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Autor: Eichenberger, Kurt / Gugerli, Felix / Schneller, J. Jakob

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Morphological and molecular diversity of Swiss common bean cultivars (*Phaseolus vulgaris* L., Fabaceae) and their origin

Kurt Eichenberger[1], Felix Gugerli[2] and J. Jakob Schneller

Institut für Systematische Botanik, Zollikerstr. 107, CH-8008 Zürich, Switzerland
[1] Current address: Pro Specie Rara, Pfrundweg 14, CH-5000 Aarau, Switzerland,
E-mail kurt.eichenberger@psrara.org
[2] Current address: Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft,
Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

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Abstract

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We investigated morphological and molecular diversity within cultivated common bean (*Phaseolus vulgaris* L.) in Switzerland and examined the origin of Swiss cultivars. We considered commercially traded cultivars and elder cultivars. These elder cultivars, often called landraces, collected and kept on farm by the Swiss non-governmental organisation *Pro Specie Rara*, are supposed to represent an endangered heritage of diversity within common bean. Morphological characters, phaseolin Sodium Dodecyl Sulfate Polyacrylamid gel-electrophoresis (SDS-PAGE), isoenzymes, and banding patterns of Random Amplified Polymorphic DNA (RAPD) showed polymorphisms and diversity among Swiss common bean cultivars. Cultivars were characterised either by the S-type or the T-type of the major storage protein phaseolin. This indicates that some Swiss cultivars have Mesoamerican origin while others have Andean origin. This hypothesis is corroborated by the Unweighted Pair Group Measured Analysis (UPGMA) clustering of the cultivars with a combined set of morphological, phaseolin, isoenzyme and RAPD data. A better knowledge of genetic and phenotypic aspects of the common bean helps to improve conservation programs for its endangered cultivars.

Key words: Phaseolus vulgaris, cultivars, genetic variability, proteins, morphology, conservation.

Introduction

The genetic variation in many crop plants is narrow. The use of wild progenitors, weedy races or cultivars is recommended to promote genetic diversity within a crop species (Berthaud 1997). The danger of an irretrievable loss of such existing genetic heritage has been generally recognised (Doebley 1989).

Many cultivars of food crops are endangered because conservation is difficult. On farm conservation is very costly. Ex situ conservation in seed banks over a long time period reduces germination capacity (Harrington 1972). Ageing of seeds may lead to biochemical and cytological aberrations (Smith & Berjak 1995) and is likely to influence the ability of plants to adapt to environmental changes.

Molecular methods offer a tool to estimate the genetic variability within an observed taxon. Morphological diversity and genetic diversity may often not correspond because domestication has led to a high variability of certain morphological features, which are in most cases coded by relatively few genes (Gepts 1993). Various studies using Random Amplified Polymorphic DNA (RAPD) have been recently carried out to investigate origin, identification, and variability of cultivars (Farnham 1996, Ortiz et al. 1997, Ferguson et al. 1998).

In Switzerland, *Phaseolus vulgaris* is nowadays commercially represented mainly by foreign cultivars. Only a few native cultivars are sold. Breeding of native cultivars was important only during war times, when Switzerland was isolated from seed deliveries of neighbouring countries. Private gardeners have grown common bean for at least 200 years. Professional seed sellers began to spread foreign selections over Switzerland in the second half of the 19th century (Eichenberger & Meile 1994). Most of the 85 endangered cultivars that are kept on farm by *Pro Specie Rara* (PSR), a Swiss non governmental organisation, probably refer to former commercially traded cultivars (Eichenberger & Meile 1994).

Phaseolus vulgaris is a genetically well examined species. A multiple domestication from wild beans (*Phaseolus vulgaris* ssp. *aborigineus*) in Mesoamerica and in the Andes has been indicated by Sodium Dodecyl Sulfate Polyacrylamid gel-electrophoresis (SDS-PAGE) studies of the major storage protein phaseolin (Gepts & Bliss 1988, Gepts et al. 1992, Gepts 1993) and with isoenzyme electrophoresis (Singh et al. 1991). RAPDs in common bean studies are used to generate markers for disease resistance (Adam-Blondon et al. 1994) to evaluate segregation between cultivars (Skroch et al. 1994, Haley et al. 1994) and to estimate the genetic diversity of different cultivars (Alvarez et al. 1997, 1998, Escribano et al. 1998, Skroch et al. 1998) and wild beans (Freyre et al. 1996). However, Swiss cultivars have not been subject of studies on their genetic diversity.

We therefore investigated the following items: 1) How diverse are common beans in Switzerland, especially pole beans (*Phaseolus vulgaris* ssp. *vulgaris* var. *vulgaris*)? Their relatively long period of cultivation in Switzerland has enabled the evolution of local cultivars. Cultivars from all parts of Switzerland have been considered. We considered commercially traded cultivars and elder cultivars, often called landraces .We used RAPDs, isoenzyme electrophoresis, and phaseolin SDS/PAGE as well as morphological data as indicators of variation. 2) Were Andean and/or Mesoamerican wild races important for the genetic composition of Swiss cultivars? 3) Can the results be used to argue about conservation of Swiss common bean cultivars?

Materials and methods

Plant material

We studied 15 cultivars of *Phaseolus vulgaris* ssp. *vulgaris* var. *vulgaris* Aschers. (pole bean), one cultivar of *P. vulgaris* ssp. *vulgaris* var. *nanus* Aschers. (dwarf bean), and three races of *P. vulgaris* ssp. *aborigineus* Burkart ex Burkart for the variation of RAPDs, isoenzymes, and phaseolin. Cultivars held in private gardens refer to the different regions of common bean cultivation in Switzerland (Table 1). The plants were grown under experimental

Table 1. Accessions of *Phaseolus vulgaris* used in molecular and morphological investigations, their source, origin, taxonomy, and status. Status indicates their economic importance in Switzerland. Cultivars are only grown in private gardens; commercial races are used at least locally in farming.

Accession	Source	Origin	Subspecies & Variety	Status
Berner Landfrauen	Samen Mauser[b], CH	Switzerland	vulgaris vulgaris	cultivar[e]
Blaue Speck	Pro Specie Rara[a], CH	Bennwil (BL)	vulgaris vulgaris	cultivar
Dell' Aquila	Pro Specie Rara[a], CH	Dangio (TI)	vulgaris vulgaris	cultivar
Huguette	Pro Specie Rara[a], CH	Penthalaz (VD)	vulgaris vulgaris	cultivar
Klosterfrauen	Pro Specie Rara[a], CH	Laufen (BE)	vulgaris vulgaris	cultivar
Meuch	Pro Specie Rara[a], CH	Oftringen (BE)	vulgaris vulgaris	cultivar
MI 1293	Gembloux[c], Belgium	Peru	aborigineus	wild race
MI 578	Gembloux[c], Belgium	Mexico	aborigineus	wild race
MI 621	Gembloux[c], Belgium	Argentina	aborigineus	wild race
Monstranz	Studerus[d], CH	Mellikon (AG)	vulgaris vulgaris	cultivar
Muttelibohne	Pro Specie Rara[a], CH	Attelwil (AG)	vulgaris vulgaris	cultivar
Pea Bean	Pro Specie Rara[a], CH	England	vulgaris vulgaris	cultivar
Sabo	Samen Mauser[b], CH	Switzerland	vulgaris nanus	commercial race
Schmalzkönigin	Baumberger[d], CH	Herrliberg (ZH)	vulgaris vulgaris	cultivar
Selma Star	Samen Mauser[b], CH	Switzerland	vulgaris vulgaris	commercial race
Selma Zebra	Samen Mauser[b], CH	Switzerland	vulgaris vulgaris	commercial race
Steimerer	Baumberger[d], CH	Herrliberg (ZH)	vulgaris vulgaris	cultivar
Vaters Dicke	Pro Specie Rara[a], CH	Rorschach (SG)	vulgaris vulgaris	cultivar
Weinländerin	Samen Mauser[b], CH	Switzerland	vulgaris vulgaris	cultivar[e]

