BIOLOGIA PLANTARUM 43 (2): 291-295, 2000

BRIEF COMMUNICATION

# Seed storage proteins in *Capsicum annuum* cultivars

R. VLADOVA, R. PANDEVA and K. PETCOLICHEVA

*Acad. D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

## Abstract

Electrophoretic analyses of non-reduced and reduced seed storage proteins from 34 cultivars of *Capsicum annuum* L. were performed on two gel systems. On the basis of clearly expressed difference in electrophoretic profiles of non-reduced proteins, the investigated cultivars were divided into two groups. The two observed phenotypes were genetically determined and related with intermediary subunits of 11S globulins and/or their oligomers.

*Additional key words*: acidic and basic polypeptides, electrophoretic spectra, intermediary 11S subunits, oligomers, pepper.

The electrophoretic profiles of seed proteins are widely used as a valuable tool for cultivar identification of breeding plants (Cooke 1984, 1995). The 7S, vicilin-like, and 11S, legumin-like globulins are the two main seed storage proteins in dicotyledonous plants. 7S globulins (molecular mass about 150 kDa) consist of three identical or not identical subunits joined via weak interactions. 11S globulins (molecular mass about 360 kDa) are built up of 6 subunits (so-called intermediary subunits). Each subunit has an acidic (40 kDa) and a basic (20 kDa) polypeptide joined by disulfide bridges and hydrophobic bonds (Shewry *et al.* 1995).

Five cultivated species of *Capsicum* are currently recognized: *C. annuum, C. chinense, C. frutescens, C. pubescens,* and *C. baccatum*; *C. annuum* is the most widely grown today. Some phylogenetic relationships in this genus were clarified on the basis of electrophoretic analysis of the soluble protein fractions (Panda *et al.* 1986). The polymorphism of *Capsicum annuum* seed proteins was studied by two-dimensional electrophoresis (Posh *et al.* 1994). In previous experiments, some peculiarities of seed storage proteins in *Capsicum* species and cultivars were revealed (Vladova and Pandeva 1994). In the present paper, seed storage proteins were identified by electrophoretic spectra and used for characterization of *Capsicum annuum* cultivars.

Seeds of 34 pepper (*Capsicum annuum* L.) cultivars and 9 $F_1$ hybrids (Table 1) reproduced in the Institute of Genetics, Sofia, were used. For protein extraction, seeds (0.01 g) were crushed into fine powder and mixed with 1 $cm^3$ of extraction medium: either 7 M urea dissolved in water:glycerol (2:1, v/v), or 0.05 M Tris-HCl buffer, pH 8.0, containing 0.2 % sodium dodecyl sulphate (SDS) and 5 M urea. The slurries were vortexed periodically and after 2 h at room temperature centrifuged in an *Eppendorf 5402* centrifuge at 15 800 *g* at 4 °C for 30 min. The clear supernatants (10 $mm^3$ per well) were used for electrophoretic analysis. Reducing conditions were achieved by addition of 2-mercapto-ethanol (2-ME), to the protein extracts. Two gel systems for electrophoretic analysis were used: *1*) 7.5 % acidic polyacrylamide gel, pH 4.3 (Reisfield *et al.* 1962), and *2*) 12.5 % SDS-PAGE, pH 8.8, according to Laemmli (1970). Both gels contained 5 M urea (Vladova and Pandeva 1994, Vladova *et al.* 1989). The proteins were stained with Coomassie Brilliant Blue R-250. Home made, dual gel unit for vertical slab gel electrophoresis (gel size 70/90/1 mm and 12 wells) was used. The following proteins were used

*Received* 18 February 1999, *accepted* 19 August 1999.

*Abbreviations*: SDS-PAGE - sodium dodecyl sulfate polyacrylamide gel electrophoresis; 2-ME - 2-mercaptoethanol; Mmm - molecular mass markers.

*Acknowledgements*: The financial support of the NSF of Bulgaria, Grants Nos. 509/95 and 510/95, is appreciated.

