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Similarities and differences between Amaranthus species and cultivars and estimation of outcrossing rate on the basis of electrophoretic separations of urea-soluble seed proteins

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Summary

The methods of distinguishing Amaranthus species and cultivars and assessment of outcrossing rate in grain amaranths are proposed. The collection of 20 cultivars and accessions of 7 species (*A. tricolor, A. mantegazzianus, A. cruentus, A. caudatus, A. hypochondriacus, A. hypochondriacus* × *A. hybridum* and *A. paniculatus*) was examined. Urea-soluble proteins both from bulked samples (20 seeds) of particular cultivars, and samples which were obtained by mixing together cultivars of each species were extracted. To estimate the outcrossing rate, the proteins were extracted from each of 100 seeds of cultivar A10 (*A. caudatus*). In this case seeds were collected both from isolated inflorescences and from flowers exposed to open-pollination. The electrophoretical separations of proteins (SDS-PAGE) were performed. The protein pattern of 56–64 bands was detected. The samples were classified to three groups: 1- *A. caudatus* and *A. cruentus*, 2- *A. tricolor* and *A. mantegazzianus* and 3- *A. hypochondriacus, A. hypochondriacus* × *A. hybridus* and *A. paniculatus.* All species can be distinguished from each other. The protein pattern of *A. tricolor* (leafy type of amaranths) is considerably different from other species. The present study suggests a closer similarity between *A. caudatus* and *A. cruentus* than between the pairs of species *A. hypochondriacus* / *A. caudatus* and *A. hypochondriacus* / *A. cruentus.* Only small differences were seen between cultivars, nevertheless they were sufficient for distinguishing all the cultivars. It is possible to detect outcrossing between cultivars, by observing that 30% of 'A10' seeds performed other patterns of proteins. Electrophoretic analysis of seed proteins proved to be useful for distinguishing species and cultivars of Amaranth, for describing similarity between species and for estimation its outcrossing rate.

Introduction

Amaranth is one of the few nongrass species which potentially can become a cereal-like grain crop or source of dietary proteins. Amaranth seeds are known to have proteins with a better balanced content of the essential amino acids than the proteins of cereals and legumes. The proteins from amaranth seeds contain two to three times more lysine than those of cereals. The nutritional value of amaranth proteins measured as Net Protein Utilization (NPU) is 75, compared with 44,57 and 62 correspondingly of maize, wheat and barley (Amaranth. Modern prospects . . ., 1984).

Amaranths are grown as a minor crop over extensive areas in India, Mexico, Central Africa, Kenya and Tanzania. Recently, amaranth has been described as an 'alternative crop' or as a 'new crop' thanks to possibilities of using its seeds and leaves in food industry and pharmacy.

The genus *Amaranthus* consists of approximately 60 species, of which, however, only a limited number are of the cultivated types, while most are considered weedy species. Three species of cultivated grain amaranths were recognised (*A. caudatus, A. cruentus* and *A. hypochondriacus,* Sauer, 1950, 1967). Associated with these are two weedy species: *A. powelli* and *A.*

hybridus. The weed amaranth *A. retroflexus* is considered one of the worlds worst weeds. The species grown as vegetables are represented primarily by *A. tricolor, A. dubius, A. lividus* and *A. cruentus.*

There are at least five levels of the grain amaranth gene pool corresponding to 1/ a diverse group of wild and weedy relatives of grain amaranths, 2/ a group of three cultivated grain species, 3/ races or grain types within each cultivated species based on geographic and morphological patterns of diversity, and 4/ individual landrace populations collected from unique localities. The final level corresponds to genetic stocks, breeding lines, and cultivars. New cultivars of grain amaranth were registered in USA (MT3, Plainsman, Amont), in India (Annapurna, Suvarna) and in Poland (Aztek, Rawa). Between each level there are recent (or dating from the past) cases of hybridization that blur the boundaries between groups.

The cultivated grain species are distinguished by characteristics of the female flowers and inflorescences according to the taxonomic key developed by Sauer (1967). Grain type amaranth plants have a main stem axis that terminates in an apical large branched inflorescence. The wild and weedy amaranth species are profusely branched and have a small terminal inflorescence. The cultivated grain species differ from their wild and weedy relatives by having pale-yellow rather than black seed coats. The seed weight within the genus ranges from 0.37 to 1.21 g per 1000 seed, the low values corresponding to wild and weedy species and the high values to cultivated grain species (Nowe Rośliny, 1995).

