Electrophoretic patterns of reserve seed proteins with reference to the taxonomic comparison of bean cultivars

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Abstract

The seed albumins and seed globulins of five Polish cultivars of *Phaseolus vulgaris* and one cultivar of *Phaseolus multiflorus* were electrophoretically separated in polyacrylamide gel using the disc-technique of Orstein and Davis. To compare any two cultivars, the percentage of similarity coefficient (S) was calculated based on the R_p values of protein bands. It was found that the electrophoretic mobility of the bean globulins is in general lower than albumins. Cultivar 'Piękny Jaś', belonging to *Phaseolus multiflorus* species showed large qualitative and quantitative differences in the albumin and globulin patterns as compared to other cultivars belonging to *Phaseolus vulgaris* species.

INTRODUCTION

Cultivar differences have been observed in the electrophoretic patterns of storage proteins of beans (Adriaanse et al., 1969; Sasek, 1974; 1976). Dunnill and Fowden (1965) have pointed out that seed composition is unlikely to be affected by variation in nutritional or environmental factors during growth. This is in agreement with results of Adriaanse et al. (1969) who showed that the protein pattern of *Phaseolus vulgaris* cultivar is independent of external growth conditions. Starch-gel and disc-electrophoresis in polyacrylamide gel are capable of discerning different cultivars or groups of cultivars and may find application in certain genetic and breeding aims (Sasek, 1976).

The present paper reports on a protein analysis by disc-electrophoresis in polyacrylamide gel of six Polish cultivars of bean seeds with reference to their taxonomic comparison.

MATERIALS AND METHODS

Five cultivars of *Phaseolus vulgaris*: 'Bomba', 'Słowianka', 'Igołomska', 'Biała Wyborowa', 'Jubilatka' and one cultivar *Phaseolus multiflorus* — 'Piękny Jaś' were investigated.

The albumin and globulin fractions of seeds were extracted and purified according to Pant and Tulsiani (1969). Both fractions were then electrophoretically separated in 7.5% polyacrylamide gel using the disc-technique of Orstein and Davis (Sargent, 1969; Gordon, 1969). The protein samples (200 µg) in a 5% sucrose solution were placed directly on top of the "spacer gel". Electrophoresis was run at +2°C in Tris-glycine buffer at pH = 8.3 for 1.5 h at 4.5 mA constant current per gel tube. The exact length of a run was determined with the bromophenol blue marker. The gels, after being removed from their tubes, were stained with 0.5% Amido-Black 10B in 7% acetic acid for 1 h. They were then destained by diffusion against frequent changes of 7% acetic acid. The gels were photographed and the positions of the bands after electrophoresis were drawn and expressed as Rp values, which are the relative positions compared to the bromophenol blue marker. To compare two cultivars the percentage of similarity coefficient S, was calculated (Whitney et al., 1968).

$$S = \frac{c}{c + d} \cdot 100,$$

where:

c — number of pairs of similar bands,

d — number of different bands.

Protein bands were considered to be similar and given the same Rp value when there was at least a 50% overlap. Each tube drawing was based on two replicates.

RESULTS AND DISCUSSION

Figure 1 is a drawing of the electrophoretic patterns of the albumins of cultivars of beans. The different intensities of staining were accepted on an arbitrary basis. Seven proteinic zones were discerned for all cultivars of *Phaseolus vulgaris* and 9 zones for *Phaseolus multiflorus* ('Piękny Jaś'). All of the investigated cultivars appeared to have one intense protein band in common with a mobility $R_p = 0.79-0.84$. Some cultivar differences can be observed on the electrophoregrams, especially in the zones of lower mobility of albumin bands ($R_p = 0.02-0.40$). Besides the characteristic of band mobility, staining intensity may have signifi-

cance for comparison of cultivars, but the present investigation was restricted to comparison of R_p value.

The globulin patterns differ from those of albumin (Fig. 2). Four to seven globulin bands were discerned for the tested cultivars and the 'Piękny Jaś' cultivar has most these bands.

Fig. 1. Electrophoretic patterns of bean seed albumins. Cultivars: 1 — 'Bomba', 2 — 'Słowianka', 3 — 'Igołomska', 4 — 'Piękny Jaś', 5 — 'Biała Wyborowa', 6 — 'Jubilatka'

Fig. 2. Electrophoretic patterns of bean seed globulins. For explanation see Fig. 1

The electrophoretic mobility of the globulin proteins is in general lower than that of the albumins. All of the cultivars have highly mobile and poorly visible narrow bands at Rp: 0.73 ('Bomba'), 0.75 ('Biała Wyborowa'), 0.77 ('Piękny Jaś') and 0.79 ('Słowianka' and 'Igołomska'). It can be observed (Fig. 2) that the globulin band patterns for 'Słowianka' and 'Igołomska' cultivars are almost identical.

The percentage of similarity coefficients between all investigated cultivars were calculated on the basis of the Rp values obtained for albumins (Table 1). The *S* values oscilate between 14% and 100%. No differences were observed (*S* = 100%) between the pairs of the following cultivars: 'Bomba' and 'Igołomska', 'Bomba' and 'Jubilatka', 'Igołomska', and 'Jubilatka'. High similarity was also shown by 'Bomba' — 'Słowianka' (*S* = 75%), and 'Słowianka' — 'Jubilatka' (*S* = 56%). 'Piękny Jaś' cultivar (*Phaseolus multiflorus*) differed markedly from the others in albumin patterns. This is shown by the lowest similarity coefficients 14-23% (Table 1).

