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Acid phosphatase polymorphism within and among populations of Cauliflower (*Brassica oleracea* var *botrytis*)

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Abstract

Eighty-one lines of cauliflower (*Brassica oleracea* var. *botrytis*) from 12 populations used to produce commercial hybrids in Brazil were screened for polymorphism in the acid phosphatase system, in order to evaluate the usefulness of this marker for the determination of the parental contamination level in hybrid seeds. Little polymorphism was detected in the examined lines, but the system appeared to be very useful for hybrid identification, since the only condition required was polymorphism between the two parental lines. If the analyzed lines were used for hybrid production, 8.4% and 12.3% of the possible crosses would result in hybrids which can be positively identified using the APS-1 and B_1 loci, respectively. If only one plant of each homozygous type (SS or FF) was analyzed in each population, 41% and 50% of the possible crosses would result in hybrids which can be positively identified using the APS-1 and B_1 loci, respectively.

Key words: isoenzyme, polymorphism, cauliflower.

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Introduction

The use of F_1 hybrids is a very common procedure in breeding programs of *Brassica* species. Hybrids are grown in more than 90% of the Brazilian cauliflower fields (Ikuta, 1974; Ikuta and Paterniani, 1979). These hybrids are produced by crossing self-incompatible lines under open-field conditions. In spite of the self-incompatibility system found in the lines, a certain degree of self-crossing may happen, producing an undesirable number of non-hybrid seeds, which will determine their quality and price (Thompson, 1957; Haruta, 1962).

Isoenzymatic markers have been successfully used to identify hybrids and cultivars in many plant species (Heidrich-Sobrinho, 1982; Cardy and Kannenberg, 1982; Arús and Orton, 1983; Grossi *et al.,* 1997). The isozyme method has proven to be one of the simplest ways to assess the level of parental contamination of seeds within a hybrid cauliflower seed lot (Nijenhius, 1977; Wills *et al*., 1979; Arus *et al*., 1985). Isoenzyme analysis is a low-cost and accessible technique (Kephart, 1990). The acid-phosphatase isoenzyme system has proven to be the most useful enzyme system for the detection of contamination within hybrid cauliflower seeds (Nijenhius, 1971; Woods, 1976; Wills *et al*., 1980).

The aim of this study was to analyze acid phosphatase polymorphism in cauliflower lines used to produce hybrids in Brazil, and to evaluate the usefulness of this enzyme system to identify hybrids between different lines.

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Material and Methods

Plant material

Eighty-five lines from 12 cauliflower (*Brassica oleracea* var. *botrytis*) populations cultivated in Brazil were analyzed (Table I). The number of times the lines were self-crossed is listed in Table I. Hybrids resulting from the crossing of seven Piracicaba Precoce lines and one line from the Spring Snow population were analyzed to determine the genetic control of one acid phosphatase zone.

Isoenzyme analysis

Ten-day-old cotyledons were grounded in liquid nitrogen and transferred to 1.5 mL tubes, to which 50 µL of 0.01 M Tris-glycine buffer, pH 8.3, were added. The samples were centrifuged for 30 min at 4 °C, and 20 µL of the supernatants were loaded into a 10% polyacrylamide gel

Table I - Cauliflower lines analyzed using acid phosphatase isozymes.

Population	Number of progeny	Number of selfcrosses	Origin
1 - Wase Ataria	7	S_1 Sibcross	Japan
2 - Pa Shi Tien	8	S_3 Sibcross	China
3 - Tainung	7	S_3 Sibcross	Thailand
4 - Main Crop Benares	8	S_4 Sibcross	India
5 - PI 163485	7	S_4 Sibcross	Pakistan
6 - PI 183214	2	S_3 Sibcross	Egypt
7 - Kangaroo	3	S_1 Sibcross	Australia
8 - Jaragua	7	S_3 Sibcross	Brazil
9 - Piracicaba Precoce	8	S_3 Sibcross	Brazil
10 - Piracicaba Precoce n. 1	8	S_6 Sibcross	Brazil
11 - Piracicaba Precoce 439	8	S_5 Sibcross	Brazil
12 - Teresópolis Gigante	8	S_8 Sibcross	Brazil
Total	81		

prepared in 0.37 M Tris-HCl, pH 9.1. The electrode buffer was 0.001 M Tris-glycine, pH 8.3. After 5 h of electrophoresis at 4 °C, acid phosphatase activity was detected in the gels using the histochemical techniques described by Allen *et al.* (1963).

