Medicinal Plants

Identification of plant taxons by isoelectric focusing

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Summary: Differences were demonstrated in esterasei soenzyme pattern of some essential oil producing plants belonging to the Apiaceae family – fennel (Foeniculum vulgare Mill.), angelica (Angelica archangelica L.), lovage (Levisticum officinale Koch.), dill (Anethum graveolens L.), coriander (Coriandrum sativum L.), anise (Pimpinella anisum L.), caraway (Carum carvi L.) – as well as differences between two varieties of fennel seed by using isoelectric focusing. That method provides quality control in essential oil plants and is suitable to describe isoenzyme pattern characteristic for taxons.

Based on our findings, isoelectric focusing seems to be suitable for identification and differentiation of different plant samples, providing an easy tool for further processing as well as for breeding.

Our further aim is to apply that method to differentiate among samples belonging to the same species according to their value of inner content.

Introduction

The importance of plant breeding is getting more and more pronounced with increasing environment pollution. Plants, which are able to contribute to the improvement of the biosphere or have high nutritional value may claim the special attention of breeders. Plant analysis at the protein level – combined with genetics – can provide information for selection of plants carrying desired characters.

Since proteins are products of gene transcription and translation, the protein patterns are characteristic for plant taxons. Thus the analysis of protein patterns can be considered to be an analysis of gene expression and a comparison of a particular set of proteins means a comparison of the genetic differences among individuals *(Bergmann,* 1987, *Patterson* and *Payne,* 1989)

There are many highly polymorphic proteins in plants, such as proteins and enzymes of seeds and of vegetative parts, especially seed storage proteins.

Although plants contain a lot of different proteins only a few of them are targets of genetic modifications. The method of gel electrophoresis and isoelectric focusing (IEF) can be

applied for the identification of different species and clones if for a given species breeding or other genetic modifications are reflected in changes of an isozyme pattern *(McMillin,* 1983, *Stegemann* 1983).

Isozyme markers have been utilized in a broad array of genetic studies ranging from basic studies concerned with the nature and range of naturally occurring genetic variations to applied studies involving the genetic characterisation and discrimination among cultivars within a given species *(Righetti,* 1983).

Isozymes differ from each other either in their net charges, and/or molecular sizes and/or shapes, thus they can be separated by electric field according to their different migration velocities through a gelatinous matrix. Following separation, identification of isozymes is accomplished by staining with specific substrate-containing solution that reveals enzyme activities that are visualized as spots or bands on a gel.

The constituents of samples applied to an isoelectric focusing gel will move along a pH gradient to that pH value where their surface charge is compensated, e.g. to their isoelectric points, where they stop moving. At this point the proteins have minimal solubility and precipitate into a sharp band.

Gel electrophoresis and isoelectric focusing have been used for species identification such as maize, rice *(Abdel-Tawab,* 1993/a,b), barley *(Sykorova,*1995), citrus *(Zhend* et al., 1993), olive *(Trujillo* et al., 1995), potato *(Poppr,* 1993), tulip *(Booy* et al., 1992), watermelon *(Cao,* 1994), grapevine *(Scienza* et al.,1994, *Walker,* 1995), cherry *(Beaver* et al., 1995).

Up to now, only few data were available for species belonging to the Apiaceae family *(Ross* and *Murphy,* 1992, *Agarwal* and *Kaul,* 1993, *Pierre* et al., 1990). Some data regarding relationship between inheritance and isozyme pattern of carrots have been published quite recently *(Lalleman* and *Briand,* 1995, *Dodeman* and *Ducreux,* 1996).

The aim of this study was to find any difference in esterase isozyme patterns of some essential oil accumulating plant species belonging to the Apiaceae family as well as differences between two varieties of fennel seeds by using isoelectric focusing in the range of pH 3–9.

Material and methods

Seed samples were as follows: dill - *Anethum graveolens* L., angelica – *Angelica archangelica* L., caraway – *Carum carvi* L., coriander – *Coriandrum sativum* L), fennel – *Foeniculum vulgare* Mill., *Foeniculum vulgare* var. dulce, *Foeniculum vulgare* var. azoricum, lovage – *Levisticum officinale* Koch., anise – *Pimpinella anisum* L.