Fax: (+359) 2 757087, e-mail: toshev@chem.uni-sofia.bg

as molecular mass markers: myosin (205.0 kDa), galactosidase (116.0 kDa), phosphorylase b (97.0 kDa), fructose-6-phosphate kinase (84.0 kDa), albumin (66.0 kDa), glutamic dehydrogenase (55.0 kDa), ovalbumin (45.0 kDa), glyceraldehyde-3-phosphate dehydrogenase (36.0 kDa), carbonic anhydrase (29.0 kDa), tripsinogen (24.0 kDa), trypsin inhibitor (20.0 kDa) and α-lact-albumin (14.2 kDa).

Electrophoretic protein spectra of the investigated cultivars were rather uniform with respect to the slowly moving components. That is why the spectra of only 12 representative cultivars are shown (Fig. 1*A*,*B*). When 7 M urea was used as extraction medium all studied cultivars could be divided into two groups: phenotype *a* containing two slowly moving proteins and phenotype *b*, containing three slowly moving proteins (Fig. 1, Table 1). With urea at lower concentrations (up to 5M), components slowly moving to the cathode were missing in the gel (data not shown). At high concentrations (7 M and above) urea disrupted secondary bonds without disulfide bridges. So it could be assumed that more slowly moving components were storage proteins: subunit of 7S globulins or/and intermediary subunits of 11S fraction.

After reduction of protein extracts with 2-ME, the slowly moving components disappeared and other components, moving fast to the cathode, appeared (Fig. 1). The differences between two pepper phenotypes were in the zone of the fast moving components. These results imply that the two phenotypes are determined by polypeptides of 11S globulins.

In order to check whether the two phenotypes are genetically determined appropriate crosses between cultivars have been performed and $F_1$ hybrids obtained were investigated (Table 1). It was found that all $F_1$ hybrids contain three slowly moving proteins in their electrophoretic spectra (Fig. 3C). This result is in accordance with the hypothesis that the additional protein component (in phenotype *b*) is dominant inheritable genetic trait.

Table 1. List of *C. annuum* cultivars and hybrids used in the present study and distribution of the *a* and *b* phenotypes amongst them.

| Design. | Cultivar | Phen. | Design. | Cultivar | Phen. | $F_1$ hybrids | Phen. |
|---|---|---|---|---|---|---|---|
| 72 | Royal Purple | *a* | 93 | Gorogled | *a* | 83×84 | *b* |
| 73 | Fiesta | *b* | 94 | Vibo | *b* | 84×83 | *b* |
| 74 | Minito | *a* | 105 | Mulato | *b* | 84×86 | *b* |
| 76 | Chorbadjiiski | *a* | 106 | Pazardjishki Edar | *a* | 84×90 | *b* |
| 78 | Kozi rog | *a* | 110 | Nelyut Chorbadjiiski | *a* | 87×88 | *b* |
| 79 | Djoulyunska Shipka | *a* | 111 | Julta Shipka | *b* | 87×89 | *b* |
| 80 | Bjala Shipka | *a* | 115 | Feherozon | *a* | 90×89 | *b* |
| 81 | Nigrum | *a* | 117 | Feherozon synthetic | *a* | 105×94 | *b* |
| 82 | Zulu | *a* | 121 | Taltos | *b* | RLO×94 | *b* |
| 83 | Sivriya | *a* | 144 | 144 | *b* | | |
| 84 | Zlaten Medal | *b* | 200 | 200 | *a* | | |
| 85 | Albena | *b* | SK | Selska Kapiya | *a* | | |
| 86 | Pazardjishka Kapiya | *a* | OK | Oranjeva Kapiya | *a* | | |
| 87 | Kourtovska Kapiya | *a* | Ch | Chereshki | *a* | | |
| 88 | Bjala Kapiya | *b* | N Sh | Nelyuta Shipka | *a* | | |
| 89 | Sofiiska Kapiya | *b* | Cay | Cayenne | *b* | | |
| 90 | Kalinkov | *a* | RLO | Rouge long ordinaire | *b* | | |

Taking into account the subunit structure of 11S globulins, SDS-PAGE analyses were carried out to elucidate the reason for the observed differences in the electrophoretic spectra of investigated cultivars. Seed storage proteins of 14 pepper cultivars were dissociated by 2-ME into their constituent units (subunits of 7S globulins, acidic and basic polypeptides of 11S fraction), and subjected to electrophoretic separation (Fig. 2*A*,*B*). The protein components were divided into three groups according to their molecular masses (Fig. 2). Using electrophoretic spectrum of soybean as a standard and molecular mass markers (Vladova *et al.* 1997), constituent units of pepper storage proteins were identified along the gel. Two slowly moving components are subunits of 7S globulins, components from the next two zones belong to the acidic and basic polypeptides of 11S fraction. Electrophoretic spectra of investigated cultivars were similar in the zone of 7S subunits and varied in the zones of acidic and basic polypeptides. However, it is impossible to connect the two phenotypes with any clearly manifested peculiarity of the protein profiles.

