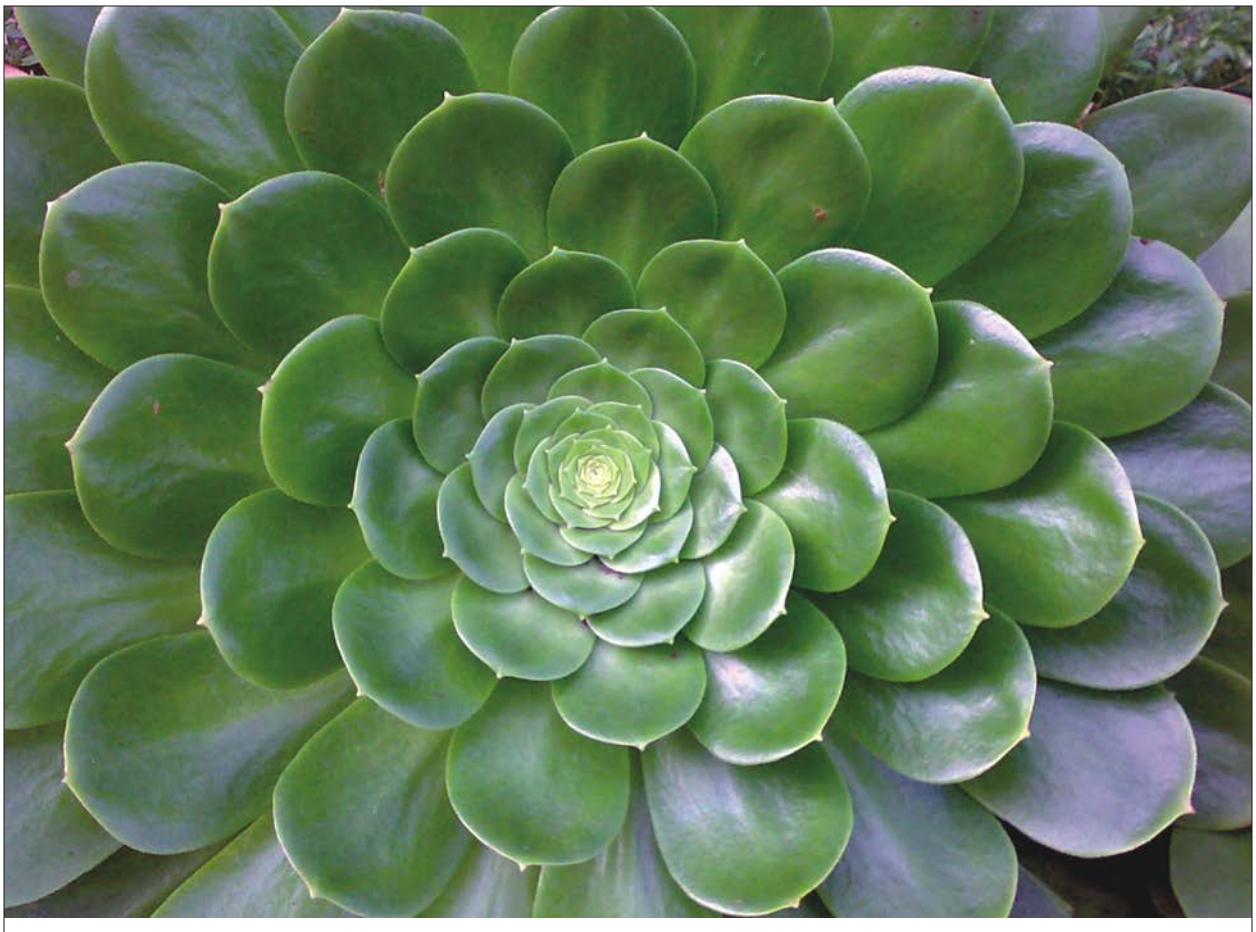

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Identification of *Cymbopogon* Species and *C. flexuosus* (Nees Ex. Steud) Wats Cultivars Based on Polymorphism in Esterase Isoenzymes

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Abstract: Esterase isozyme electrophoresis was used as a method for identification of five *Cymbopogon* species and seven cultivars of *C. flexuosus* Nees ex Steud. Two regions (slow and fast moving) of banding pattern were observed in all the material tested. A maximum of nine esterase isozymes were observed in different *Cymbopogon* species. species. Isozymes in different species ranged from 2 (*C. flexuosus* and *C. citratus*) 5 (*C. martinii*). The study revealed that all the *C. flexuosus* cultivars tested could be categorized as geraniol or citral rich by resemblance of their esterase isozymes pattern either with geraniol rich mutant cv. GRL-1 or normal citral rich cv. OD-19. All the *Cymbopogon* species had unique banding patterns, the only common feature being that all of them, except *C. pendulus* was isozyme Rf = 0.41. Only three species *C. pendulus*, *C. winterianus* and *C. martinii* could be uniquely identified on the basis of their esterase isozyme patterns. *C. flexuosus* and *C. citratus* showed identical esterase isozymes patterns.

