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A comparative study between molecular and agro-morphological methods for describing genetic relationships in Tunisian faba bean populations.

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Abstract - This study aimed to compare the genetic diversity of nine Tunisian faba bean populations by using 27 agro-morphological traits and sequence-specific amplification polymorphism (SSAP) markers. Results showed that faba bean populations exhibited a significant amount of variation for their agro-morphological studied parameters. Different populations were assigned into three groups based mainly on seed size. Furthermore, genetic variability among populations using SSAP markers showed that the average percentage of polymorphic loci for all population was 49.5% and the average Shannon's diversity index was 0.21. The relationships between agro-morphological traits variation and SSAP markers variation were estimated using Mantel test. Experimental results showed low correlation between them (r=0.08). Therefore, these two techniques reveal different aspects of the diversity of faba bean, demonstrating that agro-morphological characters are not good markers for overall genetic variation and SSAP markers cannot resolve plant groups defined by visible traits.

Keywords: Vicia faba, populations, SSAP, genetic diversity, correlation, Mantel test.

1. Introduction

Faba bean (Vicia faba L.; 2n = 12) is a major legume that is used as food owing to the high nutrient components in seeds (Duc 1997). Vicia species are genetically separated from any other species in its family (Hebblethwaite 1983) according to differences in some of the seed characters such as weight, shape and size.

Different approaches have been used to assay genetic diversity in crop plants including morphological traits and isozyme markers, however, these techniques are insufficient to serve as accurate markers due to environmental influences on morphological traits which influence the phenotype and insufficient polymorphism produced among closely related genotypes (Matus and Hayes 2002). DNA-based molecular markers are free of environmental influence and could readily be detected at any stage and part of the plant, through direct genome analysis. Therefore, DNA markers provide an efficient method for genetic resources characterization, through which genetic diversity and organization at different levels can be assessed (Lanaud and Lebot 1997; Loerz and Wenzel 2004).

The sequence-specific amplification polymorphism (SSAP) marker method is an anchored PCR approach derived from AFLP (Vos et al. 1995), which amplifies the region between a transposon insertion and an adjacent restriction site approach (Waugh et al. 1997). SSAP is the most popular

transposon-based molecular marker method. Most studies, using long terminal repeat (LTR) retrotransposon based SSAP marker systems have suggested that the performance of an SSAP marker system in a species depends upon the particular retrotransposon chosen. Knowledge of genetic variation and relationships between accessions or genotypes is important: (i) to understand the genetic variability available and its potential use in breeding programs, (ii) to estimate any possible loss of genetic diversity, (iii) to offer evidence of the evolutionary forces shaping the genotypic diversities, and (iv) to choose genotypes to be given priority for conservation (Thormann et al. 1994). The aim of this study is to compare the genetic diversity of nine Tunisian faba bean populations by using agro-morphological traits and sequence-specific amplification polymorphism (SSAP) markers and evaluate the potential of these markers in assessing the genetic variation of populations in order to understand the degree of congruency.

2. Materials and methods

2.1 Plant material

Nine Tunisian populations were used in this study (Table1). They belong to the major and minor botanical classes of faba bean.

Table1. Population common names, origins/pedigree and botanical class of faba bean

Populations Common name	Origins/pedigree	Botanical class
Malti	Local population	Major
Batata	Local large seeded landrace collected from Boussalem (Tunisia)	Major
Chahbi	Selection from cross S83182-22 / (New Mamoth x Local Tunisian faba bean) – Commercial variety (INRAT) Commercial variety	Major
Super aguadulce	Commercial variety	Major
Aguadulce	Local population	Major
Chemlali	Selection from Tunisian population – Commercial variety (INRAT)	Major
Badï	Pure line developed from FLIP84-59FB (S82166) – Commercial variety (INRAT)	Minor
Bachaar	Local small seeded landrace Collected from Boussalem (Tunisia)	Minor
Masri		Minor

