Diversity of Common Bean Landraces Collected in the Western and Eastern Carpatien

Oľga HORŇÁKOVÁ, Monika ZÁVODNÁ, Mária ŽÁKOVÁ, Ján KRAIC and František DEBRE

Gene Bank of the Slovak Republic, Research Institute of Plant Production, Piešťany, Slovak Republic

Abstract: The study of diversity in common bean was based on morphological and agronomical characteristics, differentiation of collected accessions by morphological and molecular markers, detection of genetic variation, and duplicates detection in bean landraces. The analysed 82 accessions of common bean (*Phaseolus vulgaris* L.) were collected in the Western and Eastern Carpatien as landrace mixtures. Their seeds were segregated and pooled according to their characteristics; they were further multiplicated, and introduced into the collection. An extensive variation in plant and seed traits was discovered in thirty-three morphological and agronomical characteristics. Nevertheless, some of the accessions were identical in these characteristics. Cluster analysis grouped genotypes into two main branches, reflecting the growth type, seed size parameters, and thousand-seed weight. Molecular differentiation studies were performed by multilocus polymorphism detection in microsatellite and minisatellite DNA regions. Cluster analysis based on molecular data also grouped genotypes but no linkage to morphological traits was revealed. Bean accessions with very similar or identical morphological characters were clearly distinguished by DNA banding patterns. The presence of duplicates was excluded.

Keywords: bean; *Phaseolus vulgaris* L.; landrace; morphological traits; microsatellite; minisatellite; polymorphism; duplicates

The common bean (*Phaseolus vulgaris* L.; $2n = 2x = 22$) is a food crop of a high nutritive value for people on five continents. Based on archaeological observations from Peru and south-western United States in the late 19th century, it was concluded that the common bean originated from the New World; two centres of origin were identified – Andean and Mesoamerican. Domestication and subsequent evolution of the common bean affected changes in morphological, physiological, and other traits. A reduction of genetic variation of cultivated beans, in comparison with wild beans, accompanied this process (Gepts & Debouck 1993). The common bean was introduced into other regions over the world. Bean is a traditional grain legume cultivated and bred also in Slovakia. Until 1949, only old original landraces (e.g. Slovenská ľadvinka biela krajová, Slovenská perlová biela krajová, Slovenská sírovožltá krajová) were cultivated here. An intensive bean breeding programme in Slovakia started from 1948 and the first result was the cultivar Kočovská biela released in 1959. More than 20 cultivars have been released by Slovakian bean breeders to the present time. Traditional landraces and old cultivars played a very important role in their effort. The collected bean germplasm maintained in the collection of genetic resources should be important for a new advanced cultivar creation in the future. An abundant and rich source of valuable genes and genetic diversity are landraces grown by small farmers and collected by collecting missions. Nevertheless, the information about their origin, pedigree, and other characteristics is usually not known or is not available and their identity and difference from the previously obtained genotypes is also questionable.

This study and other activities in plant genetic resources were supported by the Ministry of Agriculture of the Slovak Republic, Grant No. 05-514-31.

The occurrence of duplicates within the maintained collection can not be excluded either.

The knowledge about the extent of genetic diversity, identification, differentiation, and characterisation of genotypes and populations, respectively, provides an information tool for the detection of duplicates in the collection, their effective extension, a better characterisation and utilisation in breeding. The common bean is primary characterised by a variation in seed characteristics – shape, size, colour, drawing, glance, and others. The Centro International de Agricultura Tropical (CIAT) classifies beans according to 9 colour classes, seed size, and seed shape (Hidalgo 1991). Pedigree analyses and the coefficient of parentage studies were also used for the differentiation of bean accessions (Voysest *et al*. 1994). Nevertheless, these approaches are usually not as sensitive as required for the identity determination and genotype differentiation.

We expected that the collected set of the Eastern Carpatien bean landraces would possess broad variations in morphological and agronomical traits but the presence of duplicates was also expected. Therefore, the objectives of this study were: (1) to characterise the morphological and agronomical traits of bean landraces, to select pure lines from landrace mixtures, to segregate genotypes into groups with similar morphology; (2) to test the efficacy of microsatellite and minisatellite polymorphism for the differentiation of genotypes; (3) to detect duplicates within the set of bean landraces collected in the Eastern Carpatien.

MATERIAL AND METHODS

Bean (*Phaseolus vulgaris* L.) landraces (Table 1) were collected during missions from different locations of the Western (Slovakia) and the Eastern (Ukraine) Carpatien in the years 1992–1996. Most of them were collected as landrace mixtures, i.e. mixtures of seeds differing by seed size, shape, colour, and drawing. The seeds from every mixture were segregated according to these basic characteristics, pooled, and multiplicated as pure lines. Altogether 33 morphological and agronomical characteristics of plants and seeds (Table 2) were evaluated in 80 landraces and cultivar Jutta, according to *Phaseolus* L. descriptor (Horňáková *et al*. 1991). Two additional genotypes (210/97 005/1, 258/97 405/9) were included in the molecular analysis studies.

