CHARACTERISTICS OF A YELLOW-SEED LINE BELONGING TO Brassica napus L.

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Introduction

In the last two decades, genetical improvement has managed to substantially increase the quality of rapeseed by-products, and notably approach a product which was technically defined as triple zero rapessed. First and foremost, the erucic acid was eliminated, which is an undesireable component of rapeseed oil and which constituted one of the main fatty acids of this oil (Stefanson et al. 1961). Secondly, efforts were aimed at improving the quality of flour, by reducing glucosinolates, incorporating genes of the Polish cultivar Bromoswki. Both targets have been covered and have permitted double zero cultivars to be used in many rapeseed producing countries. The third zero is related to obtaining yellow coat seeds. In phytogenetically close species, such as Brassica campestris L., experimentally showed that the level of fibre was smaller in yellow seeded types, than in those which had black colour testa. A traditional taxon in India, B.campestris var. yellowsarson, has yellow testa. Although this form is today considered at sub-specific level or also like botanical variety, in the past it was occasionally used with specific rank. The high level of fibre present in rapeseed flour is an unfavourable factor compared with proteic flours of other sources. The reduction in fibre implies an increase in the content of protein + oil. The change in colour of the seed, from black to yellow, increases the palatibility of the protein flour, because cattle do not like the black remains of the testa so much.

According to VAUGHAN and WHITEHOUSES (1971), BOUMAN (1975), BENGOECHEA and GOMEZ-CAMPO (1975), there are basically four seed coat layers, which correspond to the following:

a) Epidermic coat, made up of thick cells which may be mucilaginous.
b) Sub-epidermic coat.
c) Coat of pallisade cells or sclerotic layer.
d) Coat of parenchymatous cells.

Pigments derive from the most internal cell coat (BOUMAN, 1975).

Althoug in other species of Brassica and Sinapis the presence of seeds with yellow testa has been obtained (B.campestris, B.juncea, B.carinata, Sinapis alba), greater difficulties have been found in obtaining this type in rapeseed (B.napus), even though various methodologies have been used: Intra and inter-specific crossings, including B. napus re-synthesis from B.oleracea L. and B.campestris L. (BARCIKOWSKA et al., 1987; MIROSLAV, 1987). HOU-LY and YONG-TONG (1987) report that in 1975 they obtained yellow seed in Brassica napus from the 13th generation of the inter-specific hybrid B.napus cv. 363 x B.chinensis cv. Qi Xin-jian.

In the last ten years we have developed an exhaustive screening for total or partly yellow seed types in rapeseed, using material from various sources, and have tried to explore the possible inter-specific variability for this character.

A new line of yellow seed is presented in this study, which has appeared from mutation in the winter cultivar SA-401, and its morphological, productive and quality characteristics.

2.- Material and methods.

The plant material has consisted in the new line of yellow testa, SA-PB and in the line which originated it, CE-401, belonging to *Brassica napus* L. subsp. *oleifera* f. *biennis* . For comparative purposes yellow and black coat lines have also been used, of the following species: *Brassica campestris* L., *B.juncea* , *B.carinata* , *Sinapis alba* .

All seed samples belong to the Seed Bank which Cecosa maintains at its Plant Breeding Station in Malpica (Toledo).

Analysis of oil, protein and glucosinolate content were determined on intact seed, in collaboration with the Departamento de Fitotécnia de la Universidad Politécnica de Madrid, by the NIR method (Near Infrared Reflectance) after gauging the equipment. Amino-acid analysis were performed with an automatic analyser and the rest were carried out under the official standards (MAPA, 1986).

Evaluation of productivity was made in fieldwork, with four random repeats, on plants grown under identical farming conditions, with the following parameters: Height (cm.), height of the first ramification (cm.) nº of primary ramifications, nº of secondary ramifications, total nº of siliqua, length of siliqua, nº of grains/pod, weight of 1000 grains, marketable biomass.

Photosynthesis activity was measured on a LICOR-6000 unit on all leaves of a representative plant under fields conditions.

