Restriction fragment length polymorphisms of the phytohemagglutinin genes in *Phaseolus* and *Vigna*

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Introduction

In *Phaseolus* and *Vigna*, up to 10% of the seed storage proteins belong to the lectins, particulary the phytohemagglutinins (PHA). Proteins of the phytohemagglutinin gene family (e.g. α-amylase inhibitor and arcelin) are thought to play a major role in resistance to pests (reviewed by Chrispeels and Raikel, 1991). Restriction fragment length polymorphism (RFLP) analysis have been used as molecular markers to construct linkage maps of crop plants, to mark quantitative trait loci, and to complement phylogenetic relationships in several plant taxa. RFLP markers show co-dominant inheritance, are phenotypically neutral and are not influenced by the environment. In the present study we show that the PHA locus is an additional useful molecular marker to assess genetic relationships among several *Phaseolinae*.

Material and Methods

The anaylzed genotypes of *Phaseolus* and *Vigna* are represented in Fig 1. Total plant DNA was isolated and digested (5μg) with *Eco*RI, *Bam*HI, *Hind*III, *Dra*I, *Rsa*I or double digested with *Eco*RV and *Xba*I, separated in agarose gels and transferred to nylon membranes. A 746bp *Eco*RV/*Xba*I fragment of the genomic subclone pTV770 (Voelker et al., 1986), containing the intron-less gene for PHA-L, was radiolabeled for the subsequent Southern hybridization. The last stringent wash was performed at [Tm-25°C] = 67.2°C. The presence or absence of a specific restricition fragment on the autoradiograms was transformed into an 1/0 (present/absent) matrix over all genotypes studied. A computer program converted this matrix into a pairwise distance matrix. These matrices were used as input files for different distance (FITCH, KITSCH, NEIGHBOR) and parsimony (MIX, DOLLOP) programs of the PHYLIP program package (Vers. 3.5) developed by Felsenstein (1989). To estimate the variation statistically, the data sets were resampled by bootstrap analyses. Further details on material and methods are described by Zink et al. (1994).

Results and Discussion

RFLPs at the PHA locus were determined among 18 genotypes of *Phaseolus vulgaris, P.coccineus, P.acutifolius, P.lunatus* and three *Vigna* species using five restriction enzymes and one double digestion, in order to provide molecular evidence for their genetic relationships. Interspecific and intraspecific variation could be detected with all endonucleases used, except with *Bam*HI. The results show that the highest polymorphisms are revealed between the genera *Phaseolus* and *Vigna*. Within the two genera, the polymorphisms among species is still rather high and of variable extent between genotypes of the same species. Intraspecific patterns demonstrate higher variability in allogamous species (e.g. *P.coccineus*) than in autogamous species (e.g. *P.vulgaris*). In *P.vulgaris*, two different DNA hybridization patterns are found, giving further evidence for two major gene pools in this species. The cultivars cv. Tendergreen (T-phaseolin type), cv. Contender (C-phaseolin type) and cv. Hilds Marona form one group belonging to the Andean gene pool, while the cultivars cv. Kentucky Wonder (S-phaseolin type), cv. Blauhilde, and cv. Neckarkönigin form the other group, evidently belonging to the Mesoamerican gene pool. The two genetically distinct groups originate from two major and independent domestication events in the Andes and in Mesoamerica, which resulted in two primary centres of genetic diversity (reviewed by Gepts, 1988). No polymorphisms at the PHA locus were detected among genotpyes within the two gene pools of *P.vulgaris*. It has been proposed that South American cultivated *P.vulgaris* forms were domesticated from the wild form *P.vulgaris* var. *aborigineus* (Berglund-Brücher and Brücher, 1976). In our study *P.vulgaris* var. *aborigineus* exhibits a higher degree of homology to the Andean gene pool than to the Mesoamerican gene pool. This correlates with the distribution of *P.vulgaris* var. *aborigineus*, which is mainly found in the Andean region (Brücher, 1988). An undefined landrace from Taiwan could be identified as a *P.vulgaris* genotype belonging to the Andean gene pool, because all hybridization patterns were identical with those found in the investigated *P.vulgaris* cultivars of this gene pool. Within the genus *Phaseolus*, the cultivars of *P.coccineus* revealed the highest, *P.acutifolius* an intermediate, and *P.vulgaris* the smallest number of hybridization fragments. The analyzed genotypes of *P.lunatus* showed a lower number of signals than *P.acutifolius*. In

comparison to the *Phaseolus* species, the *Vigna* species displayed completely different RFLP patterns. The level of polymorphism among *Vigna* species was lower than in *Phaseolus*, and the intensity of the RFLP signals was reduced.