[a] PSR, Sortenzentrale, Pfrundweg 14, CH-5000 Aarau (seed bank for endangered species in Switzerland)
[b] Samen Mauser, Industriestrasse 24, CH-8404 Winterthur (seed producer)
[c] Jardin botanique de Belgique, B-1860 Meise (seed bank)
[d] Private farmers or horticulturists
[e] Cultivar under current mass selection

conditions (20°C, 90% humidity, artificial light regime to extend normal daylight up to constant 13h) for laboratory use.

Random Amplified Polymorphic DNA

Leaves were harvested after entire unfolding. We took 50 to 200 mg plant tissue (one pair of primary leaves), froze it in liquid nitrogen, and vacuum dried it. The tissue was homogenised in a 2 ml tube with one glass pearl of 4 mm in diameter, using a shaker Retsch MM 2000. The glass pearl was removed and 1 ml of extraction buffer was added (Tris 100 mM pH 9, EDTA 50 mM, NaCl 500 mM, PVP 2%, SDS 1.25%). After incubation at 65°C for 30 min, proteins were removed adding two equivalents of chloroform/isoamylalcohol (24:1) and spinning at 20,000g. We added an equivalent of isopropanole to the supernatant, incubated for 30 min on ice, and harvested the pellet by centrifuging at 5000 g. We resuspended the pellet in 500 µl TE buffer for 30 min at 35°C and centrifuged the samples at 20,000 g to remove insoluble polysaccharides. After incubating the extracts with 5 µl RNAse (10mg/ml) at room temperature for 20 min, we determined quantity and quality of DNA electrophoretically with the help of quantitation standards (Gibco-BRL, Rockville, MD, U.S.A.), with spectrophotometry (Uvikon 860, Kontron), and flu-

orometry (TKO 100, Hoefer Scientific). Only extracts without RNA smears on agarose gels and with UV-light absorption ratios (A260/A280) between 1.8 and 2.0 were used.

We screened 84 primers (Microsynth, Balgach Switzerland), 35 of which gave banding patterns. We randomly chose 12 primers for our investigations in (5′ → 3′ order 70% GC content: TGCCGAGCTG, TGCGCCCTTC, GGTGACGCAG, TGGACCGGTG, GGGGTGACGA, AGGAGGACCC; 60% GC content: AGTCAGCCAC, AATCGGGCTG, AGGGGTCTTG, GTTGCGATCC, GGACTGGAGT, GGAGGGTGTT). Amplifications were done in 25 µl reaction mixtures containing 15.5 µl ddH_2O, 2.5 µl Taq polymerase buffer (Boehringer Expand no. 1, 10×), 1U Taq polymerase (Boehringer Expand), 2.25 µl $MgCl_2$ (final concentration 4 mM), 0.1 mM of each dNTP, 0.3 µM primer, and 10 ng DNA extract. The mix was overlaid with 25 µl mineral oil to prevent evaporation. A Techne PHC-3 thermocycler was programmed with the following cycles: (1) initial denaturation for 1 min at 94°C; (2) 2 cycles with 30 sec at 94°C, 30 sec at 41°C, and 120 sec at 72°C; (3) 20 cycles with 30 sec at 94°C, 15 sec at 39°C, 15 sec at 45°C, and 90 sec at 72°C; (4) 18 cycles with 30 sec at 94°C, 15 sec at 39°C, 15 sec at 45°C, and 120 sec at 72°C (5) 5 min at 72°C. We separated amplified fragments on 1.5% NA agarose gels (Pharmacia) in 1% TAE buffer at 50 V. Gels were stained in a 1% ethidium bromide solution for 30 min and destained in ddH_2O for 10 min. We visualised banding patterns under UV-light. All amplifications were carried out in four replicates. We repeated most of the PCRs and periodically ran negative controls. To test for genetic homogeneity among individuals within cultivars, we ran extracts of ten different individuals of the cultivars 'Vaters Dicke' and 'Berner Landfrauen'.

Isoenzymes

We took ca 60 mg of fresh primary leaves (2 to 4 weeks old) and ground the tissue in 100 µl 4% PVP grinding buffer (Soltis et al. 1983). Grinding was done on ice and within 30 min after harvesting to prevent a loss of enzyme activity. We performed the same extraction with 20 mg washed root tissue. The electrophoresis procedure followed Wendel &

Table 2. Isoenzymes used for *Phaseolus vulgaris* accessions, buffer systems (electrode buffer and gel buffer) following Soltis et. al (1983), plant organs used for extraction, and results in electrophoresis, respectively. 'Variable' = polymorphic loci, 'invariant' = monomorphic loci.

Enzymes	Abbreviation	Buffer no	Plant material	Results
Aconitase	ACO	1	fresh primary leaves	invariant
Alcohol dehydrogenase	ADH	1, 5	fresh primary leaves	invariant
Aldolase	ALD	5	fresh primary leaves	invariant
Aspertate aminotransferase	AAT	5	fresh primary leaves	invariant
Diaphorase	DIA	5, 7	fresh pr. leav. & roots	variable
Esterase fluorescent	EST	5	fresh primary leaves	invariant
Glucose-6-phosphate isomase	GPI	5, 7	fresh primary leaves	invariant
Isocitrate dehydrogenase	IDH	5	fresh primary leaves	invariant
Leucine amino peptidase	LAP	1	fresh primary leaves	invariant
Malate dehydrogenase	MDH	5	fresh primary leaves	variable
Phosphoglucomutase	PGM	5	fresh primary leaves	invariant
Phosphogluconate dehydrogenase	PGD	5	fresh primary leaves	invariant
Shikimate dehydrogenase	SKD	5, 7	fresh primary leaves	variable
Triose-phosphate isomerase	TPI	5	fresh primary leaves	invariant

Weeden (1989). We tested 23 enzymes (Table 2) following the recipes of Soltis et al. (1983) or with slight modifications (Table 1). According to the enzyme conditions, we used buffer no. 1, 5, or 7 (Soltis et al. 1983). Incubation and staining of the gels were performed following Wendel & Weeden (1989). All enzymes revealing polymorphisms among the samples were run at least three times with freshly harvested leaves, each time from different individuals, to confirm the banding patterns.

