ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(5):1091-1094

ORIGINAL RESEARCH ARTICLE

Isoenzyme Analysis of *Amomum* Species

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Received 20 Apr 2012; Revised 17 Sep 2012; Accepted 27 Sep 2012

ABSTRACT

Large Cardamom has been used in Ayurvedic preparations since ancient times. Now it is mainly used as spice and for flavoring foods. Its seeds contain 2-3% essential oil, a powerful flavoring agents very much resembling like small cardamom. Genetic variation in plants has been investigated using morphological, physiological and biochemical traits. Isoenzymes have proved to be the best method for studying genetic variation in plants compared to the use of physiological and morphological characters and molecular markers (Brown, 1990). The present investigation was aimed to carry out isoenzyme analysis of peroxidase, esterase, polyphenol oxidase and acid phosphatase of *Amomum* species namely *A.subbulatum*, *A.cannicarpum*, *A.aromaticum* and *A.Kingii*. Isoenzyme analysis did not show much variation between the species. Only peroxidase isoenzyme showed little variation.

Key words: Esterase, peroxidase, polyphenol oxidase and Acid phosphatase.

INTODUCTION

The large cardamom belongs to the family Zingiberaceae, is also known by the names 'greater Indian cardamom' or 'Nepal cardamom'. It is native to the Eastern Himalayanregion. In India, it is cultivated in Sikkim, Darjeeling and the Assam hills and it is also cultivated in Nepal, Bhutan and, to some extent, in some of the south-east Asian Countries such as Thailand, Indonesia and Laos. Isoenzymes have become very popular genetic markers in many facets of plant breeding and genetics, including studies of gene flow mating systems, levels of genetic variability and systematic and phylogeny (Slatkin, 1985). A genetic marker is a selectively neutral polymorphism at a locus wherein the alternative alleles are used to indicate genetic relationship in problems like estimation of gene dispersal or of genealogical relationship. Although isozymes can play critical roles in metabolic pathways with their variants potentially subjected to natural selection, most isozyme polymorphisms are regarded to be effectively and selectively neutral .In forest tree breeding and genetics isozymes have received wide use as genetic markers (Paule, 1990).

Genetic markers

Genes are invisible but it is possible to infer genetic constitution (the genotype) of a plant at an environmentally stable, qualitative trait from the phenotype, i.e., its direct observable appearance .The procedure to establish the relationship from phenotype to genotype is called genetic analysis and a trait, for which a genetic analysis has been successfully performed, is called a gene marker, genetic marker or gene marker locus (Hattemer, 1991). Important characteristics of a gene marker are:

- ❖ Environmental stability ("pure" genetic control)
- ❖ Qualitative variation among plants (discrete traits
- ❖ Identification of the genotype by observation of the phenotype

Types of genetic markers

Out of a variety of markers available for genetic surveys, two groups have gained outstanding importance. Biochemical markers, especially isozymes became standard tools in genetic research during the last 30 years and will continue to play a leading role in genetic marker studies both in developing and developed countries

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(Paule, 1990 and Lengsiri *etal* 1990). Isoenzymes are enzymes catalyzing similar reactions in the metabolism of an organism. They can be separated in an electric field by a simple biochemical procedure (electrophoresis), Since enzymes are polypeptidase coded directly by DNA, they are under strict genetic control ie, they reflect a high degree of environmental stability. Isozymes are currently the most frequently used genetic markers for population genetic surveys to infer patterns of genetic variation and mating system parameters in plant population.

More recently, molecular genetic markers were developed to access patterns of genetic variation at the DNA level .Two methods are most widely applied: RFLPs (Restriction fragment length polymorphisms) and methods based on PCR (Polymerase Chain Reaction, e.g. RAPD i.e. Random Amplified Polymorphic DNA).Besides the advantage of observing genetic variation at the most basic level, DNA genetic markers allow to identify a virtually unlimited amount of variation (Paule, 1990).