An attempt at the classification of some (10) edible and weedy species of the family *Amaranthaceae* was made by Feine-Dudley (1980).

The genus *Amaranthus* is known to be a taxonomically difficult group (Joshi & Rana, 1991). Synonyms are a basic problem. According to Sauer (1967), Pal et al. (1982) and Coons (1982) there are following pairs of synonyms: *A. cruentus* L./ *A. paniculatus* L., *A. caudatus* L./ *A. mantegazzianus* Passerini, *A. caudatus* L./ *A. edulis* Spegazzini, *A. tricolor* L./ *A. gangeticus* L., *A. edulis* Spegazzini/ *A. mantegazzianus* Passerini, *A. hybridus* L./ *A. quintensis* HBK. We would like to verify the identity of some mentioned above species. Are these species identical or we can consider them as separate species?

Phyllogenetic relationships among the three major species of grain amaranths need investigation to provide information useful in breeding programmes. Sauer (1967) developed two hypotheses concerning the origin of grain amaranth. In one scenario, the three cultivated species were independently domesticated from weedy species in three regions: *A. cruentus* from *A. hybridus* in Central America, *A. hypochondriacus* from *A. powellii* in Mexico, and *A. caudatus* from *A. quitensensis* in South America. The alternative hypothesis proposed a single origin of the pale seeded amaranths where *A. cruentus* arose from *A. hybridus* in Central America. Movement of the early domesticate forms north into Mexico and south into South America, followed by subsequent hybridization with local weed populations, lead to the selection of *A. hypochondriacus* and *A. caudatus*.

Gudu & Gupta (1988) successfully discriminated three grain amaranths (*A. caudatus, A. cruentus* and *A. hypochondriacus*) with the use of starch gel electrophoresis of leaf enzymes. It was observed that there is banding homology between *A. hypochondriacus* and *A. caudatus*. Gudu & Gupta (1991) reported that it was possible to transfer genes among all three cultivated grain species. The F_1 hybrids between *A. hypochondriacus* and *A. caudatus* were relatively easy to obtain. It was suggested that *A. hypochondriacus* and *A. caudatus* were genetically closely related. According to Gudu & Gupta (1991) this may support Sauer's (1967) hypothesis of common ancestry of these two species.

As known, the phenomenon of protein variation was widely used in the study of phylogenetic relations (Konarev, 1983). According to opinion of Zheleznov et al. (1997) electrophoretic analysis (acid PAGE) of storage proteins is a very useful method of describing phylogenetic relationships between 10 species of Amaranth. The homology of protein pattern between *A. hybridus* and *A. paniculatus* was detected. The homology of protein electrophoregrams can be well explained, since, according to the hypothesis of Pal et al. (1982) that *A. hybridus* is the wild ancestor of *A. paniculatus* syn. *A. cruentus*. The phylogenetic relationship between *A. paniculatus* and *A. hybridus* was confirmed. On the other hand, data of Zheleznov et al. (1997) were not consistent with the hypothesis of Sauer (1967) that A. *cruentus* moved from Central America to the south and then *A. caudatus* was selected, since this species by type protein pattern was signifficantly different from *A. cruentus.*

The results of phylogenetic studies have caused some controversy. Zheleznov et al. (1997) examined phylogenetic relationships for two grain amaranths only – *A. caudatus* and *A. paniculatus* syn. *A. cruentus*. The question of species relations is still

open. We were interested in describing of phylogenetic relationships between all three grain amaranths. We would like to verify the opinion of Gudu & Gupta (1988,1991) that *A. hypochondriacus* and *A. caudatus* were genetically closely related. If it was true, we would have additonal evidence in favour of the hypothesis of single origin of grain amaranths.

There are some works on the characterisation and composition of total proteins of seeds, especially globulin fractions (Fairbanks et al., 1990, Gorinstein et al., 1991 b, Barba de la Rosa et al., 1992, Segura-Nieto et al., 1992, Paredes-Lopez, 1994; Zheleznov et al., 1997). Most amaranth protein is composed of a globulin fraction that shares approximate weight, size and apparent multimeric dissociation characteristics with both of the two major legume proteins (Paredes-Lopez, 1994).