Phaseolin SDS/PAGE

About 1 mg of the raphe end of the seeds was ground in 50 µl cracking buffer (0.0625 M Tris-HCl pH 6.8, 2% SDS, 10% glycerol (v/v), 0.1 M dithiothreitol, 0.01% bromophenol blue) and transferred into 0.5 ml Eppendorf tubes. After heating the suspensions at 100°C for 10 min and incubating them overnight at 4°C, we centrifuged them at 15000 g for 5 min. We loaded 5 µl of the supernatant onto the gel for one-dimensional SDS polyacrylamide gel electrophoresis (SCIE-PLAS). The separating gel contained 0.75 M Tris pH 8.8, 0.1% SDS, 10% glycerol, 30% (v/v) acrylamide/bis-solution 37.5:1 (Gibco BRL, Rockville MD, U. S. A.), 0.02% ammonium persulfate, and 0.025% TEMED. The stacking gel contained of 0.0125 M Tris pH 6.8, 0.1% SDS, 15% (w/v) acrylamide/bis-solution 37.5:1, a trace of bromophenol blue, 0.2% ammonium persulfate, and 0.15% TEMED. The lower chamber was filled with 0.2 M Tris buffer pH 8.9, the upper chamber with 0.1 M Tris-HCl pH 8.25, 0.1 M tricine, and 0.1% SDS. A standard protein solution from Gibco-BRL (16001-018) was used as a marker. We initially ran the gel at 100 mA. When the bromophenol blue had reached the separating gel we increased the current to 200 mA and the voltage to 200 V. We fixed the gels in 7% acetic acid in 40% methanol and stained them in Brillant Blue G – Colloidal Solution (Sigma B-2025), followed by destaining in 10% acetic acid in 25% methanol for four hours.

The commercial *P. vulgaris* lines 'Contender', 'Sanilac', and 'Tendergreen' (National Germplasm Resources Lab., USDA, ARS, Beltisville, MD 20705, USA) served as references for phaseolin types C, S, and T, respectively (Gepts 1988). A protein molecular weight standard from Gibco BRL was used as a size marker. To test the consistency of the results, all cultivars were run three times with extracts of different seeds.

Morphology

We used a total of 26 morphological characters from vegetative and reproductive organs measured as averages of ten plants (Table 3). Seed characteristics were obtained from 100 seeds (metric traits) or ten seeds (qualitative traits), respectively. The characters were selected from the descriptor list for *P. vulgaris* (IBPGR 1982).

Data analysis

RAPD data were scored as presence (1) or absence (0) of amplification products. Bands with a broad range of intensities between different samples were excluded. Results were analysed by visual inspection of photographs. With this quantitative data matrix, we used the NTSYS-pc package (Rohlf, 1993) to generate the dissimilarity matrix (Euclidian distances algorithm). The SAHN clustering programme grouped the accessions using the Unweighted Pair Group Measured Analysis (UPGMA) (Sneath & Sokal, 1973).

Isoenzyme data included two loci from malate dehydrogenase (MDH-1, MDH-2), each with two different alleles coded as binary characters, diaphorase and shikimate dehydrogenase each produced two banding patterns, but it was not possible to identify specific alleles. These enzyme phenotypes were therefore also coded as binary characters.

Table 3. Characters used for morphological and phenological descriptions of *Phaseolus vulgaris* accessions according to the descriptor list of the IBPGR (1982) or slightly modified. Metric characters are measured as averages of ten plants. The classes show the full range of used characters. 1 – 8, seed characters; 9 – 10, seedling characters; 11 – 17, flower and bracteole characters; 18 – 26, pod characters.

Character	Variable type	Classes
Seed coat patterns	unordered	absent, constant mottled, striped, rhomboid spotted, speckled, circular mottling, marginal colour pattern, broad striped, bicolor, pattern around hilum
Seed coat darker colour	unordered	black, brown (pale to dark), maroon, grey (brownish to greenish), yellow to greenish yellow, pale-cream to buff, pure white, whitish, white (purple tinged), chlorophyll green, green to olive, red, pink, purple
Seed coat lighter colour	unordered	black, brown (pale to dark), maroon, grey (brownish to greenish), yellow to greenish yellow, pale-cream to buff, pure white, whitish, white (purple tinged), chlorophyll green, green to olive, red, pink, purple
Seed shape	unordered	round, oval, cuboid, kidney shaped, truncate fastigiate, both cuboid and tr. fastigiate
Seed weight	metric (mg)	
Seed length	metric (mm)	
Seed width	metric (mm)	
Seed height	metric (mm)	
Hypocotyl pigmentation	unordered	purple, green
Emerging cotyledon colour	unordered	purple, red, green, white, very pale green
Colour of standard	unordered	white, green, lilac, white with lilac edge, white with red stripes, dark lilac with purple outer edge, dark lilac with purplish spots, carmine red, purple
Colour of wings	unordered	white, green, lilac, white with carmine stripes, strongly veined in red to dark lilac veins, purple
Wing opening	ordered	parallel closed wings, wings moderately diverging, wings widely diverging
Size of bracteole	ordered	small, medium, large
Shape of bracteole	ordered	lanceolate, intermediate, ovate
Bracteole/calyx length	ordered	bracteole shorter than or equal to calyx length, bracteole up to 1/3 longer, bracteole twice as long
Calyx/bracteole colour	unordered	green, pale violet, dark purple, lilac
Pod colour	unordered	dark purple, carmine red, purple stripe on green, carmine stripe on green, pale red stripe on green, dark pink, normal green, shiny green, dull green to silver grey, golden or deep yellow, pale yellow to white, green with carmine sprinkling
Pod length	metric (mm)	
Pod cross section	unordered	very flat, pear shaped, round elliptic, figure of eight
Pod curvature	unordered	straight, slightly curved, curved, recurving
Number of locules per pod	ordered	
Pod width	metric (mm)	
Pod beak length	metric (mm)	
Pod beak position	unordered	marginal, non-marginal
Pod beak orientation	ordered	upward (to dorsal side), streight, downward (to ventral side)

Combined analysis

We used all 70 characters evaluated with the methods described above to create a common similarity matrix based on Gower's similarity index S_G. The matrix included 39 RAPD products, two isoenzyme loci, two isoenzyme patterns, the phaseolin patterns, and 26 morphological characters. SDS-PAGE revealed two phaseolin-types (T-, S-type), coded as binary

