GENETIC DIVERSITY OF FABA BEAN (*VICIA FABA* L.) POPULATIONS ESTIMATED BY ISOZYMIC AND MOLECULAR MARKERS: RELATIONSHIP BETWEEN THE TWO METHODS

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Abstract

In our previous studies, the genetic diversity among nine Tunisian faba bean (*Vicia faba*) populations was analysed using isozymes and sequence-specific amplification polymorphism (SSAP) markers. The objectives of this study were to compare the application and utility of isozymes and sequence-specific amplification polymorphism (SSAP) techniques for analysis of genetic diversity among nine Tunisian faba bean (*Vicia faba*) populations. A high genetic diversity within populations was detected by both isozymes (SOD, 6-PGD, ME, EST, SKDH, FDH and GDH) and (SSAP) markers (*PDR1*, Tps19 and Tvf4). For all populations, the genetic diversity revealed by SSAP was more pronounced than that detected with isozyme, based on polymorphic profiles. The analyses of correspondance between the tow methodes based in Mantel test revealed a low correlation (r=0.177). The low correspondance indicated the absence of correlation and therfore the complimentarity

Keywords: *Vicia faba* populations, Isozymes, SSAP, genetic diversity, Mantel test, Tunisia

Introduction

Faba bean (*Vicia faba* L.) is a diploid legume (Fabaceae family). It is the most isolated species of the genus *Vicia* (Hanelt et al. 1989). Indeed, karyological studies showed differences between *Vicia faba* (2n=12) and other species belonging to the genus *Vicia* (2n=14) and all attempts at interspecific hybridization between *Vicia faba* and these other species have failed. Thus, the gene pool of *Vicia faba* is still restricted to itself. There are several classifications proposed for faba bean. Based on differences in seed weight, shape and size, Muratova (1931) recognized two subspecies *paucijuga* and *eu faba*. The latter gathered three types, *minor*, *equina* and *major*. Floral biology in faba bean is intermediate between allo and autogamous (Hanelt and Mettin 1989). Natural out crossing varies widely among cultivars and it is reported to be 35% (Bond and Poulsen 1983) to 60% depending on the genotype and environmental conditions (Suso and Moreno 1999).

Isozymes markers are often used to study the systematic of species or to measure levels of variation within and among populations (Hamrick and

Godt 1990). Furthermore, an understanding of the way genetic variation partitioned among populations is of primary importance for the conservation of genetic diversity of plants species. Isozymes have also proved to be useful markers to distinct different gene pools (Gepts et al. 1992), to study mating systems (Brown et al. 1989) and to evaluate the potential value of germplasm accessions in plant breeding programmes (Murphy and Phillips 1993). In *Vicia faba*, isozyme markers has been used for taxonomic studies (Yamamoto et al. 1982), for varietal identification (Kaser and Steiner 1983), to measure levels of variation within and among populations (Ouji et al. 2011) and for other aspects of breeding activities such as genetic control of some enzymatic systems (Mancini et al. 1989) and assessment of heterozygosity and out-crossing rate (Carre et al. 1998).

Recently, molecular markers; which are supposedly free of environmental influence and readily detected at any stage in any part of the plant, through direct genome analysis; provide an efficient method for genetic resources characterization, genetic diversity study and organization at different levels (Loerz and Wenzel 2004). The sequence-specific amplification polymorphism (SSAP) is an anchored PCR approach derived from AFLP (Amplified Fragment Length Polymorphism) (Vos et al. 1995), which amplifies the region between a retrotransposon insertion and an adjacent restriction site approach (Waugh et al. 1997); seem to be one of the most popular retrotransposon -based molecular marker methods. Retrotransposons are mobile genetic elements capable of changing their location in the genome with the potential to produce a wide array of changes in the genomes of their hosts (Kidwell and Lisch 2001). They are the most abundant class of transposable elements and they outnumber the genes in the eukaryotic genomes (Sabot and Schulman 2006). Plant genomes contain hundreds of thousands of these elements, which constitute a significant fraction of plant genomes (Schulman and Kalendar 2005). Most of studies, using LTR (Long Terminal Repeats) retrotransposon-based SSAP marker systems developed in faba bean (Sanz et al. 2007), barley (Waugh et al. 1997), pea (Flavell et al. 1998; Pearce et al. 2000), common bean (Galindo et al. 2004) and cashew (Syed et al. 2005), suggested that the performance of an SSAP marker system in a species depends upon the particular retrotransposon chosen.