Seven protein components were observed in the electrophoretic spectra of investigated cultivars when 2-ME was omitted in the protein extracts (Fig. 3*A*,*B*). Taking into account their molecular masses they could be

referred to the following protein classes, considering from the most fast moving proteins: subunits of 7S globulins (components 7, 6) and intermediary subunits of 11S fraction (components 5,4). The most slowly moving components (3,2,1) might be considered as oligomeric 11S intermediary subunits which are either linked by intermolecular disulfide bridges or not fully accessible to SDS and urea when 2-ME is omitted in the protein extracts. Similar findings were reported for legumin subunits from castor bean (Gifford and Bewlly 1984) and from incense cedar (Hager and Dank 1996). In two phenotypes *a* and *b*, electrophoretic spectra showed some differences: molecular mass of the second protein component (considering from the upper edge of the gel) in cultivars of phenotype *a* was about 94 kDa while in cultivars of phenotype *b* was about 84 kDa (Fig. 3*A*). $F_1$ hybrids contain the both components in their protein spectra (Fig. 3*B*).

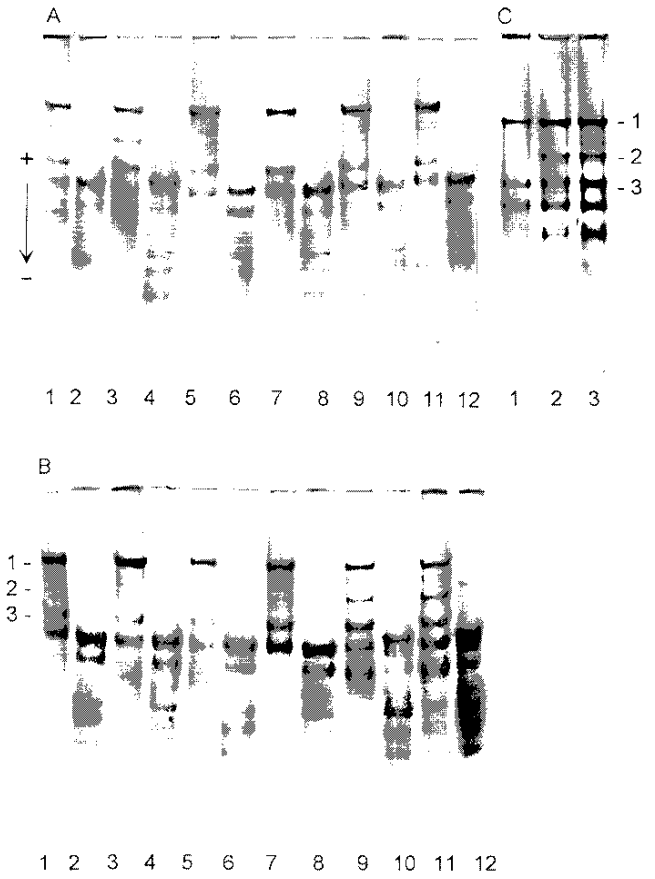


Fig. 1. Electrophoretic spectra (7.5 % acidic PAGE containing 5 M urea) of seed proteins from *C. annuum* cultivars. *A*, *B*: odd numbers - without 2-ME, even numbers - with 2-ME; *A*: *1,2* - 72, *3,4* - 73, *5,6* - 74, *7,8* - 76, *9,10* - 78, *11,12* - 79; *B*: *1,2* - 80, *3,4* - 81, *5,6* - 82, *7,8* - 83, *9,10* - 84, *11,12* - 85; *C* - without 2-ME: *1* - 87, *2* - 88, *3* - $F_1$ hybrid 87×88.

