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Comparative Electrophoretic Studies of Proteins and Enzymes of Some *Brassica* Species

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Summary. A considerable amount of variation with respect to soluble proteins and esterase isoenzyme pattern was observed between different species of *Brassica*. Naturally occurring amphidiploids had comparable proteins and isoenzyme patterns to either one or both of the parental species. The species relationship based on percentage homology of protein and esterase bands revealed that *B. nigra* and *B. campestris* are the parental species of *B. carinata* and not *B. nigra* and *B. oleracea*, as suggested on the basis of cytological studies. Elimination of a pair of chromosomes might have resulted into 2n = 34 in the case of *B. carinata*. Further studies are needed to confirm this view. The peroxidase and catalase isoenzyme patterns did not show much variation in different species and amphidiploids.

Key words: Homology – Electrophoresis – Isoenzyme

Introduction

Phylogenetic relationships of different taxa in various groups of crop plants has been worked out in the past, primarily on the basis of crossing and cytological data. Recently, electrophoresis of crude proteins and enzyme extracts has been successfully used as an additional tool to establish these relationships in *Triticum* (Johnson and Hall 1965; Nainawatee and Das 1972); *Solanum* (Desborough and Peloquin 1966); *Nicotiana* (Hart and Bhatia 1967; Sheen 1972); *Gossypium* (Cherry et al. 1970); *Hordeum* (Mitra et al. 1970) and *Oryza* (Siddiqi et al. 1972). The present study provides further insight into the taxonomy of monogenomic and amphidiploid species of *Brassica* by examining the electrophoretic spectrum of soluble protein fractions and isoenzymes of esterase, peroxidase and catalase.

Material and Methods

Present studies were conducted on six species of *Brassica* representing different genomes (Table 1). Dry seeds were surface sterilized with mercuric chloride and soaked on moist filter paper in a refrigerator for 2 hours. Soluble proteins and enzymes were extracted from these seeds by grinding them in 0.2 M phosphate buffer, pH 7.0, containing 5 mM 2-mercaptoethanol. The homogenate was centrifuged at 10,000 × g for 30 minutes at 4°C and the supernatant was used for electrophoresis. The proteins were estimated by the method of Lowry et al. (1951).

A suitable aliquot of supernatant (200 ug protein) was layered on 7.5 per cent polyacrylamide gels and subjected to electrophoresis (Davis 1964). For detection of proteins the gels were stained in Amidoschwartz and destained in 7 per cent acetic acid. In order to study catalase, 0.3 per cent starch was added to the gels and the isoenzymes were subsequently detected by negative staining in potassium iodide (Scandalios 1969). Esterase isoenzymes were stained by using alpha-napthylacetate as a substrate and Fast Blue RR as a dye coupler (Reddy and Garber 1971), while isoenzymes of peroxidase were detected using O-dianisidin as the donor (Scandalios 1969).

The Rf value of each band was calculated as the migration of each protein band or isoenzyme relative to the bromophenol marker dye movement.

Table 1. *Brassica* species included in the present study

Species	Genome	chromosome number	Remarks
1 – *Brassica nigra*	*BB*	16	Primary or elementary species
2 – *B. oleracea*	*CC*	18	
3 – *B. campestris*	*AA*	20	
4 – *B. carinata*	*BBCC*	34	Amphidiploids or secondary species
5 – *B. juncea*	*AABB*	36	
6 – *B. napus*	*AACC*	38	

Results

The electrophoretic patterns of soluble proteins and isoenzymes are presented in Fig. 1. Protein spectrums in gen-

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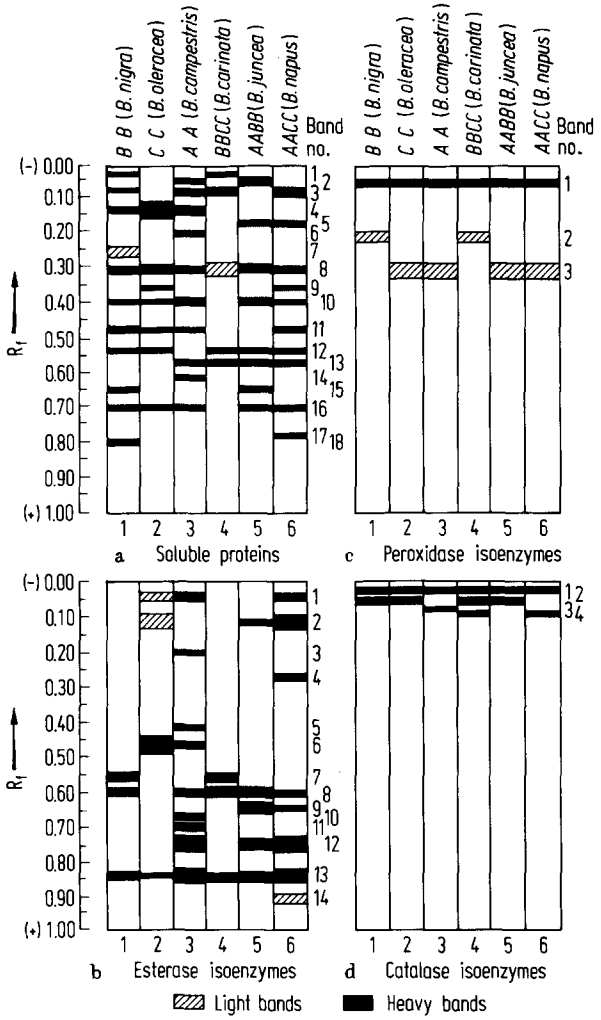