Applications of isoenzyme analysis

Studies of genetic variation and organization of plant species by isozyme analysis was studied by Moran *et al* (1989). Isozyme markers have been widely used extensively to identify and discriminate cultivars in many agricultural and horticultural crops, in forestry or identifying clones. Isozyme analysis has been used for hybrid, cultivar and clonal identification (Sedgley *et al,* 1982). It helps in the prediction of quality characteristics of forest species, sex differentiation. There are several applications of isozymes in studying the pollination process in tree breeding. They include the effectiveness of supplemental mass pollination, determining the magnitude of first pollination primacy, pollination order and the transmission of male genes to seed and determining male bias in the polycross mating design, which is controlled by crossing of several seeds (Woessner and Franklin, 1973).The use of mating designs in tree breeding requires conducting extensive controlled cross program on an operational scale. Isozymes can be used also in conjugation with other types of molecular variation to confirm validity of crosses (Shaw and Allard, 2006).

MATERIALS AND METHODS

Selection of species

The species chosen for study for biochemical analysis were *A.subulatum* (Wild type and tissue culture species) and *A.cannicarpum* (Wild type and tissue culture species). The species chosen for isozyme analysis were *A.subbulatum, A.cannicarpum, A.aromaticum and A.kingii.*

Collection and preparation of leaf samples

The leaf samples were collected from spices board farm at Gangtok, India. The selection was made from fresh leaves. The leaves were repeatedly washed in Distilled water and kept moist and cooled at 4^oC. It was cut into small pieces. Grinding and homogenizes of the sample were done following different methods as given below for each enzyme. Extraction of the enzyme was carried at 4^oC and the electrophoresis at 10^o**.**

Extraction of plant tissue in alcohol

The plant tissues were extracted in alcohol for the estimation of proteins, total phenols and phenol. The method of extraction was done according to the method given by Mahadevan and Sridhar, 1986.

Electrophoresis of Isoenzymes using PAGE

Non denaturing gel without SDS was run to separate the enzymes from leaf samples of different *Amomum* species. Four isoenzymes systems (i.e.) Peroxidase, Esterase, Polyphenol Oxidase and Acid Phosphatase were used.

Sample extraction

The compositions of extraction buffers used for various enzymes are given in (**Table 1**).

Table 1: Composition of extraction buffers

Composition of extraction buffer used for isoenzymes	Extraction buffer
Polyphenol oxidase Peroxidase	Sodium tetraborate buffer 50mM,pH
Esterase	8.3 containing all the cofactors
Acid phosphatase	Citrate buffer, pH 5.3

One gram fresh young leaf tissue was ground using a chilled mortar and pestle in an ice bath with 2 ml respective extraction buffer for each isoenzyme. The ground paste was transferred into microfuge tube and centrifuged at 10,000 rpm for 15 minutes. The supernatant served as the enzyme source which was loaded in the wells of polyacrylamide gels.

Statistical Analysis

The data were subjected to statistical analysis by by DMRT analysis (1967).

RESULTS AND DISCUSSION

Isoenzyme markers for the identification of *Amomum* species

Peroxidase

In higher plants, peroxidase have a wide distribution and exhibit broad substrate specificity. The peroxidase isozyme profiles of A.*subbulatum, A.cannicarpum, A.aromaticum and A.kingii*) were analyzed and the isoenzyme pattern is shown. The R_f values of peroxidase isoenzyme

bands in different *Amomum* species are presented in (**Table 2**) .

Table 2: The R_f values of peroxidase isoenzyme bands in different *Amomum* species

Bands	*A.subbulatum*	*A.Cannicarpum*	*A.aromaticum*	*A.kingii*
I	0.33	0.33	0.33	0.33
II	-	-	-	-
III	-	0.45	0.42	0.42
IV	0.50	-	-	-
V	0.58	0.58	0.58	0.58

The bands as depicted in (**plate 1**) revealed after staining in the region of medium mobility, except *A.subbulatum* ,all other species showed intense bands. Bands of R_f value of 0.33 and 0.58 were intensely stained in all the species. The banding pattern in *A aromaticum and A.kingii* were the same. *A.subbulatum* showed variation in isozyme pattern. A slight faint band was seen , which is not observed in other three species. Kumari *et al* (1985) reported variation in peroxidase isozyme pattern in 13 different species of Bamboo. Peroxidase isozyme profile showed six bands during somatic embryogenesis of *Bambusa vulgaris* (Rout and Das, 1995). Variation in peroxidase isozyme pattern of seed tissue of different provenances in sandal is reported by Angadi *et al* (1998).He observed nine bands.