The total plant DNA was isolated according to Dellaporta *et al*. (1993) from leaves of young seedlings. DNA sample of each genotype represented DNA collected from 10–15 individual plants. Seven 15–16-oligonucleotide primers (Table 3) derived from the core sequences of microsatellites and minisatellites, respectively, were used for DNA amplification. The PCRs were performed in 20 μl volumes, and were programmed as follows: 1 min at 94°C followed by 35 cycles, each of 1 min at 94°C, 1 min at annealing temperature (each G/C = 4°C, each A/T = 2°C), 5 min at 72°C. The extension step in the last cycle was 8 min at 72°C (PTC-200 Peltier Thermal Cycler, MJ Research). Reactions contained Taq-DNA polymerase buffer, 0.25mM each of dNTPs, 1mM primer, 0.8 U Taq-DNA polymerase, and 25 ng DNA. The amplified products were separated in 1.5% agarose gels and stained with ethidium bromide.

Statistical analyses, clustering of genotypes by Ward's method, and dendrograms (UPGMA method) were performed by software SPSS 8.0.1 (SPSS, Inc.).

RESULTS

Variations in morphological and agronomical characteristics

The phenotypes identical in seed characteristics were used for the creation of pure lines from the original landrace mixtures. Due to this, the level of similarity between the pure lines within the landrace was lower than between landraces.

The original set of 33 variables (Table 2) was reduced by factor analysis to ten, indicating about 76% of the total genetic variation. The first factor attributed with 14%, the second and third with 10%, others below 10%. The first factor included the plant characteristics – growth type, growth habit, and plant height. The second factor characterised the pod – the presence of fibre, parchment coating and colour, the third factor characterised the seed – size, length, width, height, and the weight of thousand seeds. The fourth factor characterised the secondary colour and drawing of seed. The fifth one characterised the flower – the colour of vexillum and wings, the sixth one pointing of the pod. One of the factors correlates, mainly in the climbing beans, with the shape of the middle leaflet and the seed shape. The period from sowing to maturity was included

Table 1. The list of landraces and landrace mixtures and their origin

Landrace (mixture)	Pure line	Country of origin	Landrace (mixture)	Pure line	Country of origin
Maslová královna		CZE	423	294/97 423/2	SVK
KP Vrbovce		SVK	423	295/97 423/3	SVK
KP Nitra II		SVK	437	312/97 437/1	SVK
KP Nitra III		SVK	437	313/97 437/2	SVK
KP Nitra IX		SVK	452	325/97 452/2	SVK
Veličná 13 KP		SVK	452	326/97 452/3	SVK
KP Zaježová		SVK	452	327/97 452/4	SVK
KP Vrbové II		SVK	453	330/97 453/1	SVK
Oravka 9/2 KP		SVK	453	331/97 453/2	SVK
KP Turá Lúka		SVK	469	355/97 469/1	SVK
KP Šípkové I		SVK	469	356/97 469/2	SVK
KP Šípkové II		SVK	479	363/97 479/1	SVK
Gem. Jablonec 3BK		SVK	479	364/97 479/2	SVK
Gem. Jablonec 3FK		SVK	480	365/97 480/1	SVK
KP Sovinec I		SVK	480	366/97 480/2	SVK
KP Sovinec II		SVK	502	378/97 502/1	SVK
KP Kežmarok		SVK	502	379/97 502/2	SVK
KP Grnča		SVK	005	410/97 005/1	SVK
0112 H/I		SVK	005	411/97 005/2	SVK
KP Sokolovce		SVK	005	412/97 005/3	SVK
KP Stará Myjava I		SVK	011	413/97 011/1	SVK
KP Stará Myjava II		SVK	011	414/97 011/2	SVK
KP Prašník I		SVK	011	415/97 011/3	SVK
KP Prašník II		SVK	038	442/97 038/1	SVK
KP Prašník Zbehy		SVK	038	443/97 038/2	SVK
243	156/97 243/4	UKR	038	444/97 038/3	SVK
243	157/97 243/5	UKR	038	445/97 038/4	SVK
243	158/97 243/9	UKR	048	457/97 048/1	SVK
260	165/97 260/2	UKR	048	459/97 048/3	SVK
260	166/97 260/3	UKR	048	460/97 048/4	SVK
325	209/97 325/2	UKR	048	461/97 048/5	SVK
344	213/97 344/2	UKR	053	473/97 053/1	SVK
344	214/97 344/3	UKR	053	474/97 053/2	SVK
405	250/97 405/1	SVK	053	475/97 053/3	SVK
405	251/97 405/2	SVK	053	476/97 053/4	SVK
405	252/97 405/3	SVK	013	493/97 013/1	SVK
405	253/97 405/4	SVK	013	494/97 013/2	SVK
405	258/97 405/9	SVK	013	495/97 013/3	SVK
405	260/97 405/11	SVK	013	496/97 013/4	SVK
405	261/97 405/12	SVK	013	497/97 013/5	SVK
423	264/97 423/2	SVK	Jutta		GER
406	268/97 406/5	SVK			