It could be assumed that this difference in the electrophoretic spectra of non-reduced proteins reflects the changes in content of intermediary subunits of 11S globulins or/and their oligomers. After reduction of proteins, in acidic gels where the resolution of components is mainly according to their isoelectric points, the two groups (phenotype *a* and phenotype *b*) maintain their composition, while in SDS-PAGE where the resolution is based on molecular masses, the two groups are not differentiated. Probably the two phenotypes are determined by at least two allelic polypeptides which are distinguished mostly in their isoelectric points. Electrophoretic analysis of $F_1$ hybrids from crosses between pepper cultivars confirms the genetic nature of the observed phenotypes. Thus for the parents with phenotype *a* for the females, and phenotype *b* for the males a possible practical application of the observations described would be the assessment of the $F_1$ hybrid purity.

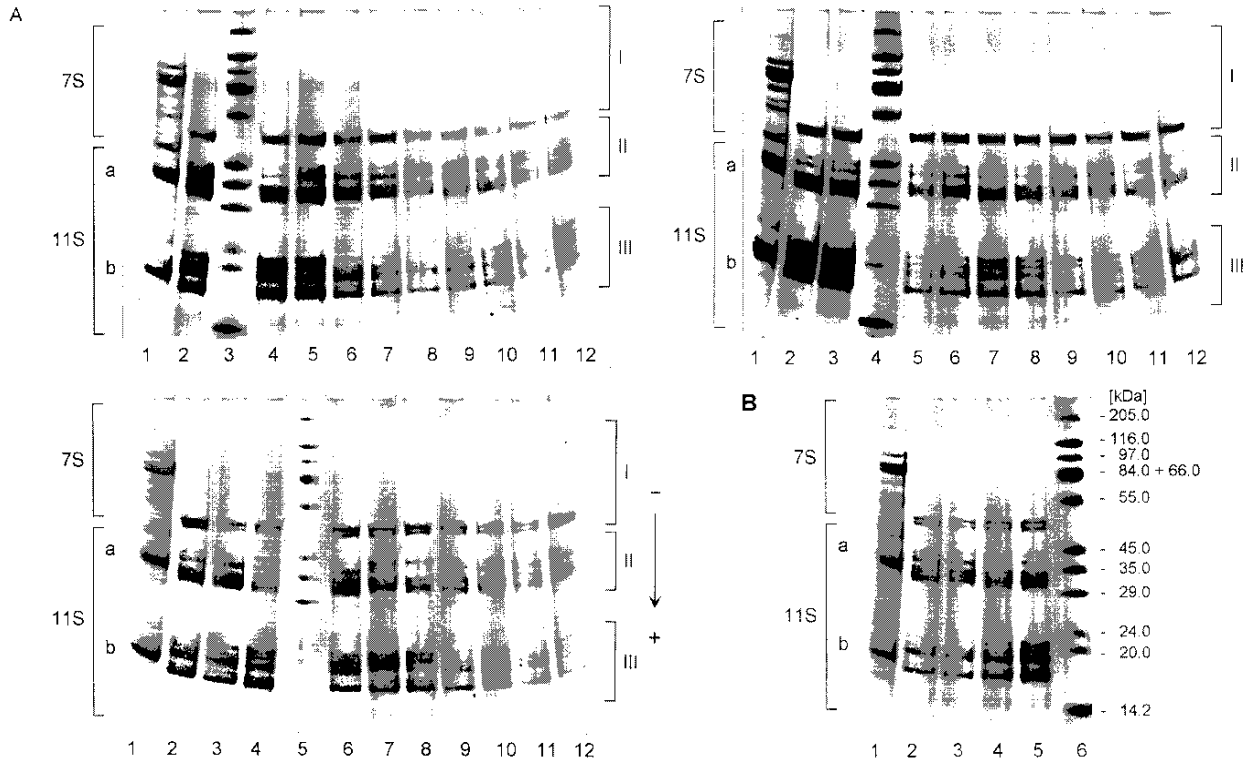


Fig. 2. Electrophoretic spectra of dissociated seed storage proteins (12.5 % SDS-PAGE containing 5 M urea and 2-ME) from soybean, used as a standard, and from *C. annuum* cultivars. 7S - vicilin-like proteins (*zone I*), 11S - legumin-like proteins: *a* - acidic polypeptides (*zone II*) and *b*-basic polypeptides (*zone III*) . *A*: *1*- soybean, *2*- 72, *3* - Mmm, *4* - 73, *5*- 74, *6* - 76, *7* - 78, *8*- 79, *9* - 80, *10* - 81, *11* - 82, *12* - 83; *B*: *1* - soybean, *2* - Ch, *3* - N Sh, *4* - Cay, *5* - RLO, *6* - Mmm.