We tested several distance matrix and parsimony methods and compared the results with the taxonomy based on morphological, genetic and biochemical data (e.g. Kloz and Klozova, 1974; Bassiri and Adams, 1978; Sullivan and Freytag, 1986). Similar cladograms were obtained (e.g. Fig. 1) with all the applied methods. The cladograms clearly exhibit the different species (i.e. *P.coccineus*, *P.vulgaris*, *P.lunatus*), while the cultivars belonging to the same species are clustered together. *P.vulgaris* cultivars belonging to the Mesoamerican gene pool are grouped together as are those of the Andean gene pool. *P.aborigineus* is clustered with the cultivars belonging to the Andean gene pool. *P.coccineus* and *P.vulgaris* genotypes form a closely related group, while *P.lunatus* genotypes form another group near to the *Vigna* species. The position of *P.acutifolius* as well as the clustering within the *Vigna* species remains uncertain, because different groupings were obtained with different algorithms. Our cladistic analyses based on RFLP-data for the PHA locus, however, are in general agreement with the classical taxonomic suggestions as given by Maréchal et al. (1978).

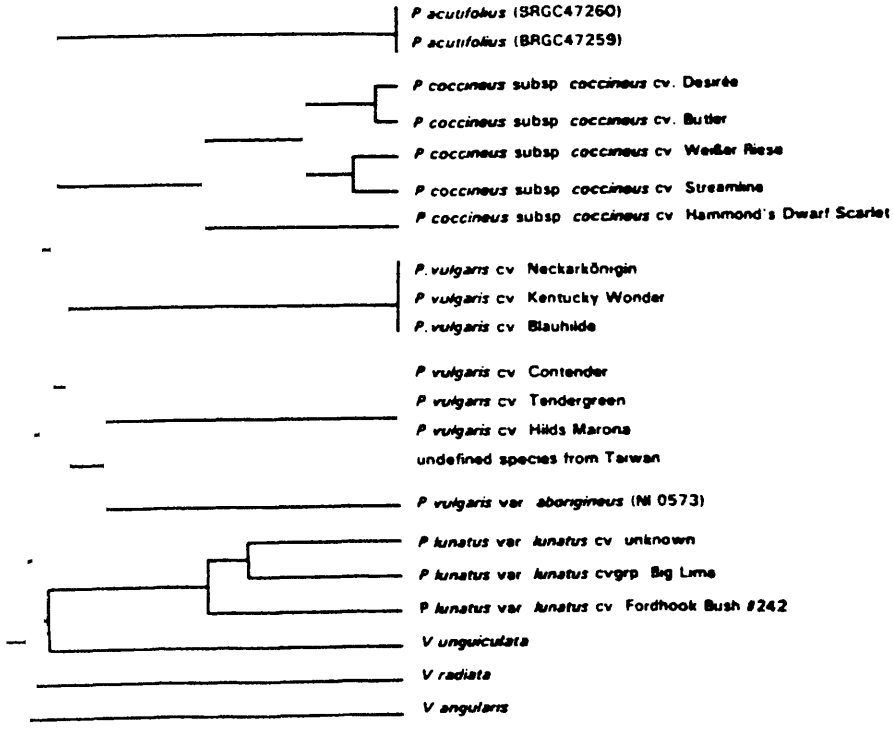

Fig. 1. RFLP-based phylogenetic tree of the PHA genes for the *Phaseolus* and *Vigna* genotypes studied, as obtained with KITSCH (options P=2, J=9). Sum of squares = 0.746, standard deviation = 4.23.

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