According to solubility amaranth proteins are divided into four classes and albumins plus globulins accounting for about 52–83% of the total proteins.

Gorinstein et al. (1991a) examined SDS-PAGE patterns of alcohol-soluble and total proteins of *A. cruentus, A. flavus, A. caudatus* and *A. hypochondriacus* seeds. Only very slight differences were found between these species.

There are only some works about use of electrophoresis for cultivar checking (Oliveras, personal communication, 1993; Szyrmer et al., 1997). Oliveras observed some differences in the protein bands of polypeptides among cultivars of *A. hypochondriacus*. Szyrmer at al., (1997) noticed slight differences in urea-soluble protein pattern (acid PAGE) among 20 cultivars and accessions of 7 cultivated amaranth species.

The cultivated amaranths have a mixed mating system with variable levels of self- and cross-pollination. The mean outcrossing rates for single landrace populations of cultivated amaranths varied from 5.8 to 28.8% for *A. caudatus*, 3.5 to 14% for *A. hypochondriacus* and 31% for *A. cruentus* (Jain et al., 1982). Within a population, outcrossing rate of individual plant varied from complete selfing to very high outcrossing rates (Hauptli & Jain, 1985; Agong & Ayiecho, 1991). Outcrossing rates vary significantly with the genotype and environment. The estimates of outcrossing rates were based usually on the red-green seedling marker.

The main objective of the study was to establish the method of distinguishing the 7 Amaranth species and 20 cultivars by using SDS-PAGE electrophoresis of urea-soluble seed proteins, also to explain similarities between these species.

No works have been reported based on electrophoresis of storage proteins for estimation of outcrossing rates in amaranth. Another questions the author hoped to answer was whether the SDS-PAGE electrophoresis of storage protein of single seeds was suitable for estimation of the outcrossing rate. Due to a possible outcrossing, both breeding programmes and seed production requirements for grain amaranth are similar to those of other cross-pollinated small seeded crops. Nevertheless, by developing a new method to estimate the outcrossing rate on the basis seed protein pattern we would acquire an additional tool to choose optimal procedures for the selection and seed production of amaranth.

Materials and methods

Plant material

Seeds were obtained from the Collection of Amaranthus at the Warsaw Agricultural University, Poland. Species and cultivars used were listed in Table 1.

The field experiments

The Amaranth plants of cv. A10 *(A. caudatus)* were cultivated on the experimental field in Warsaw, on 8 plots of 2 m^2. The plants were spaced at 6–10 cm within two rows and 30 cm between rows. Each plot was surrounded by four 2 m^2 plots of *A. cruentus* cultivars Suvarna and MT3 (two plots of each cultivar). Plot spacing was 50 cm. Two rows of plants (25 m in length, spacing design of plants and rows same as mentioned above) cv. C6 (*A.caudatus)* and unknown cultivar of *A. hypochondriacus* were grown along the both sides of the experimental field. Two experiments were conducted simultaneously. In one experiment, inflorescences of 4 plots of the cv. A10 were covered with a cellophane sheet to prevent cross-pollination (‘pure line’); in the other one flowers of 4 plots of the cv. A10 were not covered. Seeds were harvested from 200 flowers (20 flowers /mature plant), in each plot. The outcrossing rate was calculated by detecting distinct protein patterns in 100 seeds (‘open-pollinated’) in comparison with the pattern of 100 single seeds of cv. A10 (‘isolated’).

The extraction of proteins

The extraction was performed in 1.5 ml plastic centrifuge tubes. Total protein extraction solution was: 2.6