Genetic control

The genetic control of the APS-5 zone was based on information about the secondary structure of APS in other plants (Kephart, 1990) and on the analysis of the band patterns of a Piracicaba Precoce line, of a Spring Snow line, and of a hybrid between them, as well as of 112 plants of an F2 population.

Usefulness of APS loci for hybrid identification

The usefulness of each APS locus for hybrid identification was estimated by calculating the expected frequencies of crossing between pairs of lines, which would produce hybrids with bands acquired from both parents. The proportion of possible crossings for one locus that would result in hybrids was calculated as being:

$$(p + q + r)^2 = p^2 + 2pq + 2pr + q^2 + 2qr + r^2,$$

where p = frequency of genotype XX; q = frequency of genotype YY; and r = frequency of genotype XY. The desirable crossing would be XX x YY, or 2pq, according to the formula above.

Results and Discussion

Three zones of enzyme activity were detected in the gels (Figure 1), as previously described and named by Arus *et al.* (1985). The most anodic zone was named APS-1, the intermediate zone APS-3, and the most cathodic zone APS-5 (Figure 1). Two alleles were detected in APS-1, differently from Wills and Wiseman (1980), who detected four alleles in APS-1 in an analysis of 19 F_1 hybrids and inbred lines of cabbage and Brussels sprouts. Two alleles were also detected in APS-3. In the third zone (APS-5), up to five bands and three band patterns were seen (Figure 1). Pattern 1 contained three bands (e, d, and c), pattern 2 contained four bands (e, c, b, and a), and pattern 3 contained bands e, d, c, b, and a. Since the genetic control of APS-5 was not described previously, there are no reports on alleles in this zone.

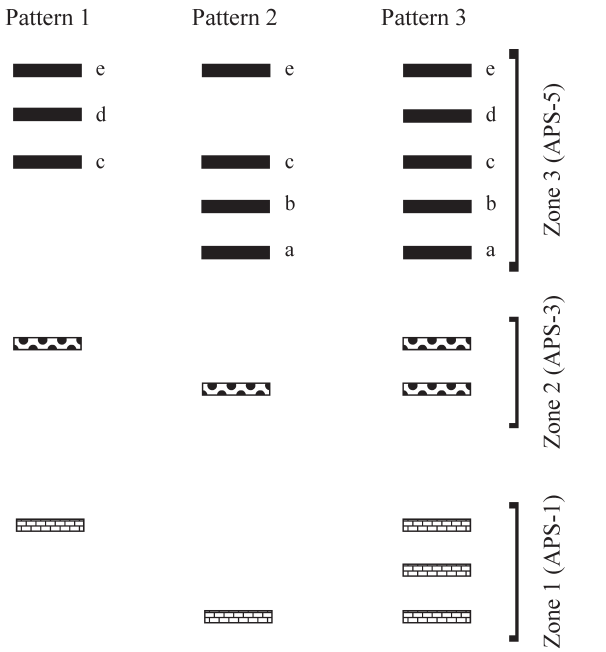

Figure 1 - Band patterns detected in each one of the three phosphatase activity zones (APS-1, APS-3 and APS-5) in cauliflower cotyledons.