2.2 Agro- morphological traits measurement

Twenty seven agro-morphological traits taken during plant cycle and based on the faba bean IPGRI descriptors were assigned. These agro-morphological traits were related to the (i) plant growth (Seed width, Leaflet width, Nodes number until first flower, Plant height at flowering, Average length of fresh pods, Plant height at maturity, Seed length and Seed width), (ii) the plant fertility (Number of stems per plant at flowering, Flowering date, Total inflorescence, Total number of flower, Petal length, Ovary Lengths, Style lengths, Number of fructufal nodes on the primary stem at maturity, Number of fructufal nodes per plant at maturity, Number of stems per plants at maturity, Number of fructufal stems per plants at maturity, Number of pods per fructufal node and the Number of pods per plant) and (iii) yield components (Average number of seeds per plant, Average number of seeds per pod, Seed number per fructufal nodes, Seed yield per plant and 100 seed weight).

2.3 DNA extraction, LTR sequences and SSAP molecular marker analysis

Plant DNAs were extracted from the leaves of faba bean 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 by Ouji et al. (2012).

2.4 Statistical Analysis

Agro-morphological data were analyzed using MVSP 3.13 software (Kovach 1993). For molecular marker, the Shannon's H index was estimated using POPGENE ver. 2.9.3.2 program (Goudet, 2001) and the percentage of polymorphic loci per population was analyzed using FSTAT ver. 2.9.3.2 program (Goudet 2001). For both Agro morphological and molecular markers, dendrograms separating populations were constructed using UPGMA metod based on matrix distance (Nei 1978).
The relationship between the agro-morphological distance matrix and the distance obtained with SSAP markers was analyzed. The correspondence between pairs of matrices based on the agro-

morphological and molecular distances was tested with Mantel Z-statistic (Mantel 1967) using MxComp procedure from NTSYS 2.02 program (Rholf 1993) with 1000 permutations.

3. Results and Discussion

3.1 Diversity analysis based on Agro-morphological traits

Analysis of variance revealed a high significant variation for all traits studied except for number of stems per plants at flowering. Results illustrated in Table 2 showed that the number of fructufal nodes per plant, the number of pods per plant, seeds number per plant, 100 seeds weight, total of inflorescence per plant, number of pods per primary stem, number of fructufal nodes per primary stem and seed width exhibited strong and positive association with yield. These traits were used as selection criteria for improving faba bean. Dendrogram, calculated using Nei's (1978) genetic distance and based on the agro-morphological data using UPGMA method, assigned the different populations into three groups based mainly on seed size (Figure 1 A). Agronomic traits in faba bean, as in other species, are usually complex characters determined by several interacting components, some of which are under polygenic control (Avila et al. 2005). This fact greatly hampers the selection process and the success of traditional breeding programs. Since only a wide genetic base gives the opportunity to select genotypes with a trait of interest, it is essential to understand the extent and distribution of genetic variation.

Table2. Agro-morphological traits correlated to seed yield per plant

	Seed yield per plant
Number of fructufal nodes per plant	0.63*
Number of pods per plant	0.59*
seeds number per plant	0.53*
100 seeds weight	0.40
Total of inflorescence per plant	0.40*
Number of pods per primary stem	0.41*
Number of fructufal nodes per primary stem	0.32*
Seed width	0.37*

3.2 Evaluation of SSAP markers for diversity estimates

Sequence specific amplification polymorphism (SSAP) markers were used to investigate the genetic diversity and population genetic structure of nine Tunisian faba bean populations. The percentage of polymorphic loci per population and Shannon's diversity index per population were determined. Results of Table 3 showed that the percentage of polymorphic loci ranged from 38.73% for the population 'Badi' to 54.91% for the population 'Malti'. The average value for the whole population was 49.52%. Shannon's diversity index per population ranged from 0.17 for the population 'Badi' to 0.32 for the population 'Chemlali'. The average value for the whole population was 0.21.