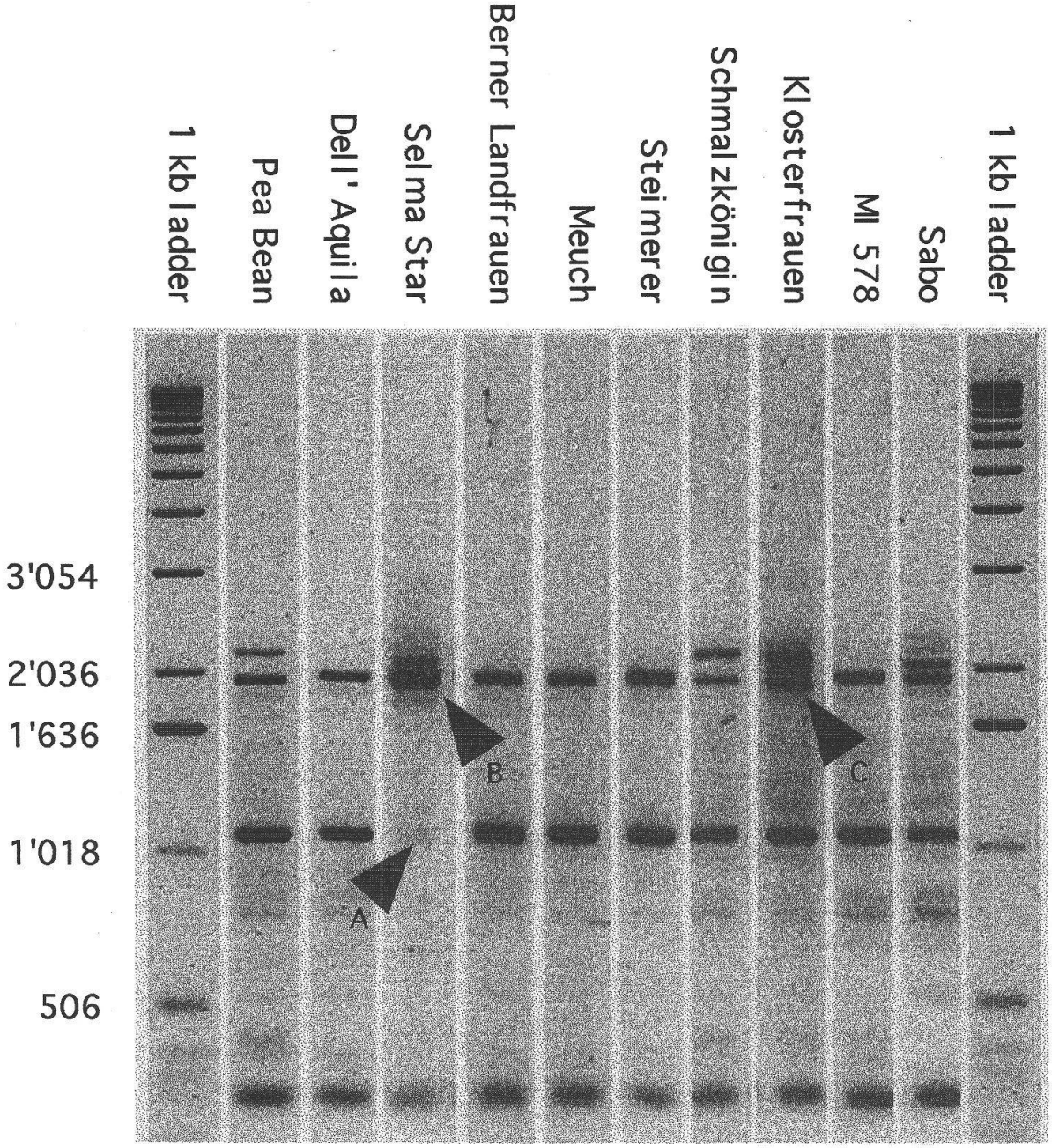

Fig. 1. RAPD PCR amplification products from ten accessions of *Phaseolus vulgaris* with primer AATCGGGCTG flanked by size markers (in bp). Arrowhead A indicates the 1.1 kbp RAPD marker absent in 'Selma Star'. Arrowhead B shows the 1.98 kbp band from 'Selma Star', in spite of its different intensity considered as monomorphic and identical to the 1.98 kbp bands of the other accessions. 'Klosterfrauen' shows a band which is not identical (1.95 kbp), indicated by arrowhead C.

characters. Morphological characters were coded as unordered, ordered, or metric (Table 3) to construct a matrix of Gower's similarity index S_G allowing the combination of the three types of variables (Gower, 1971) which was used for clustering.

Results

Random Amplified Polymorphic DNA

The 12 RAPD primers yielded 81 reproducible amplification products. Of these, 42 were monomorphic for all *Phaseolus vulgaris* accessions. Thirty-nine products showed polymorphisms (Fig. 1) and allowed to distinguish all investigated taxa. We detected no within-cultivar variation in the two tested cultivars 'Vaters Dicke' and 'Berner Landfrauen'. The closest related group of cultivars in the UPGMA tree based on RAPD variability can be seen from 'Pea Bean' down to 'Dell' Aquila' (Fig. 2). The two Andean wild races 'MI 621' and 'MI 1293' are clearly separated from the Mexican wild race 'MI 578'. The latter as well as 'Selma Star' are isolated from all other accessions. 'Selma Star' and 'Selma Zebra' are a result of a breeding programme using 'Weinländerin' as maternal plant and several dwarf beans as paternal plants, among these the commercial race 'Sabo'. The topology of the phenogram using Gower's similarity matrix did not diverge from the presented clustering (Fig. 2). Thus, transformed data could be used for the combined analysis (see Material and methods).

Isoenzymes

Six of the 23 tested isoenzyme systems gave no results, eleven gave monomorphic banding patterns, three showed unsteady bands, and three isoenzymes were polymorphic and gave

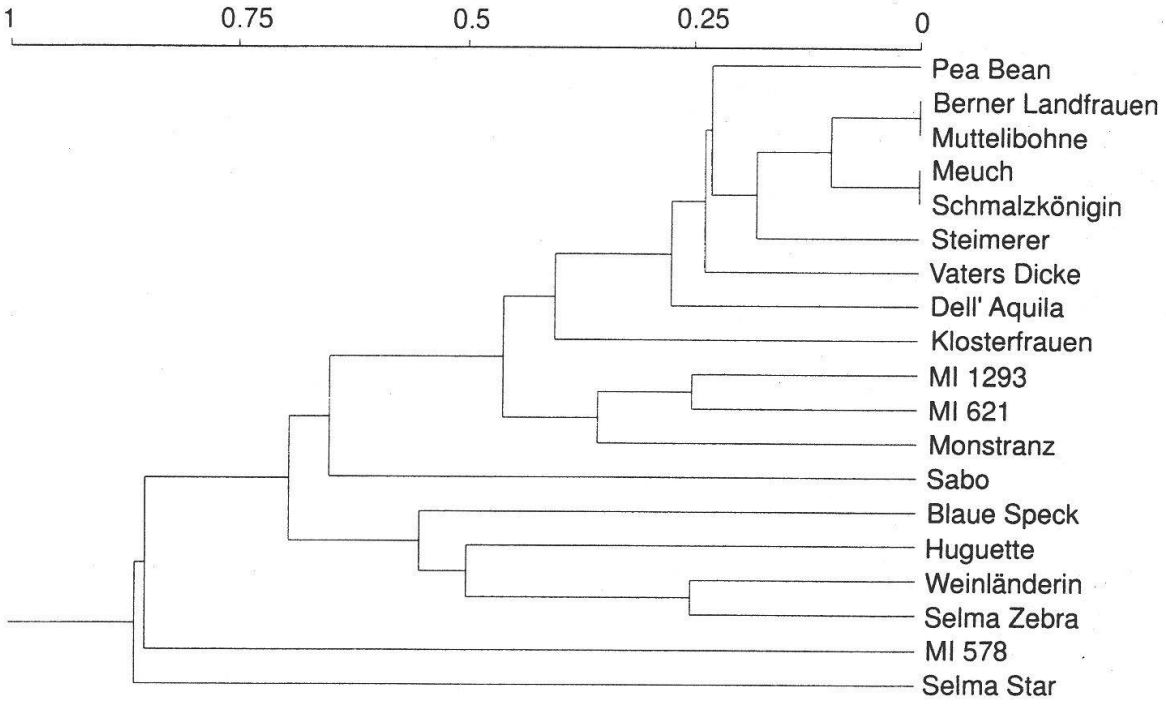

Fig. 2. UPGMA clustering based on a dissimilarity matrix of 39 RAPD PCR amplification products from 19 accessions of *Phaseolus vulgaris* (Table 1).

reproducible bands (Table 2). Malate dehydrogenase showed one main banding pattern for most accessions. Diaphorase (Fig. 3) and shikimate dehydrogenase each divided the 19 accessions studied into two groups with different isoenzyme banding patterns. Some accessions showed intermediate diaphorase isoenzyme types (arrowhead B in Fig. 3).

