PROTEIN AND ISOZYME PATTERNS IN DIFFERENT BRASSICA SPECIES AND THEIR HYBRIDS

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Abstract

Isozyme studies of proteins or enzymes provide a rapid method of ascertaining genetic hemologies and inferring phylogenetic relationship among related taxa. Keeping in mind the above objective, Brassica campestris L., B.juncea Czern. and Coss., B.napus and the two hybrids i.e. B.campestris x B.napus and B.juncea x B.napus were studied at five stages viz., at 0 and 8 hr. of imbibition and at the time of initiation of sprouting, 24 hr and 48 hr after initiation of sprounting for soluble proteins and isozyme pattern of catalase, esterase and peroxidase. Some of the protein bands which appeared at one stage and were missing at another stage can be explained by assuming that these were the proteins intimately involved in the process of germination and possibily represent the true storage proteins of the endosperm.

The variation for peroxidase enzyme at early stages of seeds imbibition was less and increased with the development of germinating seeds. Some isozymic bands of esterase showed presence at some stages while they disappeared at the next stage and reappeared at later stages which can be explained by the activity of one isozyme required in the metabolic process going on in the seedling during germination whereas the activity is switched off at the next stage where its presence is not needed. The catalase activity was found restricted at early stages while in later stages the activity was spread throughout the mobility area and variation also increased at later stages of growth. Different species and hybrids exhibited different patterns of peroxidase and esterase whereas catalase did

not show much variation at early stages of seed imbibition. The variation in general increased in later stages.

Introduction

Phylogenetic relationship of different taxa in various groups of crop plants is a fascinating study and has been attracting the attention of research workers since long. Polyacrylamide gel electrophoresis techniques used for the analysis of soluble plant proteins and enzymes have been found to be a useful tool in the new systematics. Protein and isozyme pattern is known to change during development. Therefore, soluble protein and isozyme patterns were studied in different species and their hybrids at various stages of development.

Materials and methods

In the present investigation, soluble proteins and isozyme patterns of peroxidase, esterase and catalase were studied in Brassica campestris, Brassica juncea, Brassica napus and their two hybrids viz., B.campestris x B.napus and B.juncea x B.napus at various stages of development, namely mature seeds, 8 hr after imbibition, initiation of sprouting, 24 hr and 48 hr after sprouting. Soluble proteins and isozymes of peroxidase, esterase and catalase were fractionated on polyacrylamide gel in anionic system following the procedures described by Yadava /1976/.

Results and discussion

In Brassica campestris the number of protein bands increased from ten in mature seeds to twelve in sprouted seeds /Fig.1/. The maximum number of esterase bands were found in mature seeds whereas the bands decreased in number at later stages. One esterase band at Rf 0.05 was found consistent at all the stages. Only two bands were consistent in mature and sprouted seeds but the bands increased to four in 48 hr sprouted seeds.

In Brassica juncea there was not much change in number of protein bands at different stages /Fig.2/. The peroxidase bands in mature seeds were two and three in sprouted

seeds. Esterase bands increased from five in mature seeds to seven in 8 hr imbibed seeds. Most of the bands of 8 hr imbibed seeds were also observed in 48 hr sprouted seeds. The number of catalase bands increased from two in mature seeds to five in 48 hr sprouted seeds.

In case of Brassica napus the highest number of protein bands /10/ was found in mature seeds /Fig.3/. This number did not change much in imbibed and sprouted seeds. One band /Rf 0.55/ was relatively consistent at all the stages. Mature seeds had two bands of peroxidase while the sprouted seeds had three. The number of esterase bands in sprouted seeds was less /2/. This number increased to six in 24 hr sprouted seeds.
Catalase bands increased from two in mature seeds to five in 24 hr sprouted seeds. One band /Rf 0.02/ was consistent at all the stages. The banding pattern in the hybrid B. campestris x B.napus could be studied in the last three stages of development. The number of protein bands in sprouted seeds was highest and five bands /Rf 0.05, 0.10, 0.12, 0.35 and 0.90/ were relatively consistent at all the stages /Fig.4/. The peroxidase bands in 24 hr sprouted seeds were more as compared to sprouted and 48 hr sprouted seeds. Only four esterase bands were present at each stage. The number of catalase bands was three in sprouted seeds, four in 24 hr sprouted and again three in 48 hr sprouted seeds.

