Pak. J. Bot., 40(6): 2289-2297, 2008.

EVALUATION OF TAXONOMIC STATUS OF MEDICINAL SPECIES OF THE GENUS *HYOSCYAMOUS, WITHANIA, ATROPA* AND *DATURA* BASED ON POLY ACRYLAMIDE GEL ELECTROPHORESIS

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Abstract

Seed protein profile of 42 accessions belonging to 7 species of 4 different genera (*Datura*, *Hyoscyamus*, *Withania* and *Atropa*) from the family Solanaceae were investigated through Poly Acrylamide Gel Electrophoresis. Intra and inter specific relationship was estimated using Jaccard's similarity index. A dendrogram based on UPGMA revealed the generic status and inter relationship of *Hyoscyamus*, *Atropa*, *Withania* and *Datura*. The specimens of *Withania somnifera* collected from Panjgur (109717, 109718, and 109710) not only showed the variation morphologically but also based on protein profiles. These specimens have the allelic variation at band of 66, 64 50, 42, 22 and 16 Kda. This accession is also geographically different from the rest of specimens of *W. somnifera*. These differences are enough to give it the rank of sub species of *W. somnifera*. Based on the total seed protein profile close association is found between *Withania/Datura* and *Atropa/Hyoscyamaus* but they maintain their generic status, as there is no intermixing of species was observed. The present study provides useful information for the identification of the taxa, their relationship and the delimitation of their taxonomic status.

Introduction

Atropa, *Datura*, *Withania* and *Hyoscyamus* are four medicinally important genera of the family Solanaceae. In Pakistan these four genera are represented by eleven species (*Atropa*=1, *Datura*=4, *Withania*=2, *Hyoscyamus*=4) (Nasir, 1985). The members of family Solanaceae are being used for medicinal purposes since as early as 37 A.D. (Hill, 1952). The people of that age were very well aware with the narcotic effect of *Datura stramonium* L., and *Hyoscyamus niger L.* Taxonomically these genera are quite complex and have many confusion in morphological markers.

Orthodox taxonomy more often relies on morphological markers. All taxonomists are agreed that the differences between plants and the similarities that plants may possess in common, are measurable to large degree by the morphological characters of those plants (Lawrence, 1971). When the plants are highly variable and contain large number of hybrids, identification based on morphological characters is quite difficult. *Datura* is a species complex genus, having medicinal (*D. stramonium* L. and *D. innoxia* Miller) as well as the poisonous (*D. fastuosa* L.) species. *Datura innoxia* Miller and *D. stramonium* L., show resemblance in plant height, branching pattern, flower colour, size, shape and in fruit colour, size/ shape. Hence morphological markers used in the past are insufficient for their correct and proper identification. *Hyoscyamus niger* L., and *H. pusillus* L., are

source of very important alkaloid hyoscyamine and morphologically very close to each other. Lower order taxa of the *Withania somnifera* and *W. coagulans* could not be identified on the basis of morphological markers.

Modern biological techniques are now available that can resolve these issues. In recent years SDS-PAGE of total seed proteins has found wide application in resolving systematic relationships and for inter and intra specific studies (Karihaloo *et al*., 2002). Khalifa *et al*., 1998 used SDS-PAGE to reassess the taxonomic relationships of 45 species belonging to 15 genera and 8 tribes of the Solanaceae. Based on the results he supported the conventional classification of this family. Two important species *Capsicum annum* and *Solanum melongena* of the same family were also analyzed for seed protein (Karihaloo *et al*., 2002 and Anu & Peter, 2003). SDS-PAGE was found effective for phylogenetic studies in these species. Edmonds & Glidewell (1977) confirmed the origin of *S. nigrum* from *S. americanum* and *S. villosum* by PAGE of seed proteins.

The aim of the present study was to find out the solution of existing taxonomic problems of species from *Datura*, *Atropa*, *Hyoscyamus* and *Withania*, which overlap in most of their morphological characters, and to elucidate relationship of the critical taxa by utilization of SDS-PAGE.

Materials and Methods

The taxa of *Datura*, *Hyoscyamus*, *Withania* and *Atropa* used for electrophoretic analysis are presented in Table 1.