Table 2. Morphological and agronomical traits used for bean landraces characterisation

Trait (variables)	Growth stage
Anthocyan pigmentation of hypocotyl	after in a seedling emergence
Growth type	flowering
Plant – habit	flowering
Stem – length	maturity (5 plants)
Shape of middle leaflet	flowering
Colour of triangular leaflet	flowering
Surface of middle leaflet	flowering
Inflorescence – length	flowering
Inflorescence – location	flowering
Flower – size of bracts	flowering
Flower – vexillum colour	flowering
Flower – wings colour	flowering
Pod – degree of curvature	immature pods (10 pods)
Pod – parchment coating	immature pods (10 pods)
Pod – presence of fibre	immature pods (10 pods)
Pod – ground colour (immature)	immature pods (10 pods)
Pod – pigmentation (immature)	immature pods (10 pods)
Pod – colour of pigmentation spots	immature pods (10 pods)
Pod – shape of tip	immature pods (10 pods)
Pod – wall fiber/constriction	immature pods (10 pods)
Seed – shape	mature seed (50 seeds)
Seed – ground colour	mature seed (50 seeds)
Seed – secondary colour	mature seed (50 seeds)
Seed – character of patterns	mature seed (50 seeds)
Seed – glint	mature seed (50 seeds)
Seed – hilum ring colour	mature seed (50 seeds)
Seed – length	average in mm of 10 seeds from 10 plants, measured parallel to the hilum
Seed – height	average in mm of 10 seeds from 10 plants, from hilum opposite side
Seed – width	average in mm 10 seeds from 10 plants
Seed – thousand-kernel mass	mass of 2 × 100 seeds at a moisture content of 12–14%, expressed in grams with one decimal place, as average of 800 seeds
Vegetation period	from sowing to beginning of flowering (days)
Days of flowering	from beginning of flowering to end of flowering (days)
Vegetation period	from sowing to seed maturity (days)

in the first factor. Identical statistically significant correlations ($P > 0.05$) were common for bush and climbing beans – between seed length, height, width and thousand-seed weight, and between plant height and vegetation period (days from sowing to seed maturity).

Table 3. The sequences of microsatellite and minisatellite based primers

Primer name	Primer sequence	Type of sequence	Reference
LBHB01	5'-$(ACTG)_4$-3'	microsatellite	–
LBHB02	5'-$(GACA)_4$-3'	microsatellite	–
LBHB04	5'-$(GACAGATA)_2$-3'	microsatellite	–
LBHB05	5'-$(ACAG)_4$-3'	microsatellite	–
33.6	5'-AGGGCTGGAGGAGGGC-3'	minisatellite	Jeffreys *et al*. (1985)
33.15	5'-AGAGGTGGGCAGGTGG-3'	minisatellite	Jeffreys *et al*. (1985)
M13 phage	5'-GAGGGTGGXGGXTCT-3'	minisatellite	Vassart *et al*. (1987)

Cluster analysis based on morphological and agronomical traits grouped genotypes into 2 main branches according to the growth type (bush or climbing), seed size, and thousand-seeds weight (the mean values in the subgroups ranged from 317 to 650 g). Twelve subgroups (Figure 1) can be identified in the dendrogram constructed by morphological data:

Subgroup I. Includes seven bush genotypes with a shorter than average vegetation period. Their growth habit is higher bush. Genotypes lack anthocyan colour of hypocotyl, pods are curved, seed shape is elliptic, ground colour is white and dim, seed size is bigger than the average size in all the other beans evaluated. The colour of the middle leaflet is green to dark green. They differ from the other bush genotypes by a longer period of flowering, flower colour is white to pink. All morphological and agronomical characteristics of genotypes Jutta and Oravka9/2 KP are identical.

Subgroup II. Includes 10 genotypes separated into two branches. All of them have bush habitus, seeds are big, shiny, with different colours. Pods are moderately curved. The colour of the middle leaflet is light green or green, the surface of the middle leaflet is smooth. There are genotypes with a lower bush, higher and darker seeds, and a longer vegetation period in the lower branch.

Subgroup III. This group consists of 16 genotypes in three branches.

Branch A – Lower climbing genotypes, colour of flower wings is pink to dark pink, they lack the constriction of pod, seed shape is round, seeds are bigger than average, ground colour of seed is different besides white.

Branch B – Includes bush genotypes with lower seeds and a shorter period to maturity than in subgroup A.

Branch C – Eight bush genotypes with elliptic, small, shiny, brown-yellow to black coloured seeds. Immature pod is pointed, fibre is present, with parchment coating, inflorescence is shorter and located in foliage. The colour of flower vexillum and wings is light pink to dark pink. The shape of the middle leaflet is rhomboid and oval, light green to green. Anthocyan pigmentation of hypocotyl is missing. Genotypes 364/97 479/2 and 363/97 479/1 segregated from the same landrace mixture are identical.