3.- Results

The SA-PB line of yellow testa has leaves of similar morphological characteristics to the line of origin and with a dark green colour, even though they have been subject to four generations of selfing. Photosynthesis activity was likewise similar in both lines, with mean values of 2 $\mu mol.\ CO_2\ .m^{-2}.\ s^{-1}$. The most notable difference was found in the colour of the petals which are pale yellow in SA-PB compared with a deep yellow in the original line. The study of various morphological aspects and of the components of the yield (table 1) shows that both lines have no significant differences in most of the parameters considered; significant differences were only found in the number of secondary ramifications, in the number of siliqua/plant, and the number of grains/plant. Lastly, a higher value was found in line CE-401 black testa, for productivity, but this did not reach significant levels.

In the comparison made on three species of Brassica and Sinapis alba (table 2) for yellow and black testa types, results have coincided completely and higher oil contents have been obtained in yellow testa seeds in all taxa studied, whilst the protein levels are equivalent or even lower in some of the yellow coat types. The sum of oil and protein is consistently higher in yellow testa seeds in any of the species studied, except in Brassica carinata.

It is observed that with the exception of B.napus, in all other species, the samples of black and yellow testa that were compared have different origins, and consequently the differences in protein and oil content are also affected by other factors. Even in the case of Sinapis arvensis, brown testa, a wild sample was used that was collected in the province of Toledo, which justifies the difference of an 11% in the content of oil, in comparasion with yellow coat seed, which has been subject to selection for that character.

Levels of glucosinolates are high or very high in all types used.

The fibre (Wendee) only was determinated in both types of B.napus finding a smaller content in yellow testa seed (5,53%) compared with the black coat line (7,5%).

Levels of amino-acids have been higher in flour from SA-PB than in the line CE-401, with an increase of 13,3% (table 3). In both cases, the flour has a good equilibrated aminoacids, but in the yellow seed line, the contents are higher.

4.- Discussion.

The new line of yellow testa belonging to B.napus does not show out-standard plants, as in the case reported by HOU-LI and YONG-TONG (1.98%), who find numerous anormalities in the yellow line from cv. Hurayan 3, such a dwarf plants, small flowers, yellowish cotyledons, etc. Specifically, the presence of this latter peculiarity is mentioned as having important influence on productivity and its components. Probably, this better stability is due in line SA-PB, to the fact of having been isolated B.napus, without needing to use inter-specific combinations which although stabilized, may produce some problems in the meiosis and/or in the compatibility of the nucleus-cytoplasm system.

Development of yellow seed cultivars with good agricultural properties continue being an important target for genetic improvement (DOWNEY and ROBBELEN, 1.989). Within the CEE, special interest is placed on raising the content of oil and quality of protein (THOMPSON & HUGHES, 1.986).

Under the tested conditions , this yellow line has shown stability in the colour of the testa, contrary to other lines where the yellow colour was sharply influenced by environment conditions, which has led to WANG (1.986), cited by HOU-LI and YONG-TONG (1.987), to propose the possibility of there being transposons influencing the presence of black stains in the testa, in the same sense as the mechanism described by McClintock in 1.947 for maize.

Productivity of the new line of yellow testa is inferior to the line of origin, without this difference becoming significative, but it presents significant differences in the number of grains produced per plant, deriving from a smaller number of secondary ramifications, although the number of ramifications and length of the siliqua however remain constant. Although the SA-PB yellow line offers some problems in productivity, this would not appear to be an inconvenience for farm use, within a crossing program. It has the characteristics specific of a simple zero cultivar, with no erucic acid and a content of linolenic acid which is not particularly high. The final target could therefore be to obtain triple zero cultivars with low content of linolenic acid.

None of the cultivars of yellow B.napus has been improved yet. In B. campestris has been cited a higher content of oil and protein and a 4% less fibre in the total seed than in the brown seed lines, mainly due to a finer seed coat with smaller cells (STRINGAM, et al., 1.975).

Yellow seed SA-PB contains a smaller percentage of fibre (20% less) and a higher oil content as reported for another line belongs to B.napus obtained by BECHYNE (1.987), from an inter-specific crossing, improving the oil content and reducing the percentage of crude fibre in 2,9 % on cultivar 'Jet Neuf'. This is due to a smaller thickness in the width of the testa and within this, the pallisade layer, whose fibre content is particularly marked (BECHYNE, 1.98%).