Fig. 1a-d. Diagrammatic representation of protein and enzyme bands in different species of *Brassica*.

eral showed a great variation in the number and intensity of bands in different species (Fig. 1a). Band No. 18 (Rf 0.80) and No. 6 (Rf 0.20) were specific for *B. nigra* and *B. campestris*, respectively, while band No. 12 (Rf 0.52) was absent in the later species. Other bands were common to either one or the other species. Per cent similarities between all possible pairs of species based on major protein bands were calculated as follows:

$$\text{Percentage similarity} = \frac{\text{Number of pairs of similar bands} \times 100}{\text{Number of different bands} + \text{Number of similar bands}}$$

The homology (per cent similarity) of the proteins varied from 20.0 to 54.5 per cent among different species.

With respect to the esterase isoenzyme pattern, the species were found to differ in the number and intensity of different bands (Fig. 1 b). *B. campestris* had its characteristic bands at Rf 0.20 and 0.42 and *B. napus* at 0.28 while other bands were common to one or the other species. Percentage similarities ranged from 16.6 to 100 per cent.

The number of peroxidase and catalase isoenzymes detected were low and not much variability existed for these two enzymes in different species (Figs. 1c and d). However, one peroxidase (Rf 0.04) and one catalase (Rf 0.02) isoenzyme was common to all the species investigated.

Discussion

The inter-relationship of crop forms of the genus *Brassica* were elucidated cytologically as early as the 1930's. The present investigation revealed considerable variation in different species with regards to soluble proteins and esterase bands. However, the catalase and peroxidase banding patterns did not show much variability. Some of the enzyme and protein bands were species specific. Similar types of results have been reported in *Gossypium* (Cherry et al. 1970); *Avena* (Ladizinsky and Johnson 1972); corn (Lodha et al. 1974) and wheat (Waines and Johnson 1975).

The extent of homologies or similarity percentages in the number of protein and esterase bands between all possible pairs of species and amphidiploids and the species involved in the production of amphidiploids suggests that amphidiploids had the bands which were comparable to the bands of either one or both the parental species. Other bands did not reveal this pattern. On the other hand, some species-specific bands were found to be missing in the amphidiploids. Such non-additive type of banding behaviour has also been observed in *Avena* (Murray et al. 1970) and *Gossypium* (Cherry et al. 1971) and were attributed to the evolutionary processes such as gene mutation, diploidization and species specific regulatory mechanisms.

The maximum extent of homologies amongst the elementary (Primary) species was shown by *B. nigra* and *B. campestris* with the naturally occurring amphidiploid of *B. carinata* and *B. juncea*. Similarly, *B. oleracea* and *B. campestris* showed more homology to the amphidiploid of *B. napus*. Thus, it can be suggested that both *B. carinata* and *B. juncea* have *B. nigra* and *B. campestris* as their parental species or putative genome donors and *B. napus* has *B. oleracea* and *B. campestris* as its parental species. This observation is further substantiated by the fact that the specific protein band No. 9 of genome *CC* (*B. oleracea*) did not show its presence in the genomic combination *BBCC* of *B. carinata*, which is the natural amphidiploid derived from the *BB* (*B. nigra*) and *CC* (*B. oleracea*) genome. This type of pattern was also found with respect to specific enzyme bands for *B. carinata* (*BBCC*). Such contradictory observations were not found with regard to the progenitors of other naturally occurring amphidiploids under study. These findings substantiate ancestry established from cytological and morphological data except in *B. carinata*. The cytological and morphological considera-

tions suggest *B. nigra* and *B. oleracea* as its ancestral species whereas the present study indicates *B. nigra* and *B. campestris* as its progenitors. Vaughan (1977), on the basis of serological studies of the seed proteins of three elementary species of *Brassica*, has found *B. campestris* and *B. oleracea* very close taxonomically. It is, therefore, quite possible that the *B. carinata* species includes the *campestris* genome in place of *oleracea*.

Another point to be considered here is the chromosome number in *B. carinata*. According to the current suggestion the 2n number of this amphidiploid should be 36 (*nigra* + *campestris*). However, the cytological studies indicate the 2n number as 34. It is likely that during the course of evolution the number of chromosomes has decreased due to the elimination of two chromosomes or that the centric fusion of different chromosomes has given rise to a new species and subsequently *B. juncea* and *B. carinata* have similar genomes but differ with regard to 2 chromosomes only. Further studies are needed to confirm this view by analysing the chromosome homology and homology between genomes of these species.

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