Subgroup IV. Includes 10 genotypes with higher bush, some of them with twisting apex. Seeds are small, round to elliptic, coloured from yellow-brown to black. The ground colour of immature pod is light green to green, lacks spots. The distortion of immature pod is mild with a pointed tip. Pairs of genotypes 251/97 405/2, 356/97 469/2 and 250/87 405/1, 264/97 406/1 are identical in their morphological traits.

Subgroup V. Includes three higher climbing genotypes with anthocyan pigmentation of hypocotyl. The middle leaflet is dark green, elliptic, wrinkled. The length of inflorescences is shorter than petiole, flowers are purple. Immature pod is light green or green, without parchment layer, with a pointed tip. The constriction of pod in maturity is medium to marked. Seeds are elliptic, medium size, basic colour is grey to black (no white), the secondary colour is purple and black. The period to maturity is average.

Subgroup VI: Three landraces – two climbing and one bush which is one of the highest among

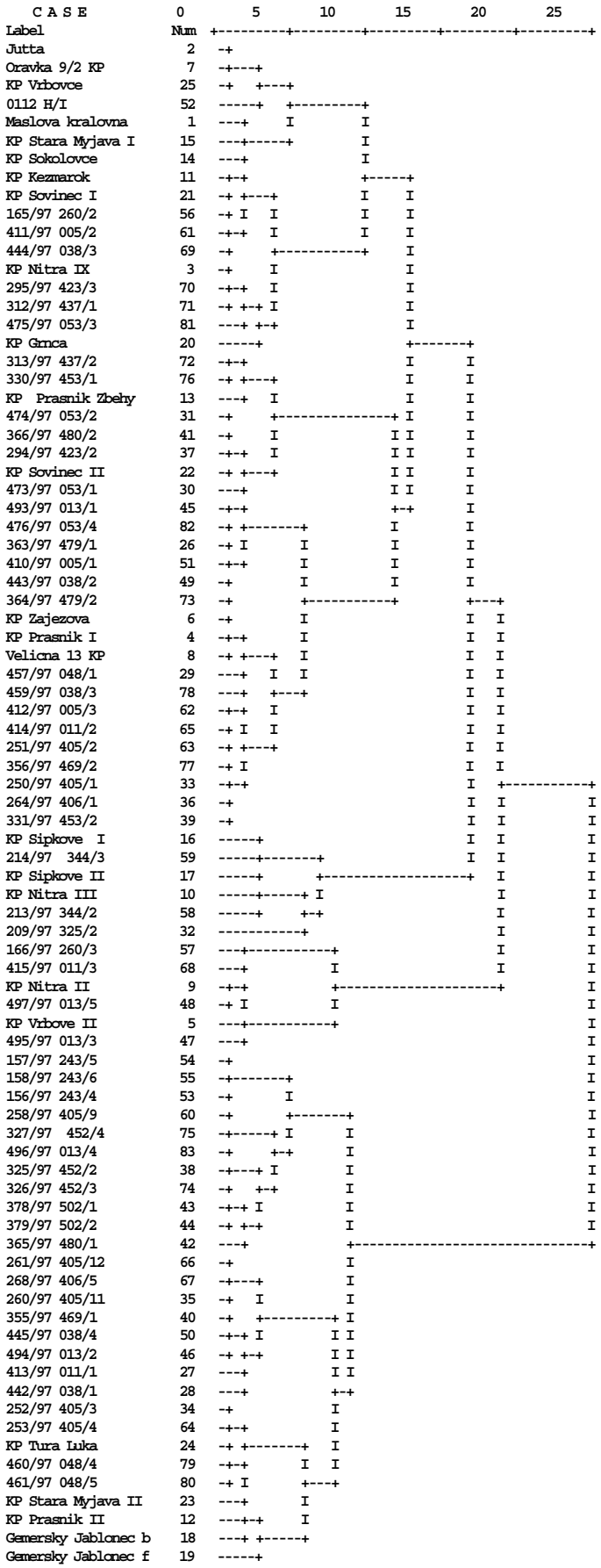

Figure 1. Discrimination of bean landraces based on morphological-agronomical characteristics

all other bush genotypes. Other traits are very similar to the climbing genotype in this group. The colour of the middle leaflet is green, smooth, inflorescence in foliage. The size of bracts is medium to high, the colour of vexillum and wings is light to dark pink. Pod is without fibre. Seed size is average, they are shiny of black colour. The time to flowering is shorter the time to maturity is longer.

Subgroup VII. Two higher bush landraces with a twisting apex, without anthocyan pigmentation of hypocotyl. Middle leaflets are elliptic, wrinkled. Inflorescence is shorter than petiole, located in foliage. The colour of vexillum and wings is pink. Seeds are of a small size, with a high shine, basic colour is grey. The colour of hilum ring is other than the colour of seed. The period to flowering and to maturity of seeds are longer.