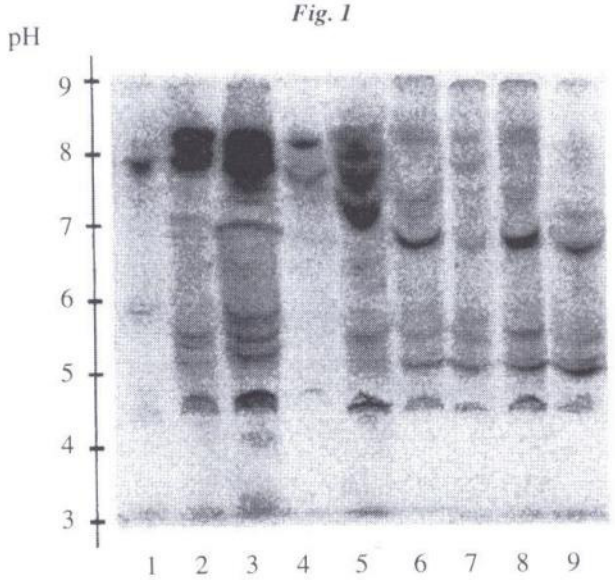
Sample preparation for isoelectric focusing: 100 mg of seeds were homogenized in an ice chilled mortar with pestle, by adding quartz powder in 500 l ice cold 20 mM Tris-HCl buffer pH 7.8, containing 100 mg/ml Triton X-100, 200 mg/ml saccharose, 2 mM phenil-methyl-sulphonil-fluorid. The homogenate was centrifuged with 15 000 g at 4 °C for 15 minutes. Supernatants were analyzed.

Isoelectric focusing was carried out on a PhastSystem (LKB-Pharmacia, Sweden). In order to develop pH gradient (pH 3-9) ready-made gels were prerun by 2.5 mA at 10 °C, for 75 Vh, and crude extracts of seed samples were applied onto the acidic (pH 4.5) end of the gel and run by 2.5 mA at 10 °C, for 700 Vh. Gels were stained for esterase activity with -naphtyl-acetate *(Vallejos,* 1983). Dried gels were scanned by Image Master Laser Densitometer (LKB, Pharmacia Sweden) and the relative positions of bands were evaluated.

Results and discussion

Esterase isozyme patterns of angelica, lovage, dill, coriander, anis, caraway and fennel are shown in *Fig. 1.*

Fig. 1



There are considerable differences in the isoelectric points of esterase isozymes of different medical plants. Differences can be seen not only in the pattern of isozymes but in their activities, too *(Fig. 2).*

The isoelectric points of each species of plants are between pH = 4.5-9.0 with one exception: those of the coriander are in the basic pH range *(Fig. 1* lane 4).

Substantial differences in the activity can be detected among the esterase isozyme patterns of Foeniculum vulgare var. dulce and Foeniculum vulgare var. azoricum as it is shown on densitometric traces *(Fig. 2)* made on the gels of *Fig. 1* lanes 7, 8, 9.

One can get more clearcut pictures *(Fig. 3)* on differences among isozyme patterns if a drawing is contructed according to *Fig. 1* where both positions and their relative intensitie of bands are demonstrated. This kind of drawing can be used for later evaluation and/or identification of any new samples.

Based our findings isoelectric focusing is a suitable method for analysing esterase isozyme patterns of medicinal seeds in order to distinquis different species providing.

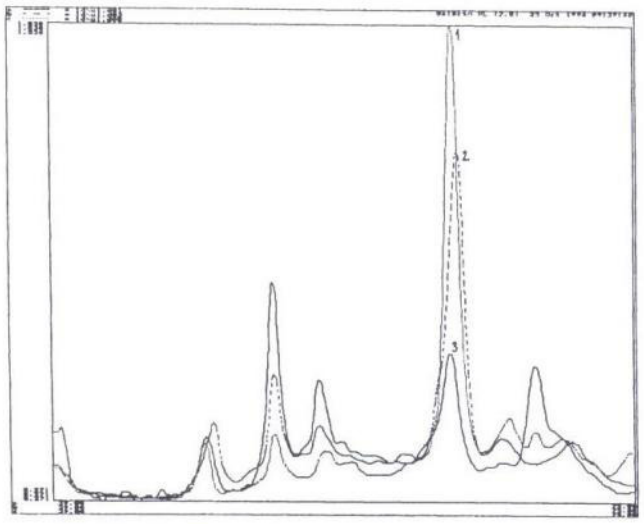


Fig. 2

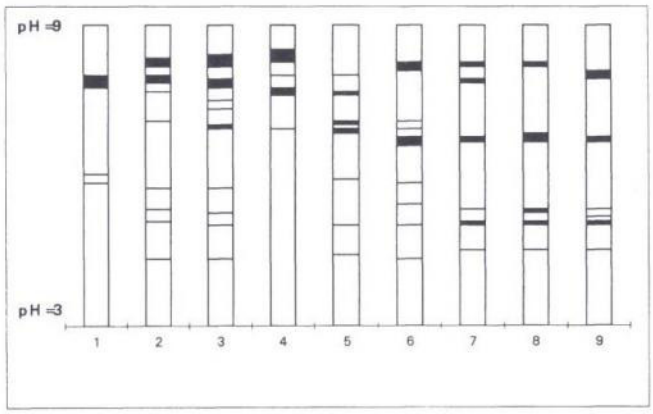


Fig. 3

References

Abdel-Tawab, F. M., Rashed, M. A., Fahmy, E. M. 1993/a: Electrophoretic characterisation of twelve inbred lines of maize (Zea mays L.). Annals of Agricultural Science Cairo. 2, 417–427.