Table 3. Polymorphic loci and Shannon's diversity index among populations

	Polymorphic Loci (%)	**Shannon's. Diversity Index**
Chemlali	53.2	0.32
Bachaar	53.2	0.23
Massri	53.8	0.21
Aguadulce	52.0	0.21
S.Aguadulce	46.2	0.19
Malti	54.9	0.2
Batata	49.1	0.25
Chahbi	44.5	0.17
Badii	38.7	0.17
Means	49.5	0.21

The dendrogram grouping the populations by unweighted pair-group method with arithmetic averages (UPGMA) method revealed three main clusters (Figure 1 B). The local major faba bean 'Batata' was the most divergent population and was separated from other population. The SSAP marker system is more informative than AFLP for studying genetic diversity, since it provides higher levels of polymorphism as in other species such as in barley (Waugh et al. 1997); in pea (Ellis et al.1998) and in tomato and pepper (Tam et al. 2005). However, we have not tested whether this is also true in Vicia faba or not.

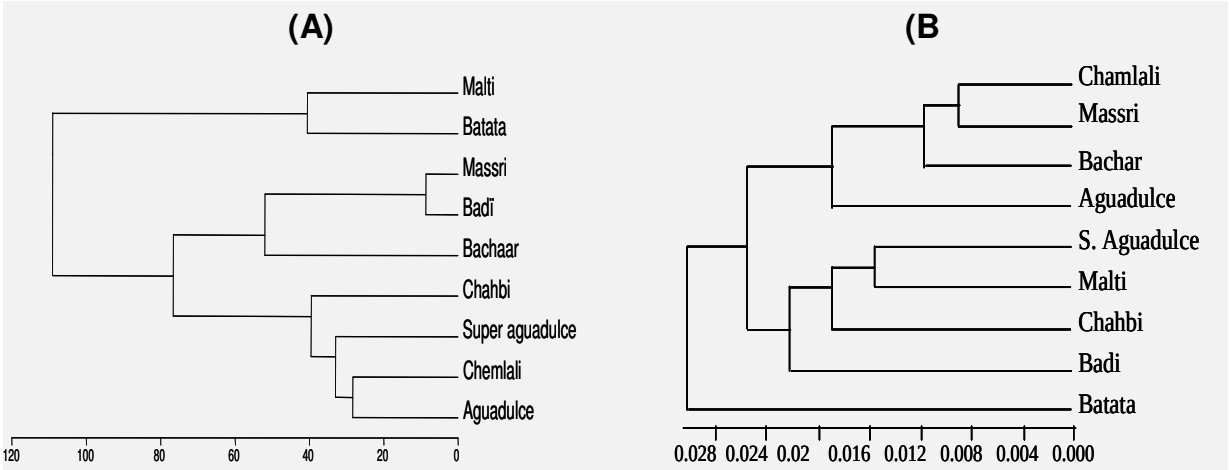

Figure 1. Dendrogram of genetic dissimilarity between the 9 faba bean populations, obtained from agro-morphological characters (A) and SSAP markers (B) by using the UPGMA clustering method.

3.3 Mantel's test

The comparison of different methods of estimating the genetic diversity could define their usefulness in plant breeding and conservation programs. In this study, in order to investigate the genetic relationships among nine faba bean populations and to compare the extent of agreement among dendrograms, derived from agro-morphological traits and molecular markers, a distance matrix was constructed for each assay and compared using the Mantel matrix correspondence test. The Mantel Z test statistics showed low correlation (r=0.08) between the matrices based on SSAP markers and agro morphological traits (Figure 2). In fact, calculated distances between populations by both methods are different. This large variation is clearly observed with the population 'Batata' that showed no genetic structure similarity with the other populations using SSAP markers.