Table 1. Samples of amaranthus used for electrophoretical examination

Sample number	Species and cultivar	Origin	Colour of seeds
1	*A. tricolor* L. cv. *gangeticus*	unknown	black
2	*A. tricolor* L. cv. Warzywny	Thailand	black
3	*A. tricolor* L. cv..Brasilia	Brazil	black
4	*A. tricolor* L. cv. A–29	India	pale yellow 'cream-coloured'
5	*A. mantegazzianus* Passerini	unknown	yellow-brown
6	*A. cruentus* L. cv. K-91	Venezuela	pale yellow 'cream-coloured'
7	*A. cruentus* L. cv. K-436	USA	pale yellow 'cream-coloured'
8	*A. cruentus* L. cv. Suvarna	India	pale yellow 'cream-coloured'
9	*A. cruentus* L. cv. MT3	USA	pale yellow 'cream-coloured'
10	*A. cruentus* L. cv. R104	Thailand	yellow, rarely light brown
11	*A.cruentus* L. cv. Don Armando	Argentina	pale yellow 'cream-coloured'
12	*A. caudatus* L. cv. A11	China	pale yellow 'cream-coloured', rarely brown
13	*A. caudatus* L. cv. C6	China	pale yellow 'cream-coloured'
14	*A. caudatus* L. cv. A10	China	pale yellow 'cream-coloured'
15	*A. caudatus* L. cv. Ozdobny	Poland?	deep-red brown
16	*A. hypochondriacus* L. cv. unknown	Chile	pale yellow 'cream-coloured'
17	*A. hypochondriacus* L. cv. Annapurna	India	pale yellow 'cream-coloured'
18	*A. hypochondriacus* L. × *A. hybridum* L. cv. K343	USA	pale yellow 'cream-coloured'
19	*A. hypochondriacus* L. × *A. hybridum* L. cv. K432	USA	pale yellow 'cream-coloured'
20	*A. paniculatus* L. cv. unknown	unknown	usually black, sometimes strong brown

M urea containing 5% DTT, 0.22 M SDS and bromophenol blue (0.02%). 1. Extraction of proteins from single seeds was made in 30μl of the extraction solution 2. Extraction from bulked samples of particular varieties was made in 100μl. 3. Extraction from 'special bulked samples' (mixture of cultivars combined in one sample, for each species separately) was made in 50μl/10 seeds for each cultivar.

Tubes were allowed to stand overnight at room temperature. Samples were mixed using a vortex mixer and heated in a boiling water bath either for 5 min. (for extraction no. 2 and 3) or 3 min. (for extraction no. 1). After cooling, the tubes were either centrifuged at 18000 × g for 15 min. (extraction no. 2 and 3) or not centrifuged (from extraction no. 1).

Electrophoresis

Electrophoresis was performed with the Hoeffer SE 600 vertical unit. The gel preparation (15% polyacrylamide, resolving gel, 5% polyacrylamide, stacking gel, 180 × 160 × 0.75 mm in size) and SDS-PAGE was carried out according to the ISTA Standard Reference Method for verification of *Pisum* and *Lolium* (International Rules for Seed Testing, Annexes 1996), with some minor changes: sample size was 25μl, the run was carried out at 20 mA per gel until the tracking dye migrated through the stacking gel and then 30 mA per gel until the bromophenol blue moved at the bottom of the gel (run was about 3 hours). The gels were stained with a solution of 0,02% (w/v) Coomassie Brillant Blue (CBB) R250 (60 g TCA, 200 ml methanol, 70 ml acetic acid, 25 ml of concentrated solution CBB (1 g /100 ml methanol), and 800 ml of water). Destaining was with 0.5% detergent solution. Gels were soaked in solution: 150 ml of 96% ethanol and 3 ml of glycerol, then dried and stored between cellophane sheets.

Results and discussion

It was established that amaranth seed proteins are heterogeneous. The protein pattern obtained from 'special bulked samples' of particular amaranth species (mixture of varieties) consists of 58–68 bands (Figure 1), that obtained from bulked samples has 46–54 bands (Figure 2), and from single seeds of cv. A10-had 45–46 bands (Figure 3). Our data are in agreement with those of Fairbanks et al., (1990), Paredes-López