In respect of the importance of the higher oil content and considering that the protein and oil tend to be negatively correlated, yellow coat seeds prove the most favourable means of obtaining an overall increase in both components.

The content of amino-acids is well balanced, being higher than in the original line.

5.- Conclusions

1) The Brassica napus line SA-PB obtained by isolation of a spontaneous mutation, does not show out-standard plants, under self-fecundation. In the field conditions used they keep always their yellow colour.

2) It has favourable agricultural characteristics but its high content in glucosynolates restricts a direct use, and its potential must be used under a crossing plan.

3) SA-PB has a lower fibre content and a better content in oil, zero type erucic acid and with 7% content in linoleic acid.

Table 1.- Mean values (standard error) of yield components of a seed line (SA-PB) in respect of a black seed reference.

VARIABLE	LINE ($\bar{x} \pm$ SE)		$F^{(a)}$
	SA-PB	CE-401	
Total height (cm)	137 ± 10	139 ± 3	0,047
Height first ramification (cm)	49,1 ± 1,1	46,1 ± 1,6	2,280
Nº primary ramifications	8,25 ± 0,85	9,75 ± 0,50	2,348
Nº secondary ramifications	4,75 ± 3,47	15,25 ± 2,10	6,699
Nº siliqua/plant	94,7 ± 10,9	181,7 ± 17,2	18,182
Length siliqua (cm)	5,50 ± 0,31	5,55 ± 0,23	0,017
Nº grains/pod	16,0 ± 2,3	17,2 ± 2,2	0,155
Weight 1000 grains (g)	3,05 ± 0,47	3,13 ± 0,25	0,020
Nº grains/plant	1528 ± 289	3065 ± 308	13,160
Productivity/plant (g/plant)	4,99 ± 1,6	9,58 ± 1,3	5,035

(a) Significance : ** $p \leqslant 0,01$
* $p \leqslant 0,05$

N.S. No significance.

Table 2.- Composition of seed of different species of Brassiceae, comparing yellow and black testa lines. (Humidity 0%, Impurities 0%).

SPECIES	LINE	PROTEIN (%)	OIL (%)	P+A (%)	GLUCOSINOLATES NIR (%)
Brassica napus	CE-401,black	18,1	37,8	55,9	145,4
	SA-PB,yellow	18,6	41,03	59,63	134,6
B. campestris	black	20,1	38,0	58,1	125,9
	yellow	19,7	41,8	61,5	183,8
B.juncea	black	21,9	35,6	57,5	231,5
	yellow	21,5	36,08	57,58	198,7
B. carinata	black	27,5	30,1	57,6	254,6
	yellow	23,4	34,2	57,6	124,3
Sinapis alba	brown	22,91	29,8	52,71	162,0
	yellow	19,7	42,10	61,8	137,4

Table 3.- Content of amino-acids of flour from SA-PB line (yellow seed) and from line of origin CE-401 (black seed).

Amino-acid (g/Kg)	CE-401	SA-PB	Δ (%)
Proline	9,9	8,6	86,7
Serine	7,8	8,6	110,3
Aspartic	12,2	15,1	123,8
Glicine	9,3	10,3	110,8
Treonine	7,7	8,6	117,7
Arginine	9,7	10,6	109,3
Alanine	8,0	9,5	118,8
Methionine	3,3	4,1	124,2
Valine	9,5	11,2	117,9
Tirosine	5,4	6,6	122,2
Phenylalanine	7,5	9,1	121,3
Isoleuzine	7,6	8,9	117,1
Lisine	11,9	12,4	104,2
Histidine	5,8	5,8	100,0
Leuzine	12,3	14,8	122,2
Glutamic	35,3	40,7	115,3

Table 4.- Content in fatty acids of oil extracted from seed of yellow rapeseed SA-PB compared with lines of origin CE-401.

Fatty Acid	SA-PB (%)	CE-401 (%)
C 16: 0	5,22	5,04
C 18: 0	1,59	1,43
C 18: 1	61,87	63,35
C 18: 2	20,67	18,72
C 18: 3	7,77	8,77
C 20: 0	0,54	0,46
C 20: 1	1,17	1,19

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