Key words: *Cymbopogons*, geraniol rich mutant cv. GRL-1, GAE, isozymes, electrophoresis, polymorphism

INTRODUCTION

The usefulness of isozymes for cultivar identification, assessing genetic distances between taxa and in analyses of systematic relationships has been demonstrated in a large number of crops (Cooke, 1984). Recently, genetic variability in cultivars of *Vitis vinifera* was determined using isozyme electrophoresis by Sandra *et al*. (2005). Previously, Kidambi *et al*. (1990) studied the genetic variability among different Sainfoin (*Onobrychis*) species using esterase isozymes polymorphism. Tissue-specific expression of esterase isozymes in *Linum usitatissimum* was studied for estimating ontogeneic variability of expression of genes controlling this enzyme by Yurenkova *et al*., (1995). Pasquet and Vandereborght (2000) have reported the use of isozymes to assess the genetic distances between 21 *Vigna* accessions belonging to *V. frutescens*, *V. membranacea* and *V. friesiorum*. Isozymes have shown their potential for rapid cultivar identification in onion and lilium (Cooke, 1986; Van Tuyl *et al*., 1986). Booy *et al*. (1992, 1993) have described an efficient procedure for identification of tulip cultivars based on polymorphism in isozymes of esterase. The usefulness of isozymes as an aid in chemotaxonomical studies of the genus *Allium* was described by Hadacova *et al*. (1983).

The genus *Cymbopogon* belongs to tribe Andropoganae of the family poaceae. It is reported to include about 100 species and almost all of them are aromatic (Soenarko, 1997). Though the species differ at the intra and inter species level, morphological differences often blurred at the intra species level. However, no information is available on the variability among different *Cymbopogon* species

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except that of Sangwan *et al*. (2001) who studied the extent of diversity in *Cymbopogon* species using RAPD. Due to the degree of multiplicity of esterases and diversity of their physiological functions in the cell, they are potential candidates for biochemical markers of morphogenesis (Everett *et al*., 1985; Coopens and Gillis, 1987; Yurenkova *et al*., 1995). Therefore we used esterase isozymes electrophoresis as method for identification of *Cymbopogon* using five different species and seven cultivars of *C. flexuosus* including geraniol rich mutant cv. GRL-1 for the first time.

MATERIALS AND METHODS

Plant Material

Five different *Cymbopogon* species *C. flexuosus*, *C. pendulus*, *C. citratus*, *C. winterianus*, *C. martinii* and seven *C. flexuosus* cultivars geraniol rich mutant GRL-1, K-7, SD-68, OD-19, Cauveri, Pragati, Kerela Local were used to study the esterase isoenzymes polymorphism. The plants were raised from slips during January, 2001 at the experimental farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow by following standard agronomic practices.

Extraction of Geranyl Acetate Esterase (GAE)

The esterases from leaves were extracted according to Dubey and Luthra (2001). The immature leaves (10-15day old) were homogenized in mortar and pestle with homogenization buffer (1:3 w/v): 0.1M NaPI buffer (pH 6.5; 50 mM sodium metabisulphite, 10 mM mercaptoethanol, 10 mM ascorbic acid, 0.25 M sucrose, 1mM $EDTANa_2$) and 50% (w/w) insoluble PVPP (polyvinyl poly pyrrolidone). The homogenate was filtered through four layers of muslin cloth, centrifuged for 60 min at 15,000 x g. Supernatant was collected and treated with purified amberlite XAD-4 resin (50% of the tissue weight) (5 min at 4°C) to remove the endogenous terpenes. The slurry thus formed was filtered through muslin cloth and the supernatant obtained was used for the study. Protein concentration was determined by (Bradford, 1976) method.