The APS-5 zone was not used for hybrid identification in previous studies (Wills and Wiseman, 1980; Arus *et al.,* 1985). The usefulness of this zone for hybrid identification was tested in this study, because of the very clear and easy to score band patterns. Crosses between plants with band patterns 1 and 2 in APS-5 always resulted in a hybrid with pattern 3, and an F_2 population in which one-quarter had pattern 1, one-half had pattern 2, and one-quarter had pattern 3. A chi-square test showed that there was no significant difference between the observed and the expected phenotype frequencies for a cross between two plants which are heterozygous for the same two alleles (Table II). This observation indicates that there is at least one segregating locus in this zone.

The genetic control of the APS-5 zone was proposed based on the band patterns detected in 112 plants from a cross between plants with band patterns 1 and 2 (Figure 1), and on hypothetical phenotypes of electrophoresed enzymes, as described by Kephart (1990). Pattern 1 would be

Table II - Chi-square test of the genetic control of the segregating loci in zone APS-5.

	Frequencies	
	Expected	Observed
Pattern 1	28	31
Pattern 2	28	27
Pattern 3	56	54
Total	112	112

DF = 2; $\chi_2 = 0.4285652$; $0.95 > \chi_2 > 0.80$.

Table III - Number of heterozygous and homozygous plants for the three loci in the analyzed lines.

	APS - 1			B_1		
Populations	SF	SS	FF	SF	SS	FF
Wase Ataria	3	4	0	5	2	0
Pa Shi Tien	1	6	1	1	0	7
Tainung	0	4	3	1	0	6
Main Crop Benares	4	3	1	3	0	5
PI - 163485	3	4	0	5	2	0
PI - 183214	1	0	1	2	0	0
Kangaroo	2	1	0	3	0	0
Jaragua	2	5	0	0	6	1
Piracicaba Precoce	3	5	0	4	2	2
Piracicaba Precoce n. 1	8	0	0	8	0	0
Piracicaba Precoce 439	1	6	1	6	2	0
Teresópolis Gigante	5	3	0	4	0	4
Total	33	41	7	42	14	25

SF = heterozygous plants for alleles S and F.
SS = homozygous plants for allele S.
FF = homozygous plants for allele F.
B1 = locus segregation in APS-5.

formed by (i) the overlapping of two homodimers, formed by the products of alleles A_1^1 and B_1^1 (band e), (ii) interlocus heterodimers resulting from the combination of chains coded by alleles B_1^1 and B_2^2 (band d), and (iii) by a homodimer coded on locus B_2 (band c). Pattern 2 would be formed by (i) a homodimer formed from the product of allele A_1^1 (band e), (ii) a homodimer formed from products coded by allele B_2 (band c), (iii) an interlocus heterodimer formed from products coded by alleles B_2^2 and B_1^3 (band b), and (iv) a homodimer formed from products coded by allele B_1^3 (band a). Pattern 3 would be formed by the overlapping of two homodimers, formed by (i) products of alleles A_1^1 and B_1^1 (band e), (ii) interlocus heterodimers resulting from the combination of chains coded by alleles B_1^1 and B_2^2 (band d), (iii) the overlapping of a homodimer coded on locus B_2 and an intralocus heterodimer formed by chains coded by alleles B_1^1 and B_1^3 (band c), (iv) a heterodimer formed from chains coded by allele $B_2^2B_1^3$ (band b), and (v) a homodimer formed by the product of allele B_1^3 (band a). Thus, the isoenzymes observed in the APS-5 zone were coded in loci A_1, B_1, and B_2, and some of them were interlocus heterodimers. B_1 was the only locus segregating in this zone. The similar electrophoretic mobility observed among the isozymes coded by the analyzed loci and the occurrence of interlocus heterodimers suggested gene duplication. Gene duplications have also been reported in other plant species (Stuber and Goodman, 1980; Massey and Hamrick, 1998).