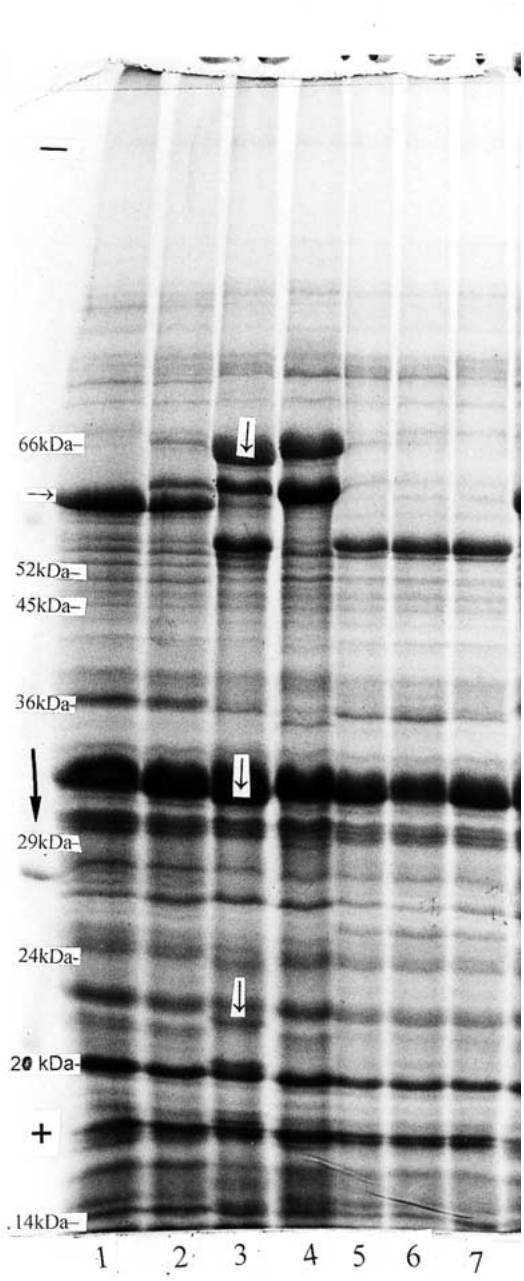

Figure 1. The SDS-PAGE electrophoresis of urea-soluble seed proteins from the mixture of genotypes/cultivars *A. cruentus* (1), *A. caudatus* (2), *A. tricolor* (3), *A. mantegazzianus* (4), *A. hypochondriacus* (5), *A. hypochondriacus* × *A. hybridum* (6) and *A. paniculatus* (7). The horizontal arrow indicates the marker band about 56 kDa which is characteristic of *A. cruentus* and *A. caudatus* species. Three vertical arrows indicate the prominent, marker bands of *A. tricolor*. Also shown are the positions and molecular weights (kDa) of reference marker proteins. The arrow on the left side of electrophoregram indicates the direction of protein migration. Abbreviations: kDa: Molecular weight of protein subunit in kilodaltons.

(1994) and Zheleznov (1997) in that amaranthus seed protein is multicomponent containing several different groups of subunits and polypeptides, thus it is characterised by essential molecular heterogeneity.

The most significant interspecific variation in seed protein pattern was noted in zone between 36 and 66 kDa (Figure 1). By their protein patterns 'special bulked samples' of particular species were classified into three groups: 1- *A. caudatus* and *A. cruentus*, 2- *A. tricolor* and *A. mantegazzianus* and 3- *A. hypochondriacus, A. hypochondriacus* × *A. hybridus* and *A. paniculatus*. The two species *A. caudatus* and *A. cruentus* (classified as the group 1) posses unique marker band at about 56 kDa (see the horizontal arrow in Figure 1). The protein pattern of *A. tricolor* is also distinguished by presence of three prominent marker bands (see three vertical arrows in Figure 1).

All the species examined can be distinguished from each other. We noticed that protein patterns of species were more similar within the group than between groups. *A. tricolor* (leafy amaranthus) is clearly different from other species. Our results confirmed the suggestion of Jain et al. (1980) that grain and leafy types of cultivated amaranths (from India) could be easily distinguishable as two genetic groups on their fixed allozyme alleles.

Our data comply with Sauer's single origin hypothesis (1967), that *A. cruentus* moved from Central America to the south and a new species, *A. caudatus* was produced by continuous selection *A. ceuntensis.* The electrophoretic pattern of *A. caudatus* was similar with *A. cruentus* (both species belong to group 1, as mentioned above). This seems to indicate similarity between these species. On the other hand, our data are not consistent with Sauer's hypothesis that also *A. hypochondriacus* arose from *A. cruentus,* or with the opinion of Gudu & Gupta (1988,1991) that *A. caudatus* and *A. hypochondriacus* were closely related, since *A. hypochondriacus* was significantly different from *A. cruentus* and *A. caudatus,* and it was asssigned to the group 3. Fairbanks at al. (1990) reported that the SDS-PAGE pattern of total seed proteins (extractable in denaturing-reducing Tris buffer pH 8,1) rarely helped to distinguish between *A. cruentus* and *A. hypochondriacus* (4 out of 38 cases): four *A. hypochondriacus* accessions possess a marker band of 52 kDa. On the other hand, Gorinstein et al. (1991) reported that two different electrophoretical patterns were recognized from SDS-PAGE of alcohol-soluble proteins of *A. cruentus* (from Brazil and Mexico), but both are different from *A. hypochondriacus.*