Subgroup VIII. Four lower bush genotypes. Hypocotyls are without anthocyan pigmentation, the middle leaflet is elliptic, light green. Inflorescence is shorter, located in foliage. Flower bracts are small, the colour of vexillum and wings is light pink. The colour of pod is yellow, immature seeds are without fibre and parchment coating. The colour of mature pod is cream and yellow-pink. Seeds are smaller, colour is different. The period to maturity is short.

Subgroup IX. Contains 11 landraces divided into three branches.

Branch A – Contains climbing landraces from Ukraine. All of them are high, climbing, without anthocyan pigmentation of hypocotyl. The middle leaflet is elliptic, green, wrinkled. The length of inflorescence is shorter than the length of petiole, located in foliage. The colour of vexillum and wings is white. Pods with parchment coating, spotting, fibre is missing. The seed shape is kidney or round, the colour is white – light yellow. Branch A belongs to beans with long periods to flowering and maturity.

Branch B – Contains climbing and one bush genotypes. All of them are smaller. The middle leaflet is green, smooth. Inflorescences are

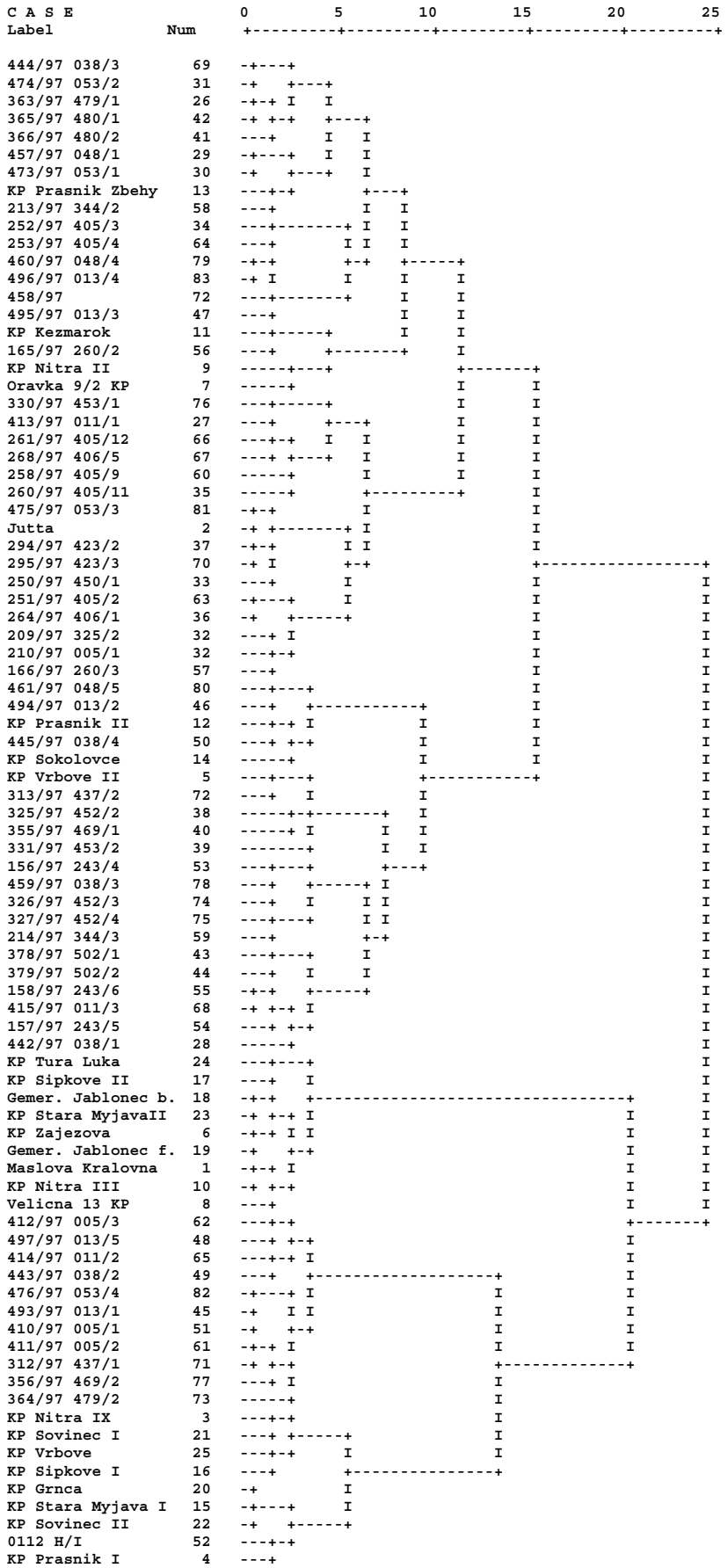

Figure 2. The dendrogram based on polymorphism in satellite DNA sequencies

partially located in foliage. The colour of vexillum and wings of flower is white to pink. Pods are of light green or green colour with parchment layer, spotting and fibre are missing. The seed shape is kidney or round, the colour is white to light yellow. The periods to flowering and maturity are long.