Abdel-Tawab, F. M., Rashed, M. A., Bahieldin, A. 1993/b: Verification of cultivar identify in rice (Oryza sativa L.) by electrophoretic fingerprints. Annals of Agricultural Science Cairo. 2, 429–440.

Agarwal, M., Kaul, B. L. 1993: Gel electrophoresis for cultivar identification in Anethum graveolens L. Indian Journal of Forestry 16, 3. 239–242.

Beaver, J. A., Iezzoni, A. F., Ramm, C. W. 1995: Isoenzyme diversity in sour, sweet and ground cherry Theor. Appl. Genet. 90, 847–852.

Bergmann, F.1987: Characterisation of multiclonal aspen cultivars using isoenzyme electrophoresis. For. Ecol. Management. 22, 167–172.

Booy, G. 1992: Polymorphism in the isozyme esterase: possibilities for an efficient identification system of tulip cultivars. Acta Horticulturae. 325, 883–887.

Cao, W. H., Zhao, Y. R. 1994: An analysis on isoenzyme and soluble protein in watermelon. Acta Agriculturae Boreali Sinica. 9, 2. 64–71.

Dodeman, V. L., Ducreux, G. 1996: Isozyme patterns in zygotic and somatic embryogenesis of carrot. Plant Cell Reports 16, 1–2. 101–105.

Lallemand, J., Briand, F. 1995: Use of electrophoresis for variety testing in carrot. Plant Breeding 1995. 114, 4. 372–374.

Mc Millin, D. E. 1983: Plant isozymes. A historical perspective. In: S.D. Tanksley, T. J. Orton (eds.) Isozymes in plant genetics and breading, Part A. Elsevier Sci. Publ.B.V. Amsterdam, 3–23.

Patterson, B. A., Payne, L. A. 1989: Zymograms of plant extracts using isoelectric focusing on ultra thin layers. Acta Horticulture 247, 163–169.

Poppr, J., Hola, Z. 1993: Identification of potato varieties by means of isoelectric focusing. Rostlinna Vyroba. 39, 11. 1057–1064.

Righetti, P. G. 1983: Some applications of IEF. In: Work, T.S.: Burdin, R.H. (Eds): Isoelectric Focusing: Theory, Methodology and applications Elsevier Biochemical Press, Amsterdam, 314–355.

Ross, J. H. E., Murphy, D. J. 1992: Biosynthesis and localisation of storage proteins, oleosins and lipids during seed development in Coriandrum sativum and other Umbelliferae. Plant Sci. Limeric. 86, 1. 59–70.

Scienza, A., Villa, P. 1994: A chemotaxonomic investigation on Vitis vinifera L. II. Comparison among ssp. sativa traditional cultivars and wild biotypes of ssp. silvestris from various Italian regions. Vitis 33, 4. 217–224.

St-Pierre, M. D., Bayer, R. J., Weis, I. M. 1990: An isozyme-based assessment of the genetic variability within the Daucus carota complex (Apiaceae: Caucalideae). Canadian Journal of Botany 68, 11. 2449–2457.

Stegemann, H. 1983: Discrimination among Intraspecific Taxa using Electrophoretic Data. Proteins and Nuclein Acids in Plant Systematics ed. by U.Jensen and D.E.Fairbrothers Springer Verlag, Berlin Heidelberg, 124–128.

Sykorova, S. 1995: Electrophoretic patterns of esterases and acid phosphatases in the grain of registered varieties of barley. (Hordeum vulgare L.). Genetika a Slechteni. 31, 2.113–122.

Trujillo, I., Rallo, L., Arus, P. 1995: Identifying olive cultivars by isozyme analysis. Journal of the American Society for Horticulture Science. 120, 2. 318–324.

Vallejos, C. E. 1983: Enzyme activity staining. In : S. D. Tanksley, T. J. Orton (eds.): Isoenzymes in plant genetics and breeding, Part A. Elsevier Sci.Publ. B.V. Amsterdam, 469–516.

Walker, M. A., Liu, L. 1995: The use of isozymes to identify 60 grapevine rootstocks (Vitis ssp.) American Journal of Enology and Viticulture. 46, 3. 299–305.

Zheng, X. H., Dotani, Y., Sawamura, M. 1993: Isozymic analysis of peroxidase and esterase in Citrus flavedo. Bioscience-Boitechnology-and-Biochemistry. 57,10.1800–1802.