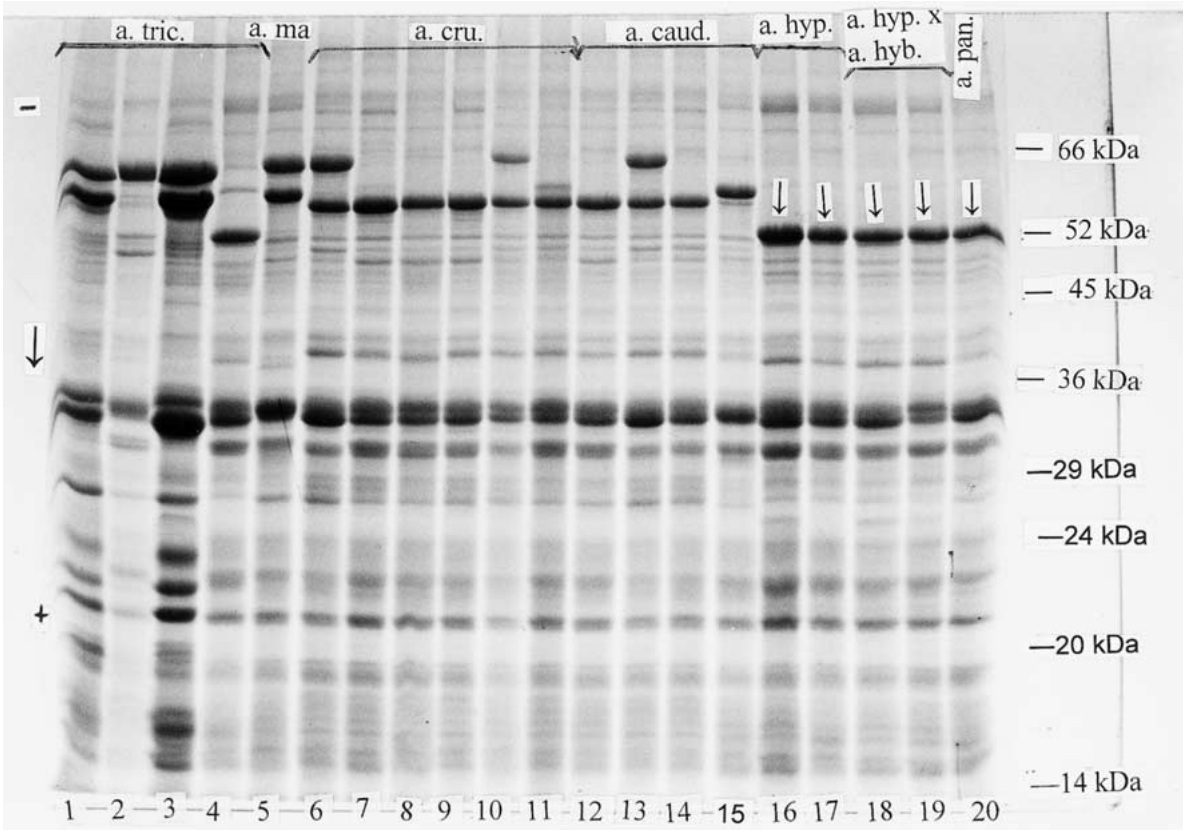

Figure 2. The SDS-PAGE electrophoresis of urea-soluble seed proteins from bulk samples of the cultivars of *A. tricolor* (a. tric., lanes 1 to 4), *A. mantegazzianus* (a. ma, lane 5), *A. cruentus (a.cru.*, lanes 6 to 11 *)*, *A. caudatus* (a. caud., lanes 12 to 15), *A. hypochondriacus* (a. hyp., lanes 16 and 17), *A. hypochondriacus* x *A. hybridum* (a. hyp. × a. hyb., lanes 18 and 19) and *A. paniculatus* (a. pan., lane 20). (L to R), lanes 1 to 20 are shown according to Table 1 (number of sample = number of lane). Also shown are the positions and molecular weights (kDa) of reference marker proteins. The arrow on the left side of the electrophoregram indicates the direction of protein migration. Abbreviations: kDa: Molecular weight of protein subunit in kilodaltons.