Branch C – Climbing, higher, without anthocyan pigmentation of hypocotyl. The middle leaflet is elliptic, of light green or green colour. Inflorescences are shorter than petiole, located in foliage. The colour of vexillum and wings is white to light pink. Immature pod is curved, light green to green, pointed, constrict in maturity. Seeds are large, colour is different. The vegetation periods from sowing to flowering and from sowing to maturity are longer.

Subgroup X. Consists of 8 climbing, shorter landraces, without anthocyan pigmentation of hypocotyl. Inflorescence is shorter than petiole, located in foliage. The colour of wings is white and the colour of vexillum is pink. Pod is of light green to green colour, pointed. Seeds are small, coloured, round to eliptic. The hilum ring colour is different than the seed colour. The periods to flowering and to maturity are longer.

Landraces 261/97 405/12 and 260/97 405/11 derived from same mixture are identical in morphological traits and can be considered as duplicates.

Subgroup XI. Six landraces, all except one are climbing. Inflorescence is shorter than petiole, located in foliage. The colour of wings is white, the colour of vexillum is white to light pink. Immature pod is light green to green, pointed. Seeds are small, differently coloured, the colour of hilum ring is different than the colour of seeds. The periods to flowering and to maturity are medium. Genotypes 460/97 048/4 and 461/97 048/5 derived from the same mixture are identical in morphological traits.

Subgroup XII. Three climbing landraces, without anthocyan pigmentation of hypocotyl. The shape of the iddle leaflet is triangular, light green to green, slightly wrinkled. Inflorescence is shorter than petiole, located in foliage. Immature pod is slightly curved, light green to green, with parchment coating, fibre is present. The size of seeds is medium, colour is varied. Periods to flowering and to maturity are longer.

Variation at the DNA level

Four from the seven primers used, based on microsatellite tetranucleotide tandem repeat sequences, and three primers were derived from the core sequences of human and M13 bacteriophage minisatellites, respectively. All primers generated reproducible, valuable, polymorphic banding patterns. Variation in DNA patterns in repeated amplifications was not revealed. Microsatellite-based primers generated 2–4 polymorphic markers with the size from 600 to 1200 bp, minisatellite based primers produced 5–9 polymorphic markers with the size from 260 to 1700 bp. The highest number of polymorphic markers – nine, was generated by the primer based on minisatellite from M13 bacteriophage. Jaccard's coefficients of genetic similarity calculated for satellite polymorphism ranged from 0.105 to 0.905. All 85 bean accessions analysed differed from one another, also the genotypes identical in morphological traits. Based on DNA analyses, bean genotypes were grouped into two main branches and several subgroups (Figure 2). Nevertheless, no correlation was detected between satellite-based grouping and plant and seed morphological and agronomical characteristics or the country of origin. The probable reason is that DNA polymorphism appears to be based on micro- and minisatellite sequences, i.e. non-coding sequences. Moreover, none of DNA markers used here is linked to loci encoding the evaluated morphological trait.

Pure lines as derived from the collected heterogenous landraces in this study covered high variations in morphological characteristics of plant and seed. This approach should be considered as one of the ways to enrich bean germplasm and to extend genetic diversity in the maintained collections; but to avoid identical samples, it should be connected with more efficient tools for genotype distinguishing than those based on morphological traits. Although the variation in the morphological traits evaluated was relatively large in this study, some of the landraces were not distinguished and can be presumed as potential duplicates. Protein and DNA analyses generally afford tools for the detection of identity, homogeneity, genetic diversity, taxonomy, genetic shift, genome stability and other studies in plant genetic resources.

DISCUSSION

Tools for the bean genotypes differentiation and biodiversity studies are usually based on agronomic traits and quality parameters. Their relationships with the seed protein diversity were described by Escribano *et al*. (1998). Phaseolins and different