Parthasarathi *et al* (1985) reported characteristic differences between sandal leaf types in their pattern of peroxidase isozyme. Kulkarni and Srimathi (1980) reported the occurrence of morphological variations such as ovate, elliptic, lanceolate and linear patterns in sandal leaves. Angadi and Ananthapadmanathan (1988) observed variations in isozyme pattern in the sandal plants affected with spike diseases. Kamala *et al* (1988) reported changes in multimolecular forms of POD associated with deficiencies of three elements in sandal seedlings.

Characteristic difference in banding pattern of peroxidase isozyme in the chlorosis affected leaves of sandal compared with control was reported by Kamala and Angadi (1986) Peroxidase isozymes activity in living bark tissue as a marker for the oil bearing capacity in sandal is reported by Parthasarathi *et al* (1986).

Esterase

Esterases are hydrolyzing enzymes which catalyze the addition or removal of water in biological reactions. Esterases are wide in nature and occur frequently in plants .Isozyme pattern of esterase is shown in plate 1. Much variation of esterase isozyme pattern was not observed among different *Amomum* species. The pattern remains the same in all the species inspite of repetitive attempts.

Esterase activity were much pronounced with 13 bands at different stages of development during somatic embryogenesis of *Bambusa vulgaris* (Rout and Das, 1995) Esterase isozyme studies on Indian Bamboo (*Dendrocalamus strietus*) in leaf revealed a light band and was found towards anodal direction in the mobility (Biswas, 1997).

Polyphenol oxidase

Polyphenol oxidase is a copper containing enzyme found in plants .In higher plants, polyphenol oxidase seems to be associated with particular portions of the cell such as plastids and mitochondria and play an important role in the respiration of plants.

Polyphenol oxidase isozyme could be visualized as low intense bands. Two banding patterns were observed in *A.Cannicarpum* and *A.aromaticum*. Only one individual band was noticed in *A.subbulatum* and *A.Kingii*. The isonzyme pattern is given in plate 1. The results are in accordance with (Egerton-warburton, 1990) who reported two banding patterns in *A.mangium and A.nilotica*.

Acid phosphatase

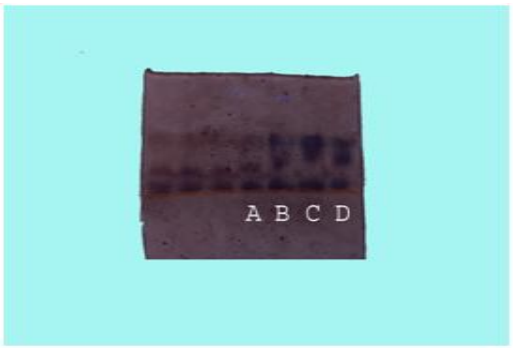
Phosphatases are vital in the metabolism of carbohydrates, nucleotides and phospholipids. In plants, acid phosphatase occurs mainly in green leaves. The pH of acid phosphatase exhibited optimum activity from 4 to 6 and hence the enzyme for electrophoresis was extracted in acid buffer. From plate 1 it is evident that Isozyme bands were same in all the four species. The bands were seen at the cathodal region of mobility. Brand (1994), reported similiar type of bands in *Trigonella foenum* and *Andrographis paniculata.*

In conclusion three peroxidase isozyme bands were revealed after staining in the region of medium mobility. Except *A.subbulatum*, all the other species showed intense bands. Bands of R_f value of 0.33 and 0.58 were intensively stained in all the species. The banding pattern in *A.Kingii* and *A.aromaticum* were the same .*A.subbulatum* showed variation in isoenzyme pattern.A slight faint band was seen, which is not observed in other three species. Much variation of esterase isoenzyme pattern was not observed among different *Amomum* species. Polyphenol oxidase isoenzyme could be visualized as low intense bands.Two banding patterns were observed in *A.cannicarpum* and *A.aromaticum*. Only one individual band was noticed in *A.subbulatum* and *A.kingii*. Acid phosphatase isoenzyme bands were same in all the species .The bands were seen at the cathodal region of mobility. The procedure should be standardized further to find out the variation.

