The recyclable nature of this type of energy, alongside the high cost and finite nature of petrol, lend support to this alternative.

Of the genus, *B. carinata,* or Abyssinian mustard, is among the species that show most promising potential. Its distribution area is fairly restricted and includes northeast Africa, Abyssinia and neighbouring countries, where it has been cultivated since ancient times isolated from the influence of modern plant improvement techniques. Local uses are multiple: once boiled the leaves are used as vegetables, the oil extracted from the seeds is used for cooking and the seed as a condiment for other foods. The species is alloploid and is not found wild in nature. It is obtained by crossing the diploid species *B. oleracea* L. (CC, 2n = 18) with *B. nigra* (L.) Koch (BB, 2n = 16) (U, 1935; Frandsen, 1947). Its genome is consequently BBCC and chromosome number 2n = 34. The high production capacity of this species, particularly in semiarid regions where rape shows problems of adaptation, has been reported by several authors (KNOWLES *et al.*, 1981; FERNANDEZ-MARTINEZ & DOMINGUEZ, 1982; FERERES *et al.*, 1983; PRAKASH *et al.*, 1984, BELAYNEH & ALEMAYEHU, 1987; SINGH *et al.*, 1988). These authors quote production values 32% higher than those recorded for *B. napus.*

The present preliminary investigation was designed to estimate the potential use of heterosis in *B. carinata* to increase the production of aerial biomass as a sustainable, non-polluting energy source, as well as grain for commercialisation and oil for transformation into biodiesel.

Materials and methods

To evaluate the potential of *B. carinata* for heterosis, a series of crosses was performed using eleven hybrid lines. Three thousand flowers from the 11 lines were crossed, by removing the anthers and placing the selected pollen on the stigma followed by isolation (SOBRINO VESPERINAS, 1988), to yield 100 hybrids (each direction of cross was considered different). The parental lines were used as controls to estimate heterosis and to morphologically confirm the hybrids obtained. The 11 lines selected were obtained from the Koipesol Semillas, S.A., Plant Improvement Scheme to represent the diversity available within this species. The following characters were considered: 1) erucic acid content: high, low; 2) height: standard, semi-dwarf; 3) flower colour: white, yellow 4) colour of the seed testa: brown, yellow; 5) leaf waxes: presence, absence.

Heterosis was estimated through an experiment involving microplots of 20 plants/m^2 performed in quadruplet. The variables subsequently determined were total aerial and commercializable (grain) biomass. These trials were conducted in Alcale de Henares (Madrid) in central Spain, of continental Mediterranean climate. The fertility of the pollen was established by collecting a sample at peak flowering time and subjecting it to microscopic examination after staining 300 grains of pollen with acetocarmine-glycerol.

Results and discussion

Pollen fertility was over 80% both for the parental lines and hybrids, with the exception of 6 hybrid combinations (BRK-146) and 1 parental line, which showed values ranging from 46% to 79%. This, however, had no effect on fertilization; in no case were there problems with the fertilization of seminal primordia and siliquae contained as many seeds as the initial primordia.

Analysis was performed by grouping all the hybrids by family, which was defined as the set of hybrids obtained when one line was crossed with each of the remaining lines. In order to detect possible cytoplasmic effects, the hybrid obtained was considered different when a line acted as a female or male parental. Tables 1 and 2 show the mean total aerial and grain biomass values corresponding to two of the families for each direction of cross. The behaviour of these lines in terms of heterosis may be summarized as three categories:

1.- High mean family heterosis values with several hybrids showing very high productivity (Table 1).

2.- Moderately high mean family heterosis values with several hybrids showing high productivity (Table 2).

3.- Slightly elevated family heterosis values with high productivity shown by isolated hybrids.

Table 1.- Heterosis in aerial (shaded) and commercializable (grain) biomass of the family obtained by crossing the BRK 81 line with 10 *Brassica carinata* lines in both directions. Mean values expressed in g/plant (d. m.).

BRK 81	P	25	27	54	60	85	95	131	146	155	159	MEAN
FEMALE	116.4	123.4	197.9	97.9	165.5	147.9	72.8	114.3	140.9	85.5	104.8	125.1
	26.8	32.3	49.7	24.5	44.1	41.6	17	28.1	36.3	22.1	28.5	32.4
MALE	-	87.7	65.6	91.7	83.7	73.6	46.6	*	81.7	56.2	53.5	71.1
	-	22.1	16.3	21.8	21.9	16.6	24.9	*	21.3	5	11.4	17.9

Table 2.- Heterosis in aerial (shaded) and commercializable (grain) biomass of the family obtained by crossing the BRK 60 line with 10 *Brassica carinata* lines in both directions. Mean values expressed in g/plant (d. m.).

BRK 60	P	25	27	54	81	85	95	131	146	155	159	MEAN
FEMALE	61.1	22.1	68.6	66.9	83.7	86.5	112.2	77.8	103.6	53.1	81	75.6
	17.8	5.2	17	16.5	21.9	25	31.9	22.3	29.2	15.5	20.8	20.5
MALE	-	94	56.8	53.7	165.5	61.1	79.3	38.4	44.2	33.1	49.5	67.6
	-	24.2	14.6	14.5	44.1	16.2	18.1	83.1	9.8	2.9	2.2	23.0

Mean heterosis values corresponding to the set of hybrids in each family were slightly elevated to high, although a greater intensity of heterosis was shown by isolated combinations both in terms of total aerial and grain biomass values. The family formed by the BRK-81 line as the female parental showed the maximum heterosis value, and in particular, the hybrid BRK-81 x BRK-60. The results obtained suggest that the direction of cross has an effect on the level of heterosis achieved. This was sometimes seen in mean family heterosis values when the line acted as the male or female parental. In other families, however, it was only shown by some of the hybrid combinations as in the case of BRK-85 x BRK-81 compared to BRK-81 x BRK-85, in which the effect was highly marked. The lines of ``type zero'' erucic acid showed no heterosis. This may possibly be explained by the real genetic proximity of these lines.

Oil content values showed general heterosis, which was particularly high in the hybrid BRK-81 x BRK-60. This hybrid showed a mean oil content of 47.1% dry weight compared to 38.0% and 40.0% for the parentals.

The levels of erucic acid in the hybrids were intermediate with respect to those corresponding to the parentals. These hybrids were classified as types:

Zero erucic acid: approx. 2%.

Intermediate erucic acid: approx. 22-28%

High erucic acid: 45.0-49.3%.

The greatest amount of erucic acid (49.3% of total fatty acids) was shown by the BRK 159 x BRK 27 hybrid, indicating the possibility of slightly surpassing (2%) parental levels in some cases. Selecting for this character may have important implications for the use of this oil as a natural, recyclable lubricant. Although, in general, the erucic acid content of hybrids obtained by crossing lines showing a high expression of this characteristic was not enhanced.

Conclusions

Although it is not possible to speak of general heterosis in the entire set of 100 experimental hybrids among lines showing variability in at least a certain number of characters, marked heterosis was observed at different levels. Heterosis was shown by mean values corresponding to all the families, with high values shown in some families. However, it was the particular hybrids which showed the greatest degree of heterosis. This occurred most frequently in families showing a generally higher degree of heterosis. Heterosis in the oil content of the seeds was less marked than that corresponding to total aerial and commercializable grain biomass. The particular combinations showing elevated hybrid vigour, indicate that *Brassica carinata* may show high potential as a recyclable energy producer in semi-arid regions where *B. napus* is less well-adapted.

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