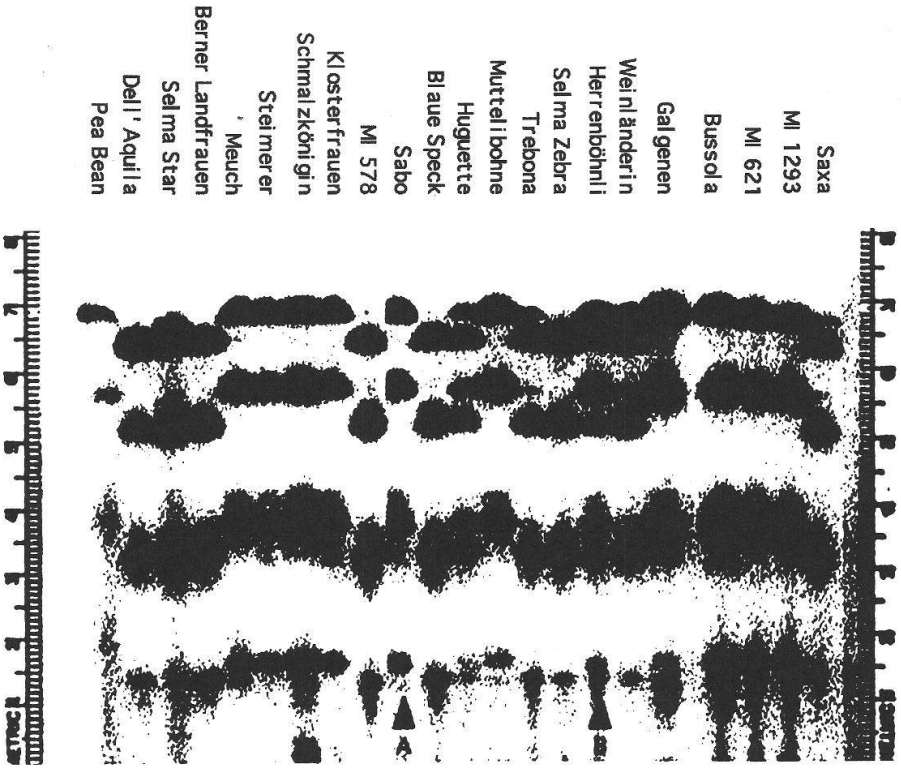

Fig. 3. Diaphorase isoenzyme electrophoresis products from 22 accessions of *Phaseolus vulgaris*. The cultivars 'Trebona', 'Herrenböhnli', 'Galgenen', and 'Bussola' were not used for analysis. The first ten accessions represent the same accessions as in Fig. 1. Two main patterns of isoenzymes are visible: one pattern with bands migrating over shorter distances is represented by e.g., 'Berner Landfrauen', the other pattern is represented by e.g., 'Meuch' (4th and 5th lane from left side, respectively). Arrowhead A points to race 'Sabo' inserted from another gel. Arrowhead B indicates an additive pattern i.e., a combination of the two main patterns. On the left and right borders, the running distance is given in centimetres. Two bands shared by all accessions on the cathodal side with distances of –0.5 cm and –1.0 cm, respectively, are not shown.

Table 4. Modified recipes, based on Soltis and Soltis (1989).

Enzyme	Abbreviation	Recipe
Diaphorase	DIA	30 ml 50 mM Tris-HCl pH 7.0, 4 mg NADPH, 15 mg Menadione, 6 mg NBT
Malate dehydrogenase	MHD	30 ml 100 mM Tris-HCl pH 8.0, 1.7 g Malic acid, adjust pH to 8.0 with Sodium acetate, 5 mg NAD
Shikimate dehydrogenase	SKD	30 ml 100 mM Tris-HCl pH 8.5, 30 mg Shikimic acid, 5 mg NADP

Phaseolin SDS/PAGE

Two types of phaseolin could be distinguished. According to investigations of Brown et al. (1981), Gepts (1988) and Gepts et al. (1992), these two forms represent the T-type and the S-type of phaseolin of cultivars 'Tendergreen' and 'Sanilac', respectively (Fig. 4). The C-type represented by race 'Contender' could not be recognized. 'MI 578' as a Mexican wild race shows the S-type, 'MI 621' and MI '1293' as Andean wild races show the T-type of phaseolin, respectively. Five of fifteen Swiss cultivars reveal the S-type of phaseolin, indicating a Mesoamerican descent. Intensive bands within the range of 43 kd to 54 kd represent the different phaseolin subunits (Brown et al. 1982, Vitale & Bollini 1995).

Morphology

The phenogram from UPGMA clustering revealed two clusters of *P. vulgaris* cultivars ('Pea Bean' to 'Muttelibohne' and 'Selma Star' to 'Huguette', respectively) (Fig. 5), mainly