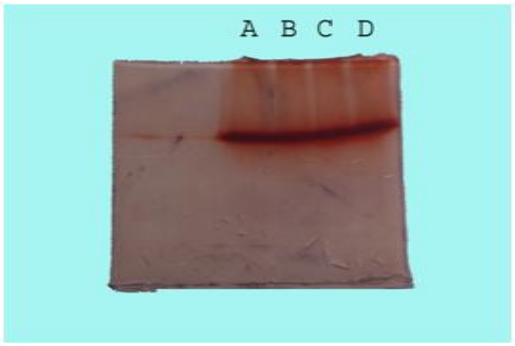
The study of four isoenzyme in four different species did not show much variation. The pattern remains the same in all the species inspite of repetitive attempts.

Plate 1: Isoenzyme Pattern in Leaf of *Amomum* Species

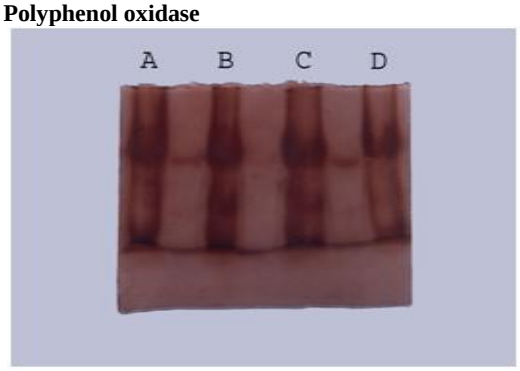
Peroxidase



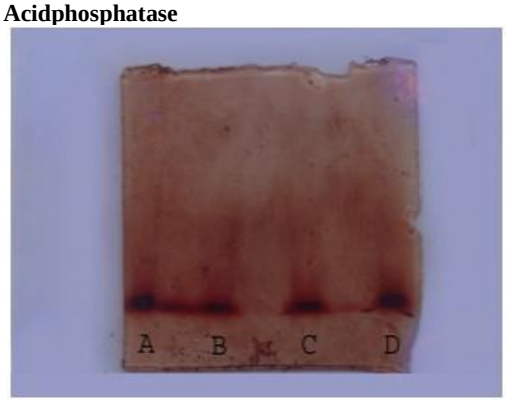
Esterase



Polyphenol oxidase



Acidphosphatase



REFERENCES

1. Angadi,V.G, Ramalaxmi,S , Rangaswamy, C.Rand Theagarajan,K.S, Isoenzyme technique – A powerful technique in research on sandal, ACIAR proceeding series, 84, 130 (1998),
2. Hattemer,H.HGenetic analysis and population genetics, Academic publishers. The Hague,The Netherlands,5-22 (1991),
3. Kulkarni,H.D and Srimathi,R.A , Variations in the foliar characteristics in sandal.A biometic analysis. Proceedings of the National Symposium on Improvement of forest Biomass. Himachal Pradesh department of forestry, Visva Vidhyalaya, 112-124. (1980),
4. Kumari, Vardana and Kalki Plant resistance to environmental stress Current Opinion Biotech 9, (1985), 214-219.
5. Moran.G, Muona.O and Bell The breeding system and genetic diversity in Acacia species, Biotropica, 21, (1989), 250 - 56.
6. Paule, Isoenzymes and Forest Trees, Report9, Sweeden.Dept of Forest Genetics and Plant Physiology,Sweedish University of Agricultural Sciences. (1990), 78-89
7. Parthasarathi .K, Rangaswamy and Angadi.V.G. Leaf Peroxidase malate dehydrogenase and esterase isoenzyme pattern in ten sandal *(Santalum album*) types showing variation in leaf pattern,Indian . J. Forester, 111, (1985), 441-449.
8. Rout.G.R and Das.P Isoenzyme profile during somatic embryogenesis and *in vitro* flowering of *Bambusa vulgaris*,J.Plant Biochem.Biotech., (1995),43-45
9. Shaw,D.V and Allard,R.W Estimation of outcrossing rate in Doughlas fur using isoenzyme markers,Theor.Appl.Genet.,62, (1982), 113-120.
10. Sedley.M, Harbard .J and Smith Reproductive Biology and interspecific hybridization of Acacia species, Aquatic Journal of Botany, 40, (1992), 37-48.
11. Wossener, R.A and Franklin,E.C Continued reliance on wind –Pollinated Southern pine seed ,In proceedings 12th South Conference for tree improvement, (1973), 64.