The aim of this study is to compare our results obtained previously (Ouji et al., 2012) using SSAP markers to those obtained by our self (Ouji et

al., 2011) using isozymes markers and evaluate the potential of these markers in assessing the genetic variation of populations. The information will be useful to understand the degree of congruency and necessary for future faba bean improvement to design conservation strategies.

Materials and methods

Plant material

Nine Tunisian populations were used in this study. These populations were described previously by ouji et al. 2011.

Isozyme analysis

Seven enzyme systems (Superoxide dismutase 'SOD', 6-Phosphogluconate deshydrogenase '6-PGD', Malic enzyme 'ME', Esterase 'EST', Shikimate deshydrogenase 'SKDH', Formate deshydrogenase 'FDH' and Glutamate deshydrogenase 'GDH' were assayed in our latest study (Ouji et al. 2011).

DNA extraction, LTR sequences and SSAP molecular marker analysis

Plant DNAs were extracted from the leaves of *Vicia faba* by the method described by Torres et al. (1993). Three LTRs were used in this study (*PDR1*, Tps19 and Tvf4). For all primers, the SSAP procedure was performed as described by Syed et al. (2005) and presented in our study (Ouji et al. 2012).

Statistical Analysis

For isozyme and SSAP markers, polymorphic intra-population parameters, genetic diversity within and among populations and genetic structure were calculated as described by Ouji et al. (2011) and Ouji et al. (2012) respectively.

In the current study, the relationships between the results of the different markers methods were analysed using Mantel's test for correlation between matrices (Mantel 1967). This test compares the elements of two matrices and estimates the degree of correlation between the matrices by means of a test criterion, Z, and a product– moment correlation, r.

Results

Isozyme genetic diversity and population structure

In our previous paper (Ouji et al. 2011) we have analyzed the phenotypic enzyme polymorphism, the intra-population polymorphic loci and the genetic diversity within and among population's. Results showed that the percentage of polymorphic loci (P), the average of allele per locus (A), total genetic diversity (HT) and intra-population genetic diversity (HS) were 59.26 %, 1.914, 0.263 and 0.206, respectively. The dendrogram based on Nei's genetic distance of the populations using UPGMA method showed some genetic drift between populations.

SSAP genetic diversity and population structure

In our latest paper (Ouji et al. 2012), results showed that PDR1, Tps19 and Tvf4 primers provided a total of 173 amplified bands, with 123 of them being polymorphic. The genetic diversity within population of 0.743 was clearly higher than that of among population genetic diversity (Dst = 0.138). The dendrogram based on Nei's genetic distance of the populations using UPGMA method showed that the local major faba bean 'Batata' was the most divergent population and was separated from other population.

Mantel's test

The analyses of correspondence between the two methods based in Mantel test revealed a low correlation (0.177). Therefore, the higher variation was observed for the population Batata that showed no genetic structure similarity with the other populations using SSAP markers and with 'Badii' that showed no genetic structure similarity with the other populations using isozyme markers

Discussion

The knowledge of the genetic diversity estimated from different genetic marker systems provides useful information to address breeding programs and germplasm resource management. In this study, isozymes data analysis was compared with molecular analyses (SSAP markers) to investigate the genetic relationships among nine faba bean populations. This information is particularly important in faba bean in which floral biology is intermediate between allogamous and autogamous (Hanelt et al. 1989) susceptible to severe inbreeding depression (Bond 1993).