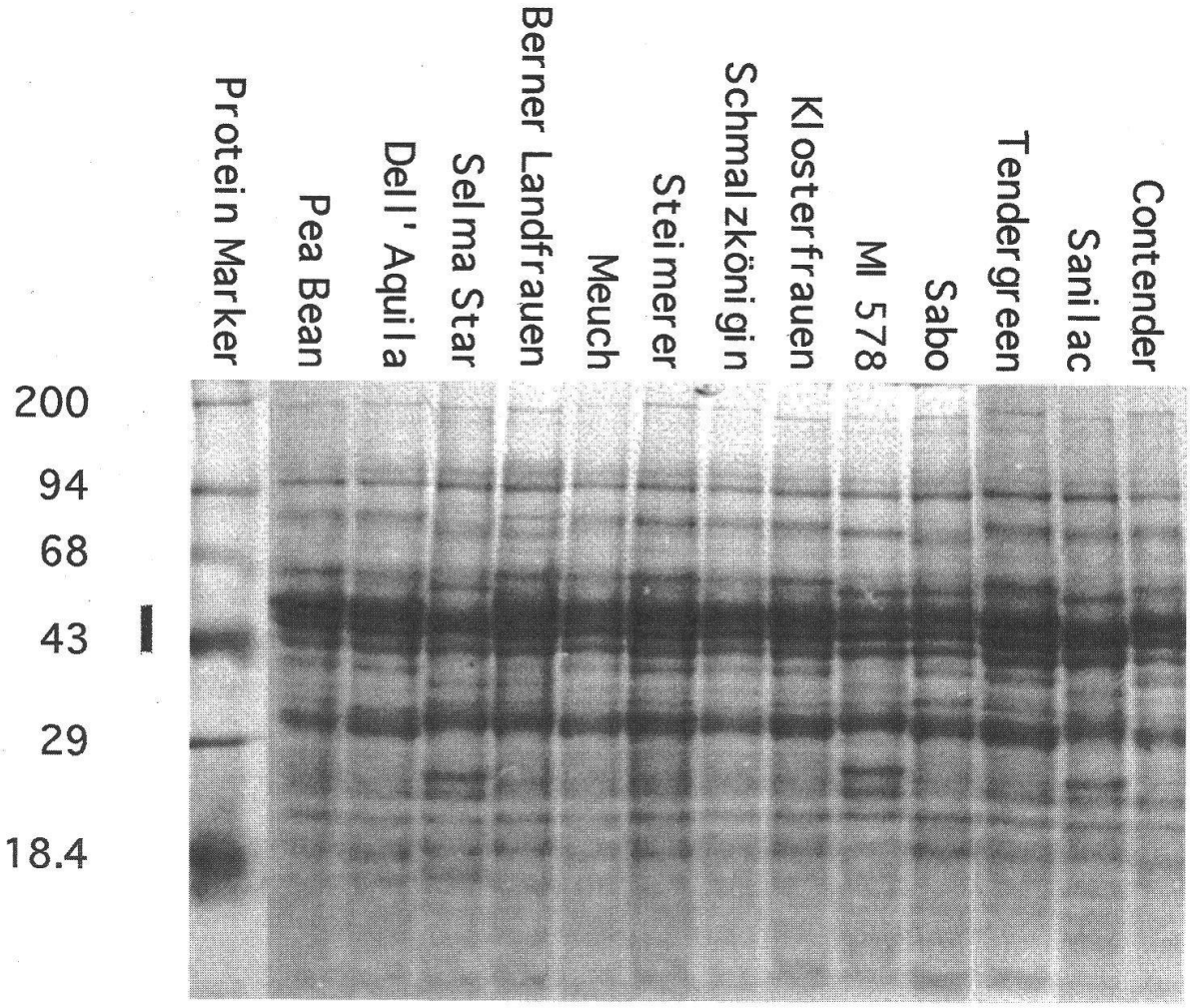

Fig. 4. Seed proteins from SDS polyacrylamid gels with 13 accessions of *Phaseolus vulgaris*. The North American cultivars 'Tendergreen', 'Contender', and 'Sanilac' serve as references to determine the Phaseolin types 'T' (3 bands in bar region), and 'S' (2 bands in bar region), respectively. Numbers on the left margin indicate the weight of protein marker bands in kilddalton [kd]. The bar indicates the region of the different phaseolin subunits (43 kd – 54 kd).

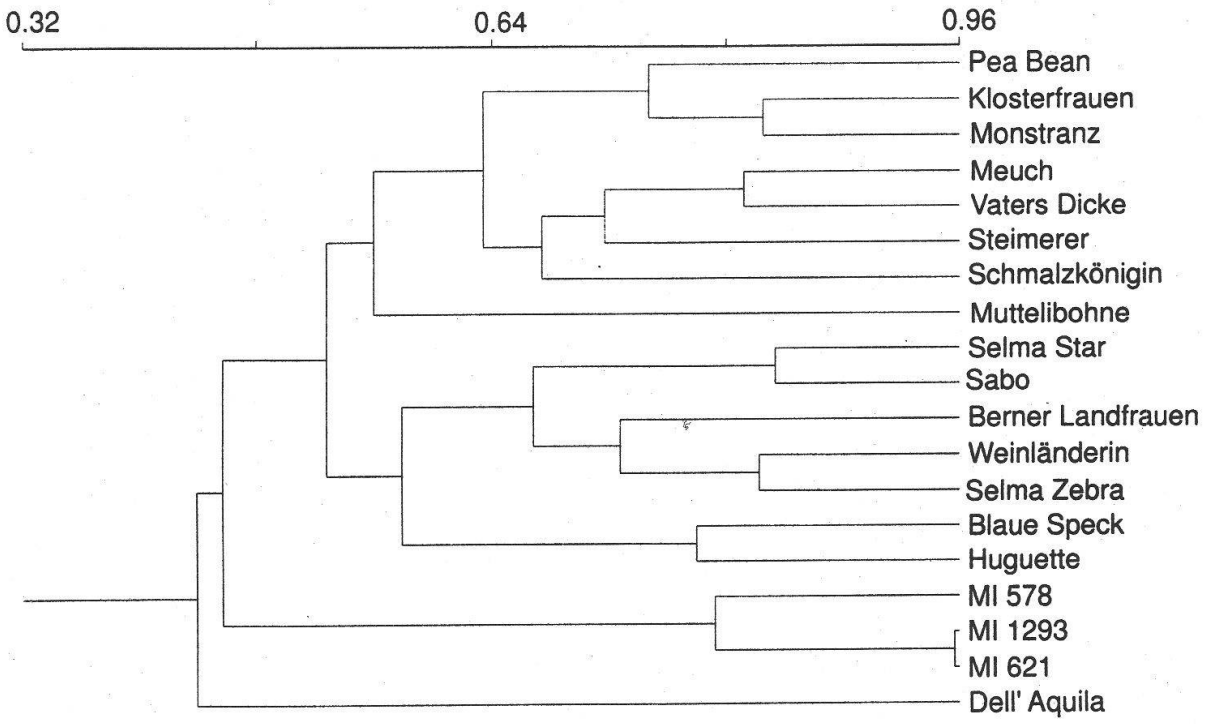

Fig. 5. UPGMA clustering from 19 accessions of *Phaseolus vulgaris* based on a matrix of Gower's similarity index S_G (Gower, 1971) with 26 morphological and phenological characters.

due to seed characteristics. In the UPGMA tree based on RAPD variability, the cultivar 'Dell' Aquila' belonged to a closely related group with a dissimilarity value of approx. 0.3. The same cultivar had the lowest similarity to all other accessions in the UPGMA tree based on morphology, which is mainly due to its tall plant organs. In contrast to the results based on RAPDs (Fig. 2) the two Andean wild races 'MI 621', and 'MI 1293' as well as the Mexican wild race 'MI 578' are grouped in a closely related cluster mainly due to seed characteristics (Fig. 5).

Combined analysis

The combination of molecular and morphological data resulted in a tree of two clusters and the isolated Mexican wild race 'M 578' (Fig. 6). The clusters correspond well to the phaseolin types. Diaphorase and shikimate isoenzyme patterns as well as the morphological traits seed shape and calyx/bracteole length relation mainly follow the clustering.

Discussion

The applied molecular techniques are appropriate for detecting genetic differences of Swiss common bean cultivars. Clustering of morphological and molecular data show a division of cultivars in two groups corresponding well with two types of phaseolin usually applied to determine the origin of common bean cultivars (Gepts 1988, 1996, Gepts et al. 1992). Conservation programs of Swiss *Phaseolus vulgaris* cultivars should be emphasized because

the present collection of accessions kept by *Pro Specie Rara* contains many known and well adapted but endangered elder cultivars woth of gardening and possibly farming, too.