Forty-two accessions belonging to 7 species of 4 different genera (*Datura*, *Hyoscyamus*, *Withania* and *Atropa*) from the family Solanaceae were used in this investigation. Total seed proteins were extracted from 0.01g of seed flour using 400µl of extraction buffer that contained 0.05M Tris-HCl pH 8.0, 0.2% SDS, 5M Urea, and 1% Mercaptoethnol. Seed flour was thoroughly mixed with buffer by vortexing. The extracted protein was separated by centrifuging the sample at the rate of 15000rpm for 10 mins. Electrophoresis was carried out in a discontinuous SDS-PAGE system of Laemmli (1970) using 15% acrylamide gel. Electrophoresis was run at 100V. The gels were stained in the staining solution containing 44% methanol, 6% acetic acid, 500ml distilled water and 2.25g of coomassie brilliant blue for 45mins. Destaining was done in a solution containing 20% methanol, 5% acetic acid and 750ml of distilled water until the background color disappeared and protein bands were clearly visible.

Data analysis: Protein bands were scored depending on their presence (1) or absence (0). Jaccard's similarity indices were determined and hierarchical clustering was constructed by unweighted pair group method with arithmetic average (UPGMA). The computer software SPSS v ll.0 was used for this purpose.

Results

Is presented profile of four genera (*Withania*, *Hyoscyamus*, *Datura* and *Atropa*) on 15% acrylamide gel concentration Fig. 1. The best polyacrylamide gel concentration to study the allelic variation among the medicinally important species *of Atropa*, *Hyoscyamus*, *Datura* and *Withania* was 15% (Fig. 1). For *Withania*, *Hyoscyamus*, *Datura* and *Atropa* altogether 25 protein bands were observed on the same concentration of gel. The intensity of bands is represented by three different colors. Band 21and 25 was

present in almost all the species so it presented the interrelationship of these four genera. *Withania somnifera* and *W. somnifera* acc. 1097187 were two specimens with certain morphological differences. They also exhibited differences in their profile. From Fig. 1 it is clear that band 1 and 2 although with low intensities but present only in *W. somnifera* and absent from *W. somnnifera* acc. 1097187. Similarly band 6 was the part of *W. somnifera* profile only. Few bands in two profiles showed variation in their intensities. Band 9 was darker in *W. somnifera* accession 1097187 whereas as band 21 were more intense in *W. somnifera*. Band 18 was absent from the profile of *W. somnifera*.

Two different species of *Hyoscyamus* (*niger* and *pusillus*) were subjected to electrophoresis. *H. niger* acc 46322, *H. pusillus* acc. 68182 and acc. 68183 were the specimens which showed variations from type specimens. These accessions were also tested for variability by electrophoresis. It was interesting to note that *H. pusillus* acc. 68182 and 68183 although had few morphological difference but their profile was exactly similar. However these accessions had certain allelic variation from *H. pusillus* (Fig. 1). Band 1 and 18 were entirely absent from profile of *H. pusillus* acc. 68182 and 68183. On the other hand band 6, 16 and 22 were absent from *H. pusillus* protein profile. Band 13 was the only band that exhibited variation in terms of intensity. Protein profile of *H. niger* and *H. niger* 46322 also had certain differences. Band 9 was only the part of profile of *H. niger* and 17 in the profile of *H. niger* 46322 although both of these bands are of low intensities (Fig. 1).

Jaccard's similarity indices were computed based on protein profile. Similarity index clarify the intra specific relationship of genus *Hyoscyamus* and *Withania*. Similarity index of *H. niger* acc 46322 with *H. niger* was 0.46. Profile of *H. pusillus* acc 68182 and *H. pusillus* acc 68183 was exactly same. These two accessions had the similarity of 0.40 with *H. pusillus*. *Withania* is another important genus of Solanaceae. Two specimens of *Withania somnifera* were examined. *W. somnifera* acc 109718 had the highest similarity index of 0.90 with *W. somnifera* (Table 2). The least similarity index of 0.04 was observed between *W. coagulans* and *H. pusillus*. From Jaccard's similarity index it was clear that *Atropa* and *Hyoscyamus* are more closer to each other as compared to other genera.