Electrophoresis

Esterase isoenzymes polymorphism was studied using native-PAGE (Lamemmli, 1970). 13% resolving gel of 1 mm thickness was prepared to obtain the esterase isoenzyme spectra. Enzyme extract (1 mg) from each species and clutivar was mixed with 10% sucrose and 0.1% bromophenol blue (tracking dye) and loaded into the gel slots. Electrophoresis was carried out in 0.25 M Tris-glycine buffer (pH 8.3) at 4°C in cold room for 3 h and at 15 mA constant current per gel.

Staining of Esterase

Esterases were stained by dark incubation of gel at 37°C for 10 min in a solution containing 3 mg β-napthyl acetate and 10 mg Fast blue RR salt prepared in 50 mL 0.025M Tris-HCl buffer (pH 7.0). After the incubation was over, the gels were washed with distilled water and then photographed.

RESULTS

Esterase Isozyme Polymorphism in *C. flexuosus* Cultivars

Only two esterase isozymes (Rf = 0.24 and 0.41) were stained enough to be scored. Isozyme Rf = 0.24, in mutant cv.GRL-1 was appeared as very faint fast single band and comparatively lightly stained in normal cv. OD-19 (lane D). Isozyme Rf = 0.24, however, appeared as double band and stained poorly in all other cultivars. Isozyme Rf = 0.41 was stained uniformly and

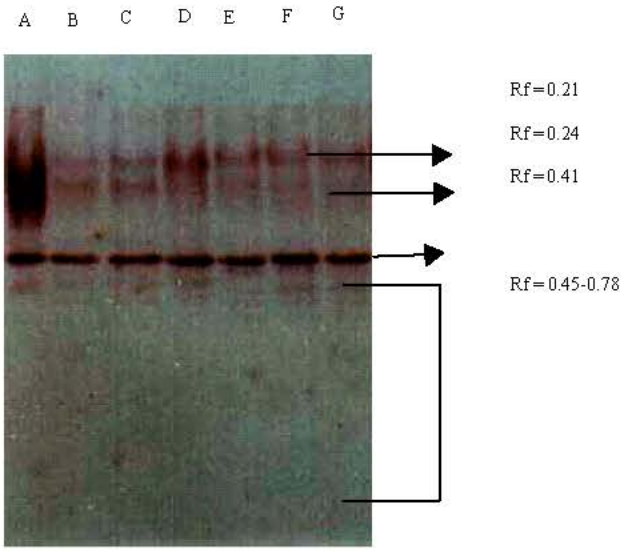

Fig. 1: The electrophoretic patterns of esterase isomzymes of *C. flexuosus* cultivars. Esterase isozymes pattern in different *Cymbopogon flexuosus* (Nees ex. Steud) Wats cultivars. (A) Geraniol rich mutant GRL-1 (B) K-7 (C) SD-68 (D) OD-19 (E) Cauveri (F) Pragati and (G) Kerela Local

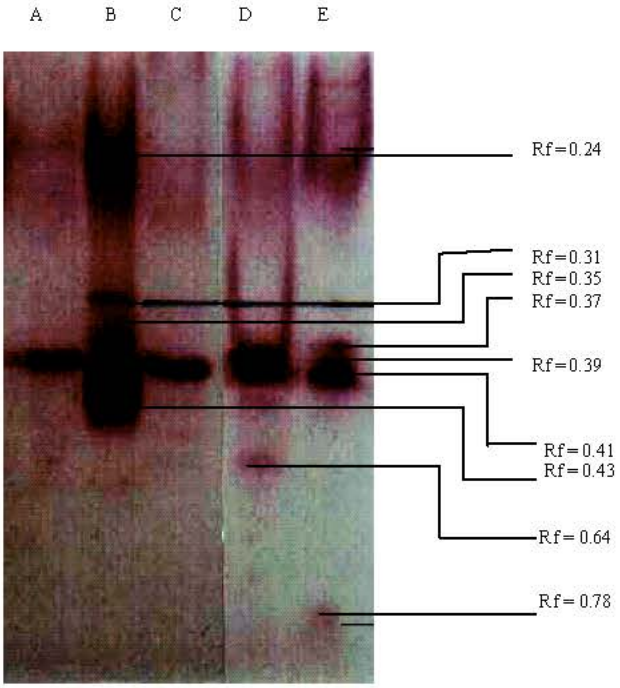

Fig. 2: The electrophoretic patterns of esterase isozymes of Cymbopogon species. Esterase isozymes pattern in different *Cymbopogon* species (A) *C. flexuosus* (B) *C. pendulus* (C) *C. citratus* (D) *C. winterianus* and (E) *C. martinii*

was common in all the cultivars tested (Fig. 1). Other isozymes (Rf = 0.45- 0.78) in the fast moving zone were not stained enough to be scored. The results indicated that geraniol rich lemongrass mutant chemotype GRL-1 could be uniquely identified on the basis of faint isozyme Rf = 0.24 from all other cultivars.