In the hybrid B.juncea x B.napus the number of protein bands in mature seeds was eleven while it decreased to six in sprouted seeds /Fig. 5/.
The bands again increased to twelve in 48 hr sprouted seeds. Two bands /Rf 0.07 and 0.45/ were found common at all the stages. Mature seeds had two peroxidase bands while they increased to five in 24 hr sprouted seeds.
Esterase bands in mature seeds increased from five to seven in 8 hr imbibed seeds. The minimum number of bands /3/ was observed in sprouted seeds. Mature seeds had two catalase bands while the number of bands increased to four

in 24 hr sprouted and 48 hr sprouted seeds. A considerable amount of variation with respect to soluble proteins was therefore observed between different species and hybrids. This variation was also observed at different stages of germination. Some of the protein bands were specific to a particular species or hybrid. The variation with respect to the number and intensity of bands between species has also been reported by several workers in various crop plants like wheat /Johnson and Hall, 1965; Mitra and Bhatia, 1971; Waines and Johnson, 1975/. Hordeum /McDaniel, 1970/, Gossypium /Cherry et al., 1970/ and Brassicas /Yadava et al., 1979/. The fact that some of the protein bands appeared at one stage and were missing at another stage can be explained by assuming that these were the proteins intimately involved in the actual process of germination and possibily represent the true storage proteins of the endosperm.

The peroxidase isozymes exhibited less variation at early stages of seeds imbibition and increased with the development of germinating seeds. The number of bands, in general, also increased in species and hybrids with the increase in stage of germination as has also been reported in Datura /Conklin and Smith, 1971/ in corn /Padma and Reddy, 1971; Lodha et al., 1974/ and in Brassicas /Yadava et al., 1979/. The species and the hybrids were differing in the isozymic pattern of esterases. A considerable amount of literature is available to support this view in many crop plants like pea /Frankel and Garber, 1965/; wheat /Cubadda et al., 1975/ and sorghum /Schechter and DE Wet, 1975/. The variation between species and hybrids was also observed at different seed germination stages under study. Some isozymic bands showed presence at one stage while they disappeared at the next stage and reappeared at a later stage. One explanation for these types of results may be that the activity of one isozyme is required in the metabolic processes going on in the seedling during germination whereas the activity is switched off at the next stage where its presence is not needed. New bands

appearing at some stages of seedling growth may be because of the synthesis of new enzymes during germination which appears as new bands at that stage.

The zymograms of catalase of different species did not show much difference at early stages of seed imbibition. Mostly the catalase activity was found to be restricted near the origin at early stages while in the later stages the activity was spread throughout the mobility area. The extent of variation also increased at later stages of growth. There are also earlier reports that catalase do not show any inter-species differences /Mitra et al.,1970, Cherry and Ory, 1973/. In most instances the heterotetramers generated by either intragenic or intergenic complementation, exhibit improved physicochemical properties over the least efficient parental molecules. This suggests that hybrid proteins may be of advantage to the organism carrying them.

To conclude, different species and hybrids exhibited different patterns of isozymes of peroxidase and esterase in different stages of development. The zymograms of catalase of different species and hybrids did not show much variation at early stages of seed imbibition whereas the extent of variation was increased in later stages.

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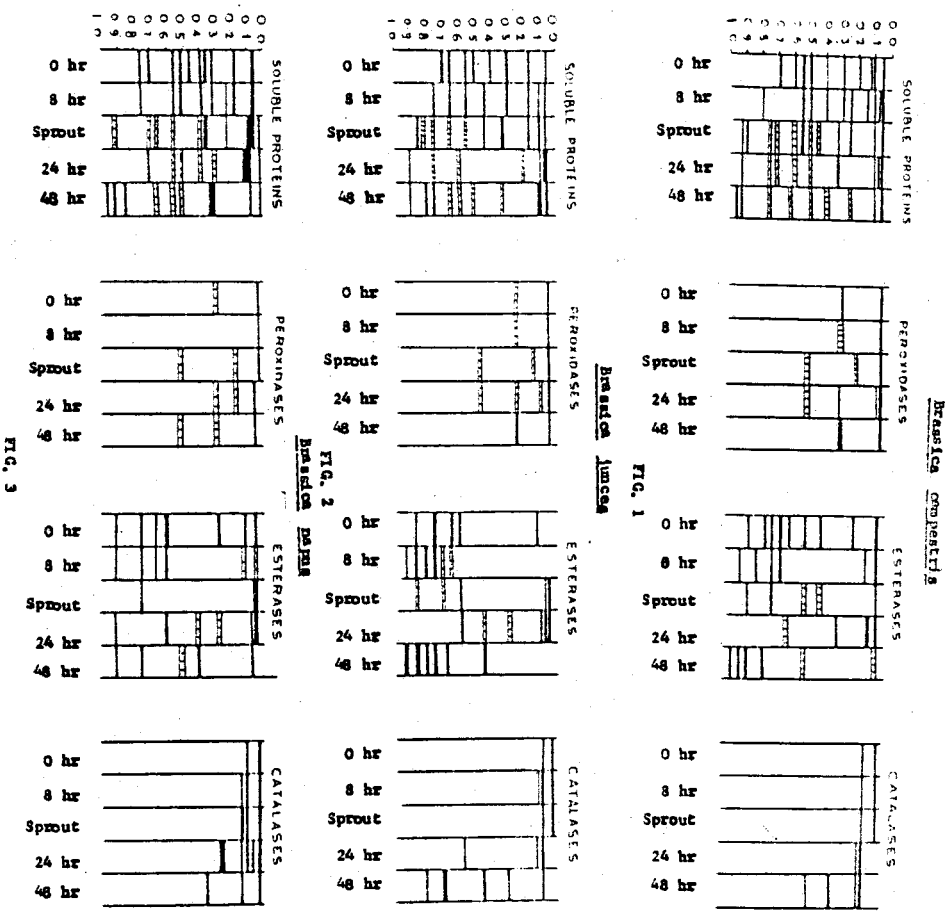

