DISCRIMINATION OF MEDICINAL ECHINACEA SPECIES BY RAPD (RANDOM AMPLIFIED POLYMORPHIC DNA)

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Echinacea, also known as "coneflower", is a perennial plant which belongs to the daisy family Asteraceae (Compositae). Nine species of Echinacea are known, and three have been shown to have medicinal properties: *E. angustifolia*, *E. purpurea* and *E. pallida*.
Antiinflammatory, immunostimulatory, antioxidant and cicatrising activities are the main properties described, which make Echinacea useful in the treatment or the prevention of different pathological status (1).
Three main classes of active constituent have been identified in the three species: a) caffeic acid derivatives b) alkylamides and polyacetylenes c) polisaccharides. They are present in roots, rhizomes, leaves but also, in minor extent, in the other parts of the plant (2).
The three species have been considered for long time very similar for their medicinal properties and used indifferently for the same therapeutic applications. Nevertheless, marked differences in the chemical composition are emerging (3,4); the different chemical profile, in some cases, correlates with different pharmacological activity (5). A better knowledge of the differences among Echinacea species will improve their use in phytotherapy. Many morphological similarities, especially between *E. angustifolia* and *E. pallida* made specimens often confused in the medicinal plant market. Nowaday, HPLC and other fisico-chemical methods are able to identify the species, while there are no studies directed to a genetic characterization of the three species.
For this reason, in the present work, we analysed genetic differences among *E. pallida*, *angustifolia* and *purpurea* using a DNA fingerprinting method: RAPD (random amplified polymorphic DNA). This technique, developed contemporaneously by Welsh and McClelland (6) and Williams et al. (7), has been already successfully used in identification of plants. It is a simple and fast process, which is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence, allowing detection of polymorphisms in the absence of specific nucleotide sequence information. This technique requires only a minute amount of DNA, it is relatively little expensive and it can be used as an aid to quality control of herbal medicine on the market, differentiating between genuine and counterfeit materials, or identifying the species and the sources of various samples (8).

Materials and Methods

Six samples of Echinacea (two individual for each species) were analysed; these samples were supplied by Aboca (Arezzo) and were kept in the botanical garden of Pisa.
We used young leaves that were rinsed with 70% ethanol and distilled water to remove surface contaminants. Then, the leaves were ground under liquid nitrogen with a mortar and pestle to a fine powder.
Leaves powder (about 100 mg) was used for DNA extraction in accordance with "Dneasy Plant Mini Kit" protocol (Qiagen). The quality and the quantity of DNA extracted were valued by electrophoresis on agarose gel (0,8%) and by UV spectrophotometer, respectively.
RAPD reaction was performed as described by Nadeau et al. (9) in a 25 µL reaction mixture, containing DNA (0,02-0,11 ng), 12,5µL of Taq PCR Master Mix (Qiagen) (2,5 U of Taq DNA Polymerase, 1x Qiagen PCR buffer and 200 µM of each dNTP), 25 pmol primer (Kit A,Operon) and distilled water up to 25 µL.
Each tube was placed in a thermocycler (PCR Sprint temperature cycling system, Hybaid) and amplification was obtained as follows: 94°C for 2', 45 cycles of 94°C for 1', 33°C for 1', and 72°C for 2', followed by one cycle of 72°C for 10'.
An aliquot (10 µL) of the amplification product was analysed by electrophoresis in 1,4 % agarose gel and detected by staining with ethidium bromide (0,5 µg/mL).

Results and Discussion

RAPD test was carried out with all the twenty primers of the Operon kit, evaluating two DNA concentrations for each individual; only the bands always present independently of DNA concentration and of the individual used were considered for the inter-species comparison. Four primers generated a total number of six species-specific fragments (table n°1): one specific for *E. pallida*, four specific for *E. purpurea* and one specific for *E. angustifolia*.

Table n° 1

Primers	pb	E.angustifolia	E.purpurea	E.pallida
OPA 10	600			+
OPA 11	1250		+	
OPA 17	750		+	
	1000		+	
	1800		+	
OPA 20	1800	+		

In table n° 2 we reported primers with common fragments to two species of Echinacea. Only the primer OPA 18 gave a band present in all three species (700 pb).

Table n° 2

Primers	pb	E. angustifolia	E. purpurea	E. pallida
OPA 4	650		+	+
OPA 9	750		+	+
	800	+		+
	1500	+		+
	2200	+		+
OPA 10	650		+	+
	1300	+	+	
OPA 14	500	+	+	
OPA 18	1400	+	+	

Data analysis

Genetic Distance between species was calculated by the similarity index (S.I.) which was obtained by the following equation, according to Nei and Li (9):

S.I.= 2Nxy/(Nx+Ny) where Nx, Ny,and Nxy are the number of bands in lane x, lane y and the number of common bands in both lanes x and y, respectively.

The genetic distance values are indicated in table n° 3.

Table n° 3

	E.angustifolia	E.purpurea	E.pallida
E.purpurea	0,894	/	
E.pallida	0,823	0,867	/

These values indicate a high degree of genetic difference for all three species and only a relative smaller distance between *E. angustifolia* and *E. pallida*. The amplification products generated by marker primers will be subjected to further sequencing analysis which should reveal their meanings on the genome.

In conclusion, the results obtained in this study demonstrate that it is possible to obtain RAPD markers which may represent a good diagnostic mean to distinguish the three species of medicinal plant in the genus of Echinacea and consequently allow their correct therapeutic employ.

References

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