It is worth emphasizing that the same marker band of 52 kDa (see vertical arrows on Figure 2) was found in an unknown cv. from Chile (grain type) and cv. Annapurna from India, both belonging to *A. hypochondriacus*. We analysed only two cultivars of *A. hypochondriacus*, which were clearly distinguishable from *A. cruentus* and *A. caudatus* cultivars. It seems that by protein pattern does not settle the controversy about phylogenetic relations among all three grain amaranths: *A. caudatus, A. cruentus* and *A. hypochondriacus.* Our data suggest that further work is necessary toward a molecular approach (DNA based molecular markers) to solve controversial problems regarding the genus.

Our data only partially supported the hypothesis that three grain amaranths originated from one progenitor (*A. hybridus*), but it must be added that the results of crosses among the three grain amaranths failed to support conclusively neither the hypothesis of a single origin of grain amaranths or the hypothesis of an independent evolution of each species from its own progenitor (Pal & Khoshoo, 1974; Jain et al., 1986; Gudu & Gupta, 1991).

We noticed that by protein pattern *A. cruentus* and *A. paniculatus* belong to different groups, so we cannot agree with Sauer (1950) and Pal et al. (1982) claiming that *A. cruentus* and *A. paniculatus* are closely related species or even synonyms. They should be considered as separate species. Neither can we accept Sauer's suggestion that *A. caudatus* and *A. mantegazzianus* are synonyms. According to the protein pattern the latter is closely related to *A. tricolor* (Figure1). Our results confirmed the opinion of Coon (1982) that *A. mantegazzianus* (or *A. edulis)* isn't a separate cultivated grain amaranth since this species