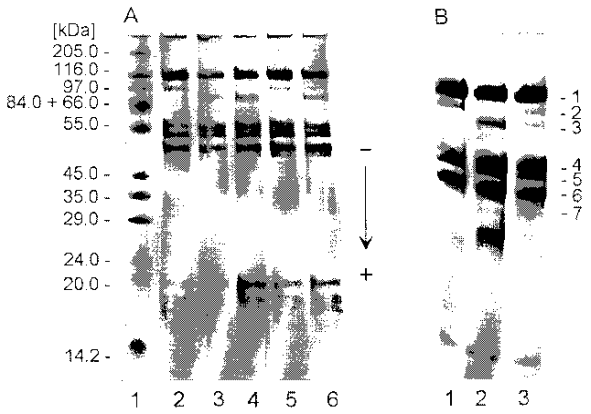


Fig. 3. Electrophoretic specra of seed storage proteins (12.5 % SDS-PAGE containing 5 M urea; without 2-ME) from *C. annuum* L. cultivars. *A*: *1* - Mmm, *2* - 87, *3* - 88, *4* - 89, *5* - 90, *6* - 94; *B*: 1 - 87, *2* - 88, *3* - $F_1$ hybrid 87×88.

## References


Cooke, R.J.: The characterization and identification of crop cultivars by electrophoresis. - Electrophoresis **5**: 59-72, 1984.

Cooke, R.J.: Gel electrophoresis for the identification of plant varieties. - J. Chromatogr. **698**: 281-299, 1995.

Gifford, D.J., Bewlly, J.B.: Interactions between subunit polypeptides of the crystalloid protein complex of castor bean endosperm. - Plant Sci. Lett. **36**: 37-42, 1984.

Hager, K.P. Dank, N.: Seed storage proteins of *Cupressaceae* are homologous to legumins from angiosperms: Molecular characterization of cDNAs from incense cedar (*Calocedrus decurrens* [Torr.] Florin). - Plant Sci. **116**: 85-96, 1996.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of head of bacteriophage T4. - Nature **227**: 680 - 685, 1970.

Panda, R.C., Kumar, O.A., Rao, R.G.R: The use of seed protein electrophoresis in the study of phylogenetic relationships in chili pepper (*Capsicum* L.). - Theor. appl. Genet. **72**: 655-670, 1986.

Posch, A., Vanderberg, B.M., Duranton, C., Gorg, A.: Polymorphism of pepper (*Capsicum annuum* L.) seed proteins studied by two-dimensional electrophoresis with immobilized pH gradients. Methodical and genetics aspects. - Electrophoresis **15**: 297-304, 1994.

Reisfeld, R.A., Lewis, U.J., Williams, D.E.: Disk electrophoresis of basic proteins and peptides on polyacrylamide gels. - Nature **195**: 281-283, 1962.

Shewry, P.R., Napier, J.A., Tatham, A.S.: Seed storage proteins: structure and biosynthesis. - Plant Cell **7**: 945-956, 1995.

Vladova, R., Pandeva, R.: Electrophoretic profiles of seed proteins in the genus *Capsicum*. - *Capsicum* Eggplant Newslett. **13**: 61-64, 1994.

Vladova, R., Petcolicheva, K., Pandeva, R., Nikova, V.: Electrophoretic study of seed storage proteins in the genera *Capsicum* L. and *Nicotiana* L. - Genet. Breed. **28**: 7-12, 1996/1997.

Vladova, R., Petrova, S., Gerasimov, R., Nikolov, C.: [Electrophoretic analysis of storage proteins in soybean (*Glycine max* L. Merril) cultivars and mutant lines.] - Genet. Selekt. **22**: 106-110, 1989. [In Bulg.]