Different frequencies were found for each allele of the two loci in the 12 analyzed populations (Table III). The frequencies of each phenotype in zones APS-1 and APS-3 were very similar, suggesting that APS-3 is most probably formed by interlocus heterodimers, as suggested previously by Arus and Shields (1983). In APS-1, the frequencies detected for phenotypes SF and SS were similar and much higher than the frequency of phenotype FF. In B_1, the frequencies of phenotypes SS and FF were a little lower than that of SF, and more similar to each other than to APS-1. The frequencies of each phenotype varied in the population, that of SS generally being higher than those of SF and FF. The polymorphism within and among populations was generally very low, since only up to two alleles were found at the two loci analyzed. The populations differed from each other in the frequencies of these alleles.

The proportion of crosses using all analyzed lines resulting in hybrids that may be identified using loci APS-1 and B_1 was calculated based on the total frequency of each phenotype (SS, FF, FS) (Table IV). The most useful locus was B_1, by which 14.3% of the possible hybrids between any pair of the analyzed lines could be detected based on their band patterns, whereas APS-1 would allow the detection of no more than 8.8% of hybrids. If the crosses were done using the lines with genotypes FF or SS of each population (Table V), the proportion of crosses resulting in hybrids that might be identified using APS-1 and B_1 would be

Table IV - Frequencies of possible crosses based on the phenotypic frequencies found at loci APS-1 and B_1 (Table III).

	APS-1	B_1
Crossings	Frequencies	Frequencies
SS x SS - p^2	0.506 x 0.506 = 0.256	0.173 x 0.173 = 0.029
SS x FF - 2pq	2 x 0.506 x 0.086 = 0.088	2 x 0.173 x 0.309 = 0.143
SS x SF - 2pr	2 x 0.506 x 0.407 = 0.82	2 x 0.173 x 0.516 = 0.268
FF x FF - q^2	0.086 x 0.086 = 0.007	0.309 x 0.309 = 0.100
FF x SF - 2qr	2 x 0.086 x 0.407 = 0.070	2 x 0.309 x 0.516 = 0.319
HH x HH - r^2	0.407 x 0.407 = 0.1656	0.516 x 0.516 = 0.266

S - slower migration allele.
F- faster migration allele.
p - frequency of homozygous plants for allele S.
q - frequency of heterozygous plants for alleles S and F.
r - frequency of homozygous plants for allele F.

Table V - Number of homozygous plants for the two loci, after exclusion of heterozygous plants. One of each kind of homozygous plants (SS or FF) were chosen from each population.

	APS - 1			B_1		
Populations	SF	SS	FF	SF	SS	FF
Wase Ataria	0	1	0	0	1	0
Pa Shi Tien	0	1	1	0	0	1
Tainung	0	1	1	0	0	1
Main Crop Benares	1	1	1	0	0	1
PI - 163485	1	1	0	0	1	0
PI - 183214	0	0	1	0	0	0
Kangaroo	0	1	0	0	0	0
Jaragua	0	1	0	0	1	1
Piracicaba Precoce	0	1	0	0	1	1
Piracicaba Precoce n. 1	0	0	0	0	0	0
Piracicaba Precoce 439	0	1	1	0	1	0
Teresópolis Gigante	0	1	0	0	0	1
Total	0	10	4	0	11	6

Table VI - Frequencies of possible crosses based on the phenotypic frequencies of loci APS-1 and B_1, when plants with pattern 3 and only one type of homozygous plant for each population are analyzed (Table IV).

	APS-1	B_1
Crossings	Frequencies	Frequencies
SS x SS - p^2	0.71 x 0.71 = 0.5041	0.45 x 0.45 = 0.2025
SS x FF - 2pq	2 x 0.71 x 0.29 = 0.4118	2 x 0.45 x 0.55 = 0.495
FF x FF - q^2	0.086 x 0.086 = 0.007	0.55 x 0.55 = 0.3025

For abbreviations, see Table IV.

41% and 50%, respectively (Table VI). These results indicate that the acid phosphatase system can be very useful for hybrid identification in the analyzed lines, and that the genotyping of these lines could increase the efficiency of this marker.

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