Dendrogram (Fig. 2) represents the division of species into two main groups (group 1 and 2). The group 1 is occupied by the species of *Hyoscyamus* and *Atropa* whereas *Withania* and *Datura* species comprise group 2. The species of these genera are going to separate from each other into subgroups of 1 and 2. It is clear from the dendrogram that close association is found between *Withania/Datura* and *Atropa/Hyoscyamus* but they maintain their generic status, as no intermixing of species was observed.

Discussion

Hyoscyamus is a uni regional genus of the family Solanaceae. This genus occupies the phyto-geographical region of Sino-Japanese. *Hyoscyamus* is a small herbaceous genus having 20 species all over the world, of these 4 species are present in Pakistan (Nasir, 1985). Two species *H. niger* and *H. pusillus* are the part of traditional medicinal system of subcontinent (Purohit & Vyas, 2004). A product of this genus is available in market in the form of tablets by the name of Hyoscine used for stomach disorders. Plant habit, pubescence, petiole status, seed shape and colour are important markers to characterize this genus (Rechinger, 1958). Leaf characters are important to establish its relation with *Atropa* and *Solanum*.

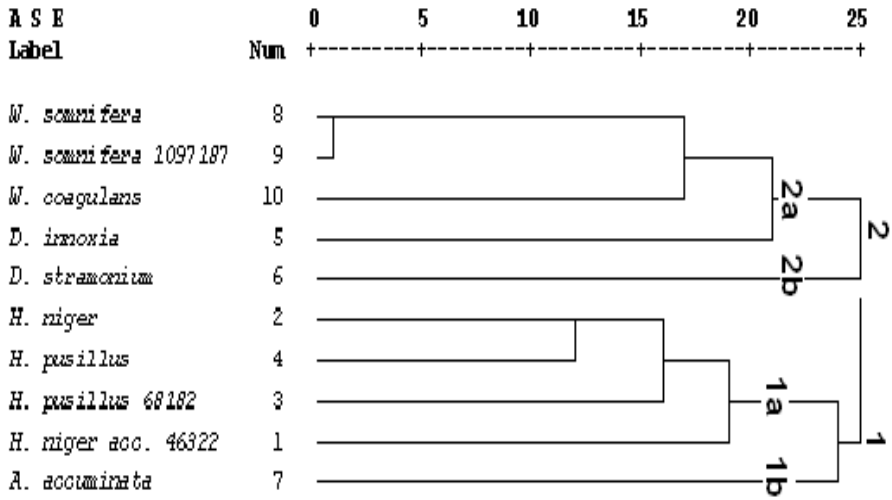

Fig. 2. A dendrogram of the medicinally important species of *Datura*, *Atropa*, *Withania* and *Hyoscyamus*.

Bamber, (1916) and Stewart, (1972) reported the presence of these species in Pakistan but they fail to provide description of morphological markers. All the available herbarium sample were studied and it was found that sample with accession no 46322 collected from Bannu showed certain morphological difference from the rest of samples. This specimen showed the differences in the anthers. Anthers are winged as usual but wings contain hairs, which is not present in other specimens of this species. Stigma is hairy and in fruiting operculum covers almost half of the fruit. Seeds are different in colour, they are yellow but in other specimens they are brown in colour. This accession was further analyzed for protein. There were certain difference in protein profile of *H. niger* and *H. niger* 46322. Band at 42 Kda was only the part of profile of *H. niger* and 23 Kda in the profile of *H. niger* 46322 although both of these bands are of low intensities (Fig. 4.25). The similarity index of 0.46 suggested the segregation of *H. niger* acc. 46322 as a new variety of *H. niger*.

Similarly the specimen of the *H. pusillus* with accession no 68182, 68183 possessed morphological variation from the other specimens of the same species. This specimen has a cylindrical petiole while in rest of specimens it is flat. Leaves densely hairy, hairs are small and straight in the rest of specimen hairs present only on midribs and lateral veins of leaves. Leaves margin are dentate while in usual pattern it is smooth. The sample was further investigated by SDS-PAGE. These two accessions have exactly similar profile. Therefore they can be considered as duplicate of one and other. However they have allelic variation for the other specimens of *H. pusillus* (these specimen had no morphological variation for type specimen). They showed variation at band no 1, 6, 16, 18 and 22 (Fig 1). The similarity index of these accessions with *H. pusillus* is 0.40. These differences suggested separating these accessions as variety *nova*.