FIG. 1 Brassica juncea

FIG. 2 Brassica napus

FIG. 3

B. campestris x B. napus

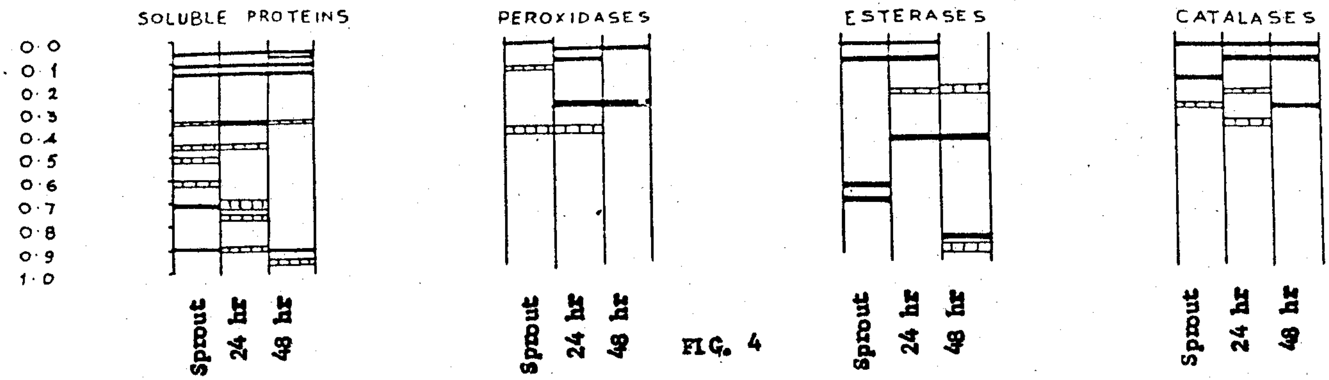

FIG. 4

B. juncea x B. napus

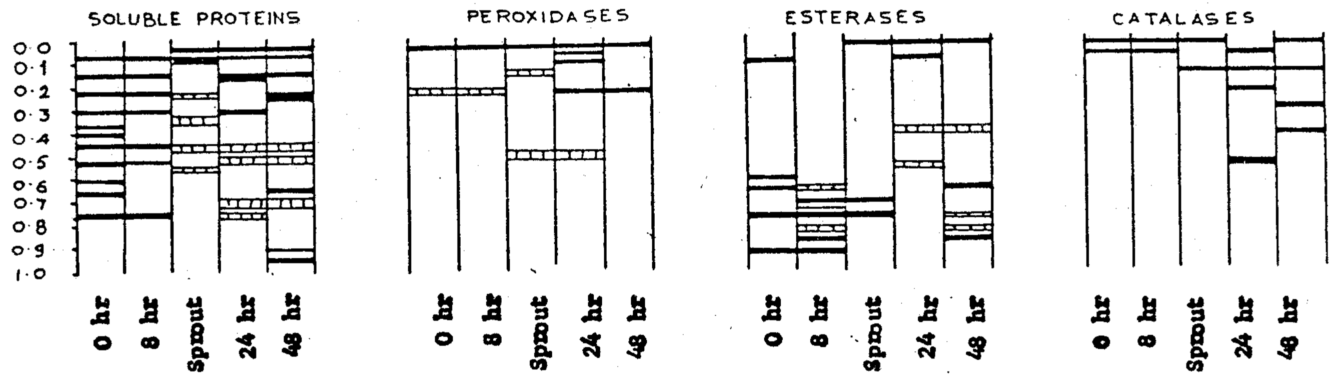

FIG. 5