isozymes still play an important role in the bean discrimination (Gepts *et al*. 1986; Singh *et al*. 1991; Driedger *et al*. 1994), nevertheless their polymorphism is limited (Limognelli *et al*. 1996; Vasconcelos *et al*. 1996) and not sufficient to differentiate large sets of bean genotypes. An example for this is the study by Limognelli *et al*. (1996) which in a set of twenty landraces originating from southern Italy revealed only two phaseolin phenotypes. This also supported the testing of two old types of DNA markers – RFLPs (Stockton & Gepts 1994) and RAPDs (Haley *et al*. 1994; Skroch & Nienhuis 1995; Fofana *et al*. 1997). Both are connected with several drawbacks, mainly time consuming in RFLP and the banding patterns reproducibility problems affected by several parameters of reaction in RAPD's, respectively (Macpherson *et al*. 1993). Eighteen years ago, satellite sequences were used in a population dynamics study in humans (Jeffreys *et al*. 1985), later in animals (Wetton *et al*. 1987), and plants (Rogstad *et al*. 1988). Minisatellite DNA clones of human and M13 bacteriophage genome were used frequently for RFLP's detection. In *Phaseolus vulgaris* genome studies, minisatellite-derived and other hypervariable probes were tested by DNA fingerprinting technique and revealed polymorphism among genotypes (Stockton & Gepts 1994). These authors concluded that not all hypervariable probes are equally useful for fingerprinting in *Phaseolus vulgaris*, but that M13 bacteriophage and 33.15 human minisatellite-derived probes generate polymorphic banding patterns. Consequently, microsatellite-derived DNA probes were used for the study of variation in the genus *Phaseolus* (Hamann *et al*. 1995). To accelerate, improve, and minimise the time of analysis, techniques based on DNA amplification by simple sequence repeats (SSR) derived single primers was presented in evolutionarily diverse genomes (Gupta *et al*. 1994). Based on this knowledge, satellite-based primers derived from evolutionarily diverse organisms were used in this study and their efficiency for bean landraces as well as for genotypes identical in morphological traits, was confirmed. Microsatellite-based primers generated 3, and minisatellite-based primers 6.3 polymorphic markers per primer on average. All seven primers used generated polymorphic banding patterns. Although Hamann *et al*. (1995) revealed that microsatellite-based DNA probe $(GACA)_4$ did not hybridise with *Phaseolus vulgaris* DNA, the primer based on the same tandem repeat motive annealed to bean DNA and amplified polymorphic DNA markers in our experiments. It confirms that the tandem repeat motive $(GACA)_4$ is present in the common bean genome.

Based on mixture landraces collected in the Eastern Carpatien, new bean genotypes were acquired and included into the bean collection. A high variation in the seed characteristics – shape, colour, drawing, and in plant characteristics – dry or snap type, bush or climbing habit, immature pods colour, and others, was discovered. Broad variations in seed shapes – round, elliptic, elliptic elongated, kidney, in seed ground colour – white, yellow, grey, brown-yellow, brown, purple, black, and others, in seed drawing – spotted, marble, pointed, striped, and in immature pods colour – white, light yellow, yellow, pink, dark pink, light green, dark green, but also in other traits can be proposed for further exploitation in breeding.

References

Dellaporta S.L., Wood J., Hicks J.B. (1993): A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep., **4**: 19–21.

Driedger D.R., Watts B.M., Hussain A., Elias L.G. (1994): Isoenzyme and cotyledon protein variation for identification of black beans (*Phaseolus vulgaris* L.) with similar seed morphology. Euphytica, **74**: 27–34.

Escribano M.R., Santalla M., Casquero P.A., De Ron A.M. (1998): Patterns of genetic diversity in landraces of common bean (*Phaseolus vulgaris* L.) from Galicia. Plant Breeding, **117**: 49–56.

Fofana B., Vekemans X., Du Jardin P., Baudoin J.P. (1997): Genetic diversity in Lima bean (*Phaseolus lunatus* L.) as revealed by RAPD markers. Euphytica, **95**: 157–165.

Gepts P., Debouck D. (1993): Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.). In: Van Schoonhoven A., Voysest O. (eds): Common Beans: Research for Crop Improvement. CAB Int., CIAT, Antony Rowe Ltd., Chippenham, Wiltshire, Great Britain, 7–53.

Gepts P., Osborn T.C., Rashka T., Bliss F.A. (1986): Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris* L.): evidence for multiple centers of domestication. Econ. Bot., **40**: 451–468.

Gupta M., Chyi Y.S., Romero-Severson J., Owen J.L. (1994): Amplification of DNA markers from evolutionarily diverse genomes using single primers of

simple-sequence repeats. Theor. Appl. Genet., **89**: 998–1006.

Haley S.D., Miklas P.N., Afanador L., Kelly J.D. (1994): Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools of common bean. J. Am. Soc. Hort. Sci., **119**: 122–125.

Hamann A., Zink D., Nagl W. (1995): Microsatellite fingerprinting in the genus *Phaseolus*. Genome, **38**: 507–515.

Hidalgo R. (1991): CIAT's World *Phaseolus* Collection. In: Van Schoonhoven A., Voysest O. (eds): Common Beans. Research for Crop Improvement. CAB Int., CIAT, Antony Rowe, Chippenham, 163–197.

Horňáková O., Hofírek P., Hájek D., Bolebruch J., Gablechová L., Teclová A., Pípalová E., Pastucha L., Mrskoš M., Bareš I., Sehnalová J. (1991): Descriptor List, Genus *Phaseolus* L. VÚRV Praha.

Jeffreys A.J., Wilson V., Thein S.L. (1985): Hypervariable "minisatellite" regions in human DNA. Nature, **314**: 67–73.