Comparison of different data sets

The comparison of the morphological and the RAPD tree shows some clear differences. The two Andean wild races 'MI 621' and 'MI 1293', well integrated in a cluster from 'Pea Bean' to 'Monstranz' in the RAPD tree (Fig. 2), are very distant from other accessions in the morphological tree (Fig. 5). This is mainly due to seed and fruit characteristics. 'Selma Star' a descendant from 'Weinländerin' and morphologically very similar to the latter (Fig. 5) appears far distant from the former in the RAPD tree (Fig. 2). 'Berner Landfrauen' and 'Dell' Aquila' are grouped into distinct clusters in the two different analyses.

Morphological characters of cultivated plants are selectively influenced by man and therefore less independent than are RAPD data. Morphological variability is usually high among cultivars as compared with their wild progenitors. On the other hand, the genetic uniformity within cultivars revealed by molecular data is in contrast to the genetic variation within wild races (Doebley 1989, Gepts 1993).

Our combined analysis including both morphological and molecular data leads to a high correspondence of the two phenogram clusters with the phaseolin types used to determine different gene pools of wild common bean. (Brown et al. 1982, Gepts 1988, Gepts & Bliss 1988). The clusters also show similarities to the variation of the isoenzyme patterns of diaphorase and shikimate dehydrogenase (Koenig & Gepts 1989), and the morphological characteristics with 'seed shape' and 'bracteole/calyx length ratio'.

The combined tree of morphological and molecular data clusters (Fig. 6) corresponds better to the phaseolin types (T/S) separating Andean and Mesoamerican cultivars more strictly than do the trees based on only one method of screening (Fig. 2, 5).

Genetic diversity among Swiss common bean cultivars

Phaseolin data show a similar range of genetic diversity within Swiss common bean cultivars as described for French cultivars in Gepts & Bliss (1988). The ratio of T-types to S-types is approximately 2:3 in both countries. Data of additionally examined 27 Swiss cultivars confirm these results (K. Eichenberger, unpublished data). We did not expect this because contrary to France, the cultivation of beans for dry seed production is hardly known in Switzerland. Such cultivars often show the S-phaseolin type (Gepts & Bliss 1988). An additional C-type found very rarely in French cultivars did not occur in any of the studied Swiss cultivars. Our RAPD data confirm the distinction of cultivars derived either from Andean or from Mesoamerican progenitors and correspond to studies of Freyre et al. (1996), Vasconcelos et al. (1996) and Escribano et al. (1998). According to our results (combined analysis), S-type cultivars in Switzerland show a similar degree of genetic diversity like T-type cultivars (Fig. 2, 6).

It is very likely that different factors have influenced the diversity of cultivars. We assume that the major factor promoting variability within common bean cultivars in Switzerland is its geographic position in Central Europe, which may have led to multiple introductions. The existing diversity within common bean in Switzerland mirrors cultivars grown in private gardens on one hand and commercially available cultivars on the other hand. The proximity of France, Italy, Germany, and Spain with their long traditions of growing common bean has led to various influences in the local choice of cultivars, types of cultivation, and consumption of common bean. Although a broad diversity of sold cultivars existed already in the 19th century (Martens 1860), breeding of cultivars in Switzerland only started

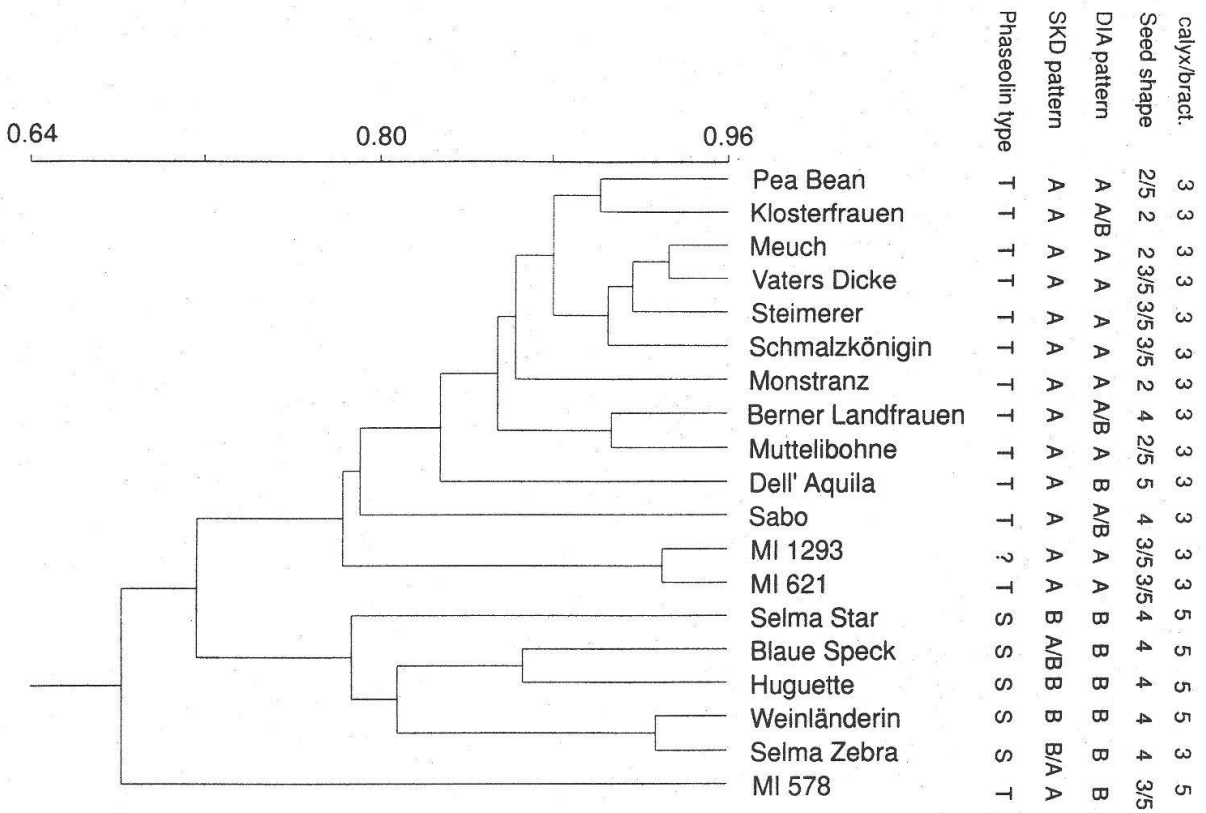

Fig. 6. UPGMA clustering from 19 accessions of *Phaseolus vulgaris* (Table 1) based on a matrix of Gower's similarity index Sg (Gower, 1971) including 26 morphological and phenological as well as 44 molecular characters (RAPDs, isoenzyme, and phaseolin data). The clusters of the resulting tree are supported by morphological characters 'bracteole/calyx length relation' (bracteole shorter than or equal to calyx; br. up to 1/3 longer than c.) and 'seed shape' (2 = oval; 3 = cuboid; 4 = kidney shaped; 5 = truncate fastigiate), isoenzyme patterns from diaphorase and shikimate dehydrogenase, and phaseolin patterns.

in the beginning of the 20th century (Eichenberger & Meile 1994) when numerous seed sellers and producers became established in the country. Industrial production and breeding programmes became important during the time of the two world wars and the economic crisis of the thirties.