Table 1

Percentage similarity coefficients of bean cultivars calculated from electrophoregrams of albumin

	'Słowianka'	'Igołomska'	'Piękny Jaś'	'Biała Wyborowa'	'Jubilatka'
'Bomba'	75	100	23	40	100
'Słowianka'		75	23	27	56
		'Igołomska'	14	44	100
			'Piękny Jaś'	23	14
				'Biała Wyborowa'	27

Heterogenity of bean seed proteins has been reported in numerous works (Adriaanse et al., 1969; Bailey and Boulter, 1970; Müntz, 1973; Sasek, 1974; 1976). Depending on the method used, different numbers of protein zones were identified on the electrophoregrams. Qualitative differences were observed between species, while marked quantitative differences in the different protein fractions (mainly globulin in character) were observed between the cultivars (Sasek, 1976). This agrees with the present results where the 'Piękny Jaś' cultivar (*Phaseolus multiflorus*) shows large qualitative differences in albumin and globulin patterns as compared to other cultivars belonging to *Phaseolus vulgaris* species. Large cultivar differences both in protein patterns and similarity coefficients also appeared among 'Biała Wyborowa' and the remaining cultivars belonging to *Phaseolus vulgaris* species. The 'Biała Wyborowa' cultivar was bred about 20 years earlier than

the other cultivars and this may imply that some proteins are easily changed or reflect minor genetic differences.

Electrophoretic protein patterns are used to identify biotypes; discover intraspecific differentiation and analyse populations (Konarev, 1979). It should be emphasized, however, that the value of data for taxonomic comparison obtained as the result of the use of the similarity coefficient can be assessed only in relation to information obtained from other systematic disciplines.

REFERENCES

- Adriaanse A., Klop W., Robbers J. E., 1969. Characterisation of *Phaseolus vulgaris* cultivars by their electrophoretic patterns. J. Sci. Fd. Agric. 20: 647-650.
- Bailey C. L., Boulter D., 1970. The structure of legumin, a storage protein of broad bean (*Vicia faba*) seed. Eur. J. Biochem. 17: 460 - 466.
- Dunnill P., Fowden L., 1965. The amino acids of seeds of the *Cucurbitaceae*. Phytochemistry 4: 933-944.
- Gordon A. H., 1969. Electrophoresis of proteins in polyacrylamide and starch gels. North-Holland Publishing Company, Amsterdam, 34-69.
- Konarev W., G., 1979. Belki kak geneticheskie markery v izuchenii prirody i proiskhozhdeniya genomov kulturnykh rastenij. Trudy po prikladnoj botanike, genetike i selekcii. 63: 3-8.
- Müntz K., 1973. Die Funktion der Hülsenschale bei der Proteinspeicherung in den samen von *Vicia faba* L. I. Gelektrophoretischer vergleich von löslichen Proteinen aus Blättern und Hülsen. Biochem. Physiol. Pflanzen 164: 357-369.
- Pant R., Tulsiani D. R. P., 1969. Solubility, amino acid composition and biological evaluation of proteins isolated from leguminous seed. J. Agric. Food Chem. 17: 361-366.
- Sargent J. R., 1969. Methods in zone electrophoresis. BDH Chemicals Ltd. 85 - 107.
- Sasek A., 1974. Elektroforeticke deleni bilkovin hrachu, fazolu a cocky. Genetika a Slechteni 10: 253-260.
- Sasek A., 1976. Porovnani bilkovin semen nekterych druhu luskovin diskelektroforezou. Genetika a Slechteni 12: 29-37.
- Whitney P. J., Vaughan J. G., Heale J. B., 1968. A disc electrophoretic study of the proteins of *Verticillium albo-atrum*, *Verticillium dahliae* and *Fusarium oxysporum* with reference to their taxonomy. J. Exp. Bot. 19: 415-426.

Rozdział elektroforetyczny białek zapasowych nasion fasoli w odniesieniu do porównania taksonomicznego odmian

Streszczenie

Stosując technikę dyskową wg Orsteina i Davisa rozdzielano elektroforetycznie na żelu poliakrylamidowym albuminy i globuliny z nasion pięciu odmian fasoli należących do gatunku *Phaseolus vulgaris* i jednej odmiany należącej do gatunku *Phaseolus multiflorus*. W celu porównania dwóch dowolnych odmian obliczano procentowy współczynnik podobieństwa (S), w oparciu o wartości R_p dla poszczególnych frakcji białkowych. Stwierdzono, że globuliny charakteryzują się niższą ruchliwością elektroforetyczną w porównaniu do albumin. Rozdział elektroforetyczny białek albuminowych i globulinowych z nasion odmiany 'Piękny Jaś' (*Ph. multiflorus*) wykazywał duże różnice jakościowe i ilościowe w porównaniu z rozdziałem białek z nasion należących do gatunku *Ph. vulgaris*.