W. coagulans and *W. somnifera* are two medicinally important species of *Withania* in Pakistan. *W. coagulans* is used to cure ailments relating to digestive systems and *W. somnifera* as aphrodisiac tonic in rheumatic pain. This genus is found in the areas, which comes under the category of Saharo-Sindian and Sino-Japanese. Leaf, flower, fruit and seed characters play key role to differentiate *W. coagulans* from *W. somnifera*. Baytop,

(1978) also give the importance to these characters for the description of *W. somnifera* of Turkey. However this species is somewhat different from the Indo-Pak *W. somnifera*. The difference lies in fruiting calyx. In Pakistani *W. somnifera*, teeth of fruiting calyx are small and triangular whereas the Turkish *W. somnifera* have longer, filiform teeth. Hawkes & Edmond, (1972) misapplied the name of *W. frutescence* for *W. coagulans*. All the morphological markers he mentioned under the heading of *W. frutescens* are exactly the characters of *W. coagulans*. He could not find even the single character for the distinction of both species.

Certain morphological differences were also found in the specimen collected from Panjgur (109717, 109718 and 109710). These specimens have the habit of herb. Hairs are very small on young branches but as the branches grow older they become quite prominent and dense while in other specimen it is equally dense in young and old branches. Leaf is small in size and hairs are not only present on mid-rib but on the whole lamina. Flowers are solitary rather than to produce in clusters which is observed in other specimens. Colour of flower is also different; it is white while in regular pattern it is yellowish green. The most important difference is in seed. Seed shape is conical and it is winged while in other case shape of the seed is rounded or oval and no wing is present. Colour of the seed is yellow. This specimen was subjected to electrophoresis for investigating the genetic variation.

Withania somnifera acc 1097187 also exhibited differences in their protein profile. From Fig. 4.25 it is clear that band at 66 Kda and 64 Kda although with low intensities but present only in *W. somnifera* and absent from *W. somnnifera* acc. 1097187. Similarly band at 50 Kda was the part of *W. somnifera* profile only. Few bands in two profiles showed variation in their intensities. Band at 42 Kda was darker in *W. somnifera* acc. 1097187 whereas as band at 16 Kda was more intense in *W. somnifera*. Band at 22 Kda was absent from the profile of *W. somnifera*. This accession is also geographically different from the rest of specimens of *W. somnifera*. These differences are enough to give it the rank of sub species of *W. somnifera*.

Atropa is known as an important source of belladonna (Chevallier, 1996). Four species of this genus are included into world flora. It is represented by a single species *Atropa accuminata* in Pakistan. Many of its products in form of tablets and injections are easily available in the market. This is a biregional species found in zones of Saharo-Sindian and Sino-Japanese. *Atropa* is segregated from the *Withania*, *Datura*, *Solanum* and *Hyoscyamus* due to the absence of pubescence (Nasir & Rafiq, 1995). This character indicates its relationship with *Capsicum.* Plant habit and floral characters make it closer to *Datura* (Pojarkova, 1997). Protein profile suggested the association of this genus with *Withania* and *Datura*.

Datura stramonium and *D. innoxia* are two medicinally important species of *Datura* genus. These species have anesthetic and sedative properties and also used to relieve muscular spasm (Hill, 1952). Seed oil can be used for soap making. *D. innoxia* and *D. stramonium* have resemblance based on morphological characters. Morphological markers used for the differentiation of these species are leaf shape, leaf margin, colour of mid-rib and the presence/ absence of pubescence (Clarke, 1885; Nasir, 1985). These characters are highly perceptive of environmental factors, creating difficulty in identification. Protein profile of these species is clearly different from each other (Fig 4.25). Cluster analysis indicated that both species of *Datura* were present in the same group.

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(Received for publication 26 March 2008)