Origin of cultivars

During the domestication process of *Phaseolus vulgaris*, several bottle necks might have occurred (Gepts et al. 1992). The first and probably strongest reduction of diversity (Doebley 1989) is supposed to have happened during the early cultivation (10,000 BP – 500 BP) in the two or three domestication centres in Mesoamerica and Southamerica (Gepts et al. 1992). The second bottle neck might have occurred five centuries ago, when the species was brought to Europe and possibly Africa after the discovery of the Americas (Debouck & Smartt 1995, Zeven 1997). A third bottle neck might have taken place within the last centuries, when the species was brought from Europe to western Asia, Middle East, Africa, and USA (Gepts & Debouck 1991, Sonnante et al. 1994, Gepts 1996). Spain can be considered as the origin of

European domestication and as the Old World centre of diversity of *Phaseolus vulgaris* because Spanish cultivars show the highest variability of phaseolin types (S-, T-, and C-types in similar proportions) among European countries (Gepts & Bliss 1988). From Spain, common bean was distributed to other European countries, which was accompanied by a decrease of Mesoamerican S- and Southamerican C-phaseolin types (NW of Southamerica) in newly colonised countries. Andean derived cultivars with T-phaseolin type seem to be better adapted to humid and colder climate (Gepts & Bliss 1988). Although the genetic variability within cultivars in NW Europe is reduced as compared to Spain, Mesoamerica, and Southamerica, it is important to concentrate also on local diversity because cultivars are in many areas the result of local adaptation over a long period.

Isoenzyme and phaseolin data give strong evidence for different origins of Swiss common bean cultivars. We expected that most of the Swiss cultivars derived from Andean progenitors (P. Gepts, Davis, CA, USA) mainly because of their advantage to grow in the climate of NW Europe (Gepts & Bliss 1988). Combined analysis clearly reveals two pools of genotypes. The first pool represents descendants of Andean progenitors due to their T-phaseolin type ('Tendergreen', Fig. 4) and their predominant diaphorase A-patterns and shikimate dehydrogenase A-patterns (Fig. 3, 6). The smaller pool of cultivars shows the S-phaseolin type and mainly isoenzyme patterns diaphorase B and shikimate dehydrogenase B which are usually found in Mesoamerican wild races (Fig. 6).

Diaphorase patterns A and B may refer to *Dia-1*, allele 100 and allele 95, respectively, described in Koenig and Gepts (1989). These two alleles are the only ones found in cultivars. Wild races showed additional three alleles. Allele 95 is found in 86% of all Mesoamerican cultivars, allele 100 occurs in 94% of all Andean cultivars (Singh et al. 1991). Patterns A and B of shikimate dehydrogenase refer to alleles 100 and 103 in Koenig and Gepts (1989). Allele 103 is only found in Mesoamerican wild races (Koenig & Gepts 1989) and is predominant in Mesoamerican cultivars (Singh et al. 1991).

Our results indicate that, in contrast to our expectations, there is a considerable proportion of Swiss cultivars associated to the Mesoamerican genepool.

Perspectives in conservation and promotion of diversity in Phaseolus vulgaris

To maintain genetic diversity within cultivated plant species, conservation efforts should concentrate on wild progenitors if such are known and still extant (Peacock 1989, Berthaud 1997). Wild races are supposed to carry broader genetic diversity and show higher disease resistance than do cultivars (Brown 1989). With some exceptions, e.g. in tomatoes and maize, it is not common to include wild progenitors or relatives in breeding programs (Ladizinsky 1989).

Progress is also made in the use of elder cultivars, commonly referred to as landraces for breeding programs (Fischbeck 1989, Moghadam et al. 1998). Domesticated plants may offer additional genetic traits that are not found in wild taxa. A reservoir of diversity often occurs in areas where cultivated descendants have been growing for a long time (Brush et al. 1994; Bellon 1996).

In Switzerland, *Phaseolus vulgaris*, especially pole beans, has lost its economic importance during the last decades but is still abundant in private gardens. Commercially traded cultivars are numerous, most of which originate from German and Dutch seed companies. At the same time, a considerable diversity of elder cultivars has been conserved in gardens. The collection of pole beans kept by the Swiss non-governmental organisation *Pro Specie Rara* amounts to 85 pole bean cultivars, all together disappeared from seed catalogues. Some of them are well known former commercially traded cultivars, such as 'Klosterfrauen', sold from

1900 up to 1970 in Switzerland (Eichenberger & Meile 1994). Others are supposed to be private selections from cultivars with unknown origin. In some cases, as for 'Pea Bean', a Swiss origin can be traced back to the end of the last century. Especially known elder cutlivars which have survived in private gardens form a diverse collection worth of conservation. The need of a diverse assortment of cultivars for gardening and initiatives for revitalisation of old cultivars, started by traders like Coop Switzerland, justify to emphasize conservation programs for *Phaseolus vulgaris*.

These 85 Swiss accessions are considered to be cultivars mainly on the basis of morphological seed characteristics. Our present study supports the assumption that a broad diversity of clearly defined cultivars is still present in Switzerland. The results also show that Swiss cultivars refer to two different gene pools in the Americas. We therefore regard it as important to continue the conservation program for *P. vulgaris* which is already started by *Pro Specie Rara*. Conservation efforts allow the survival of locally adapted cultivars which is of great interest for gardening. They may offer a potential for breeding in organic farming, too.

Zusammenfassung

Wir untersuchten die morphologische und genetische Diversität, sowie die Herkunft von Gartenbohnensorten (*Phaseolus vulgaris*) in der Schweiz. Es wurden sowohl kommerziell gehandelte als auch ältere Sorten berücksichtigt. Letztere, häuftig auch als Landsorten bezeichnet, werden von der Nicht-Regierungs-Organisation Pro Specie Rara gesammelt und lebendig erhalten (on farm). Aufgrund der intensiven langjährigen Sammeltätigkeit darf davon ausgegangen werden, daß diese älteren und vom Aussterben bedrohten Sorten weitgehend die existierende genetische Diversität der Gartenbohne in der Schweiz repräsentieren.

Unsere Arbeit zeigte, daß sich die untersuchten Gartenbohnensorten morphologisch wie auch genetisch unterscheiden lassen. Zur Anwendung gelangten neben morphologischen Untersuchungen die Polyacrylamid-Gelelektrophorese (SDS-PAGE) des Samenspeicherproteins Phaseolin, die Isoenzymelektrophorese und die Methode der Random Amplified Polymorphic DNA (RAPD).

Das Vorkommen von S- und T-Phaseolin als Samenspeicherprotein weist darauf hin, daß einige Schweizer Sorten mesoamerikanischen, andere andinischen Ursprungs sind. Diese Hypothese wurde durch die Gruppierung mittels Unweighted Pair Group Measured Analysis (UPGMA) gestützt, mit welcher ein kombinierter Satz aller aus den obengenannten Verfahren gewonnenen Daten verrechnet wurde.

Eine genaue Charakterisierung der genetischen und phänotypischen Merkmale von älteren Sorten ist notwendig, um Programme zur Erhaltung der Diversität bei der Gartenbohne zu entwickeln und weiterzuführen.

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