Limognelli G., Langhetti G., Perrino P., Piergiovanni A.R. (1996): Variation of seed storage proteins in landraces of common bean (*Phaseolus vulgaris* L.) from Basilicata, Southern Italy. Euphytica, **92**: 393–399.

Macpherson J.M., Eckstein P.E., Scoles G.J., Gajadhar A.A. (1993): Variability of the random amplified polymorphic DNA assay among thermal cyclers, and effects of primer and DNA concentration. Molec. Cell. Probes, **7**: 293–299.

Rogstad S.H., Patton II J.C., Schaal B.A. (1988): M13 repeat probe detects DNA minisatellite-like sequences in gemnosperms and angiosperms. Proc. Natl. Acad. Sci. USA, **85**: 9176–9178.

Singh S.P., Nodari R., Gepts P. (1991): Genetic diversity in cultivated common bean: I. Allozymes. Crop Sci., **31**: 19–23.

Skroch P.W., Nienhuis J. (1995): Qualitative and quantitative characterization of RAPD variation among snap bean (*Phaseolus vulgaris* L.) genotypes. Theor. Appl. Genet., **91**: 1078–1085.

Stockton T., Gepts P. (1994): Identification of DNA probes that reveal polymorphism among closely related *Phaseolus vulgaris* lines. Euphytica, **76**: 177–183.

Vasconcelos M.J.V., De Barros E.G., Moreira M.A., Vieira C.A. (1996): Genetic diversity of the common bean *Phaseolus vulgaris* L. determined by DNA-based molecular markers. Brazil. J. Genet., **19**: 447–451.

Vassart G., Georges M., Monsieur M., Brocas H., Lequarre A.-S., Cchristophe D. (1987): A sequence of M13 phage detect hypervariable minisatellites in human and animal DNA. Science, **235**: 683–684.

Voysest O., Valencia M.C., Amezquita M.C. (1994): Genetic diversity among Latin American Andean and Mesoamerican common bean cultivars. Crop Sci., **34**: 1100–1110.

Wetton J.H., Carter R.E., Parkin D.T., Walters D. (1987): Demographic study of a wild sparrow population by DNA fingerprinting. Nature, **327**: 147–149.

Received for publication February 16, 2003
Accepted after corrections April 25, 2003

Abstrakt

Horňáková O., Závodná M., Žáková M., Kraic J., Debre F. (2003): **Diverzita krajových odrôd fazule pozberaných v západných a východných Karpatoch**. Czech J. Genet. Plant Breed., **39**: 73–83.

V súbore 82 zmesných krajových odrôd fazule (*Phaseolus vulgaris* L.), pozberanej v západných a východných Karpatoch, bola hodnotená variabilita v morfologických a hospodárskych znakoch. Genotypy boli identifikované a diferencované pomocou morfologických a molekulárnych markerov. Jednotlivé línie boli zo zmesných vzoriek krajových odrôd oddelené na základe charakteristík semena, následne boli zlúčené a rozmnožené a uložené do kolekcie genetických zdrojov fazule. Súbor genotypov bol hodnotený v 33 morfologických a hospodárskych znakoch na úrovni semena a rastliny. V týchto znakoch bola pozorovaná rozsiahla variabilita, niektoré genotypy však boli vo všetkých znakoch identické. Zhluková analýza, urobená na základe morfologických a hospodárskych znakov, rozdelila genotypy do dvoch hlavných skupín, líšiacich sa rastovým habitusom, parametrami semena a hmotnosťou tisíca semien. Diferenciácia genotypov bola vykonaná následne na základe multilokusového polymorfizmu v mikrosatelitných a minisatelitných úsekoch DNA. Genotypy identické v znakoch morfologických a hospodárskych boli DNA analýzami vzájomne odlíšené a prítomnosť duplikátov nebola potvrdená. Genotypy boli zhlukovou analýzou rozdelené tak isto do dvoch hlavných skupín, ale súvislosti medzi týmto rozdelením

a rozdelením na základe morfologických a hospodárskych znakov neboli zistené. Na základe zmesných vzoriek krajových odrôd fazule, pozberaných vo východných, (Slovensko) a západných (Ukrajina) Karpatoch, boli selektované nové genotypy – genetické zdroje fazule. V študovanom súbore bola zistená široká variabilita, najmä v znakoch semena – tvar, farba, kresba, a znakoch rastliny – habitus, farba nezrelého struku, ale i v ďalších znakoch, ktorá môže byť využitá v šľachtiteľských programoch a pri tvorbe nových odrôd fazule.

Kľúčové slová: fazuľa; *Phaseolus vulgaris* L.; krajová odroda; morfologické znaky; mikrosatelity; minisatelity; polymorfizmus; duplikáty

Corresponding author:

Ing. Olga Horňáková, Výskumný ústav rastlinnej výroby, Génová banka Slovenskej republiky,
Bratislavská cesta 122, 921 68 Piešťany, Slovenská republika
tel.: + 421 337 722 311–12, fax: + 421 337 726 306, e-mail: hornak@vurv.sk