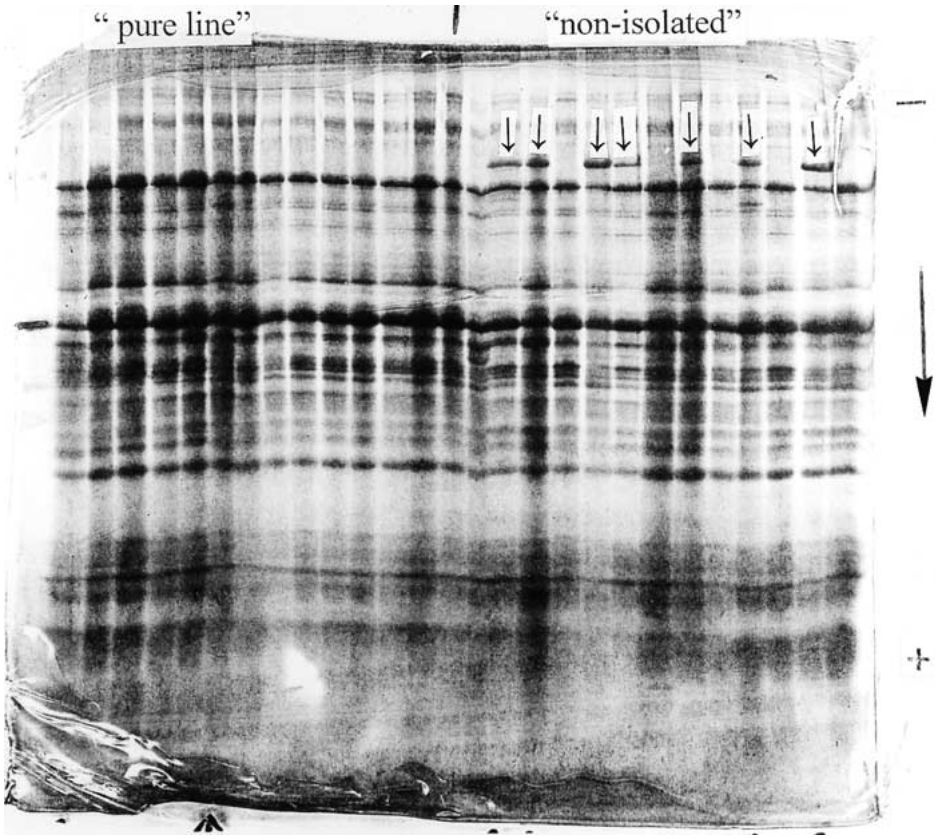

Figure 3. The SDS-PAGE electrophoresis of urea-soluble seed proteins from single seeds of cultivar A. 10 (*A. caudatus).* The 'pure line'-separation of proteins extracted from plants with the inflorescences which were covered by cellophane sheets, 'non-isolated' – separation of proteins extracted from plants which were not covered. Outcrosses are marked with the arrows. The arrow on the right side of the electrophoregram indicates the direction of protein migration.

by electrophoretic pattern was significantly different from cultivated grain amaranths.

Differences observed between cultivars, especially cultivars of cultivated grain amaranths, were very small. Nevertheless, SDS-PAGE electrophoresis was sufficient for distinguishing all cultivars, including closely related ones. The protein pattern of the following pairs of cultivars was very simillar: A11 and A10 (*A. caudatus*, both cultivars from China), cv. Suvarna (India) and cv. MT3 (USA) (both belong to *A. cruentus*). Five varieties exhibited very simillar protein pattern with a prominent, specific, very intensive band of 52 kDa (see vertical arrows in Figure 2): unknown cv. from Chile (grain type) and cv. Annapurna from India, both of *A. hypochondriacus*, cv. K 343 and K 432 from USA, (both of *A. hypochondriacus* × *A. hybridum*), *A. paniculatus*.

All seeds from isolated flowers showed the same protein pattern (it is described in Figure 3 as a 'pure line'), contrary to those with flowers exposed to open-pollination (it is described in figure 3 as a 'non-isolated"; outcrosses were marked with the arrows).

Interspecific and intraspecific outcrossing involving cv. A10 *(A.caudatus*) and other cultivars (Suvarna and MT3, both of *A. cruentus*, C6 of *A. caudatus*, cv. unknown of *A. hypochondriacus*) was a mean of 30%.

As a rule, in cross-pollinated species, individual seeds show storage protein differing from each other, and bulk seed samples are analysed. By analysing bulk samples cultivar identity can be confirmed. The result of this kind of electrophoresis test cannot be reported as percentage of 'true' seeds ('cultivar purity'). In our experiment the estimation of outcrossing rate, in other words 'cultivar purity' of a cultivar was possible only when the estimation was based on comparison of patterns of 'pure line' with the patterns of 'non-isolated' flowers.

We proved that the SDS-PAGE electrophoresis of proteins extracted even from such small dispersal units

as amaranthus seeds can be successful. In our opinion this method can also be proposed for studies of the level of genetic variability in cultivars of amaranths.

The method suggested hereby may be useful for breeders; estimation of outcrossing rate can be faster, and thus cost-effective (no planting). The genus Amaranthus is well known for its ability to form interspecific hybrids. In particular, many weedy amaranth species can cross with cultivated grain amaranth (vide cultivars K 343, K 432 and Plainsman, already registered in the USA, all of *A. hypochondriacus* × *A. hybridum*). Nowadays this is accomplished with the help of conventional methods, one of which is hybridization. We suggest examination of the electrophoretical spectrum of the proteins of hybrid forms to prove the presence of genetic material from the wild relatives in the hybrid spectrum and its localization in particular chromosomes.

Further efforts to describe all the grain and weedy amaranths based on biochemical and molecular characteristics are needed to clarify the species classification. Also estimation of outcrossing rate should be performed on the wider range of genetic diversity of amaranths species and cultivars.

Conclusions

The SDS-PAGE of urea-soluble seed proteins is suitable for distinguishing both species and cultivars of Amaranth. Samples of the seven species examined were divided into three groups. By protein patterns *A. tricolor* (leafy type of amaranths) clearly differs from other species. The present study suggest a closer similarity between *A. caudatus* and *A. cruentus* species than between the pairs of species *A. hypochondriacus* / *A. caudatus* and *A. hypochondriacus* / *A. cruentus.* Only slight differences were seen between cultivars, especially of grain amaranths. An evaluation of crossing rate on the basis of electrophoregrams of urea-soluble proteins, which were extracted from singular seeds, is proposed.

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