Esterase Isozyme Polymorphism in *Cymbopogon* Species

A maximum of nine isozymes were observed. Isozymes were categorized as slow (Rf = 0.24) and fast (Rf = 0.31 to 0.74) moving on the basis of their electrophoretic mobility Fig. 2. Isozymes in different species ranged from 2 (*C. flexuosus* and *C. citratus*) 5 (*C. martinii*). Upper slow moving isozyme Rf = 0.24 appeared only in *C. pendulus* and *C. martinii*. However, in *C. pendulus* it was strongly stained as compared to *C. martinii*. Fast moving isoenzymes Rf = 0.31, 0.35 and 0.43 were exclusively present in *C. pendulus*. Isozymes 0.37 and 0.39 were common in *C. winterinaus* and *C. martinii*. Poorly stained fast moving isozymes Rf = 0.64 and 0.74 were only present in *C. winterinaus* and *C. martinii* respectively. Isozyme Rf = 0.41 was uniformly stained and common in all the species except *C. pendulus*.

DISCUSSION

In the present article an efficient procedure for identification of *C. flexuosus* cultivars based on polymorphism in esterase isozymes described. Esterases (ESTs) hydrolyze ester linkage of different metabolites and are presented ubiquitous in all developmental stages of plants in many isoforms (Rasol *et al*., 1999) and changes in their expression and activity were observed under abiotic (de Carvalho *et al*., 2003) and biotic stress (Muarlidharan *et al*., 1996; Hassanein, 1999). All *C. flexuosus* cultivars studied showed almost identical esterase isozymes pattern indicating that they were probably originated from the same parent. Geraniol rich mutant chemotype GRL-1 was isolated during the study of genetic diversity chemogenetical improvement in citral producing lemongrass cv. OD-19 (Patra *et al*., 1997). Lemongrass (*C. flexuosus*) cultivars, however, could be categorized as citral rich and geraniol rich by resemblance of their esterase isozymes pattern either with geraniol rich mutant cv. GRL-1 or normal citral rich cv. OD-19. Studies of esterase isozymes polymorphism in different *Cymbpogon* species indicated that identification was possible only for *C. pendulus*, *C. martinii* and *C. winterianus*. The banding pattern of *C. pendulus* did not match those of *C. flexuosus*, *C. citratus*, *C. winterianus* and *C. martinii*. *C. flexuosus* and *C. citratus* seem to be closely related on the basis of their esterase isozymes pattern. The results of esterase isozymes polymorphism presented for lemongrass are analogous to those of RAPD analyses to discern the diversity in *Cymbopogons* described by Sangwan *et al*. (2001). Several reports are available based on polymorphism in the esterase and other enzymes for relationship analyses and identification viz., in grape (Sanches-Escribano *et al*., 1998; Altube *et al*., 1991; Royo *et al*., 1997; Pasquet and Vanderborght, 2000) *Lilium* (Van Tuyl *et al*., 1986) tulip (Booy *et al*., 1992) and *Onobrychis* (Kidambi *et al*., 1990) species and cultivars. Recently, Sandra *et al*. (2005) described isozyme electrophoresis as method of determination of genetic variability in constitutive genes of *Vitis vinifera* cultivars and suggested the role of regulatory genes in determining the number of molecules of enzymes in a cell and determining the berry skin polymorphism in *V. vinifera* cultivars. Thus, the esterase isozymes polymorphism used as a method for identification of *Cymbopogons* species and cultivars is similar and in addition to those reported earlier.

In conclusion all the *Cymbopogon* species studied had unique esterase isozymes patterns, the only common feature being that all of them, except *C. pendulus*, had isozyme Rf = 0.41. Identification was possible only for three species (*C. pendulus*, *C. martini* and *C. winterianus*) on the basis of their esterase isozymes patterns. Further all *C. flexuosus* cultivars tested were found to be citral rich as they

showed identical esterase isozyme pattern to that of normal citral rich cv. OD-19. This indicates a possibility for genetic relationship analyses using esterase isozyme patterns. However, expression of isozymes is dependent on the developmental stage of the plant and in lemongrass their expressions seems to be related with monoterpene compositions.

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