Our results obtained with isozym marker (Ouji et al. 2011) and SSAP marker (Ouji et al. 2012) showed a high genetic variation within populations. The high level of genetic variation could be explained by the floral biology system of tested faba bean that presented a high level of allogamy than autogamy. This biology system is useful for conservation strategies and breeding programs. Furthermore, the total genetic diversity generated by the SSAP markers was higher than that revealed by isozymes. This is in agreement with previous study based on RAPD (Random Amplified Polymorphic DNA) and isozyme markers (Torres et al. 2003). This difference could be attributed to (i) the ability of molecular markers to detect variations in both coding and non coding genes (Aagaard et al. 1998) (ii) the high rate of mutation detected by molecular markers. Indeed, rates of mutation detected by isozymes and molecular markers were 10^{-6} (Voelker et al. 1980) and 10^{-2} to 10^{-4} (Tautz 1989) respectively.

According to our estimates of genetic variation analysed by isozymes and SSAP markers, the species showed a high genetic diversity within populations and low divergence among them, as it has been reported for crossing species (Hamrick & Godt 1990). Furthermore, results obtained by Terzopoulos and Bebeli (2008) on 20 local Greek faba bean populations using four ISSR primers, suggested that the majority of the observed genetic variability was due to within population variation (75.4%). Later, Fabio (2010), based on AFLP analysis, consider a substantial level of genetic variation within faba bean accessions of 'Larga di Leonforte' landrace. Since each of applied techniques has advantages and limitations to assess genetic variations, their joint use should be necessary to better estimate the genetic diversity and population structure in order to avoid wrong conclusions.

In the present study, Mantel test slowed weak correlation between molecular and isozyme markers. The result suggests that the two marker approach give different estimates of genetic relations among populations. Although there are not any studies which compare the isozyme and the SSAP markers directly. This weak correlation shows that there is no multilocus association between molecular and isozyme markers in these populations.

Lanner et al. (1996) found moderate spearman's rank correlation (r=0.38) between RAPD and isozyme distances in *Brassica oleracea* populations. On the other hand, Smýkal et al. (2008) showed a weak correlation between isozyme and molecular markers in pea species (*Pisum sativum* L.). This correlation was 0.155, 0.035 and 0.129 respectively

between isozyme and IRAP (Inter-retrotransposon amplified polymorphism); isozyme and SSR (Simple sequence repeat) and between isozyme and RBIP (Retrotransposon-based insertional polymorphism) markers. The no correlation observed in the present work between molecular and isozyme markers can perhaps be explained by an absence of linkage between the loci that control the studied isozyme and molecular markers. It can be also due to the limited number of SSAP marker used. The level of polymorphisms revealed by molecular and isozymes also provided differing views of the amount of genetic variation present in the *E. caninus* accessions (Sun et al. 1999). Furthermore, Isozyme variation only reflects differences in protein-coding genes. Nevertheless, SSAP can detect variation in both coding and non-coding regions. Small, repeated, sequence mutations would be accumulated in non-coding sequences and the diversity can be revealed by SSAP. Other factor which needs to be considered for SSAP analysis is that bands of identical mobility may occasionally correspond to non-homologous fragments (Tinker et al. 1993).

Results showed that the two methodologies gave differing views on the amount of variation, but they all showed a high level of genetic diversity existing in faba bean populations. Thus, both techniques give complementary polymorphism information and are useful for faba bean characterization.

Conclusion

Use of isozyme and molecular markers for characterization and identification of genetic resources should be implemented so that genotypes with known and useful genes can be added to core collections to make them exploitable by breeders. This will facilitate the use of, and add value to, crop plant germplasm resources. Hence important consideration should be given to collection and conservation of local material for breeding, in order to maintain and preserve faba bean germplasm from genetic erosion.

In summary, data showed significant polymorphisms in isozyme and SSAP among faba bean genotypes. Thus, isozyme and molecular markers could be used to characterize faba bean genotypes; this will be of particular importance in the future faba bean improvement program

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