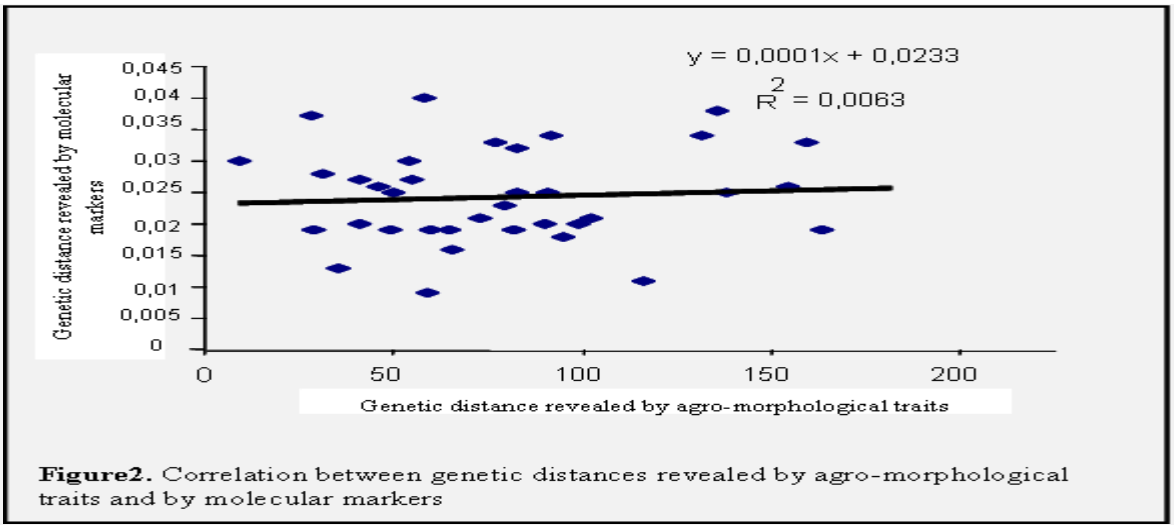

Figure2. Correlation between genetic distances revealed by agro-morphological traits and by molecular markers

Compared to molecular markers, the strong environmental evidence on agro-morphological traits makes these traits relatively less reliable and inefficient for precise discrimination of closely related populations and the analysis of their genetic relationships. However, phenotypic traits are useful for preliminary, fast, simple, and inexpensive genotype identification and can be used as a general approach for assessing genetic diversity among different cultivars (Marti et al. 2007). In the present study, Mantel test did not confirm the correlation between molecular and agro-morphological distances. The result suggests that the two marker systems give different estimates of genetic relations among populations. This is in agreement with studies of Greene et al. (2004), Bushehri, (2005) and Taran et al. (2005) who used morphological characters and Molecular markers respectively in red

clover (Trifolium pratense L.), in Barley (Hordeum vulgare L.) and in pea (Pisum sativum L.). Kiani et al. (2002) found that the cluster analysis based on molecular markers has no relationship with morphological and agricultural characters. This means that samples with the same banding pattern in one group may be completely different in morphological and agricultural characters, so molecular marker may have no correlation with morphological characters. Also, Casiva et al. (2002) didn't find a high correlation between morphological and RAPD methods in broad bean. Furthermore, Martínez et al. (2003) indicate a difference between RFLP and morphological markers in Vitis vinifera L. Spooner et al. (2005) have obtained low correlation coefficient between potato genotypes by means of AFLP and morphologic characters. This weak correlation shows that there is no multilocus association between molecular and morphological traits in these populations. Thus, in our study, SSAP markers were not efficient indicators of morphological difference. In this study, the DNA markers and morphological traits will not necessarily gain closely matching results. The low correlation observed in the present work between molecular markers and agro-morphological traits can perhaps be explained by an absence of linkage between the loci that control the studied agro-morphological characters and the evaluated markers. It can be also due to the limited number of SSAP marker used. Another possibility is that the morphological characters are determined by a few alleles, whose genotype does not correlate with the overall marker scores for the lines. It would be interesting to search for linkage between particular SSAP bands and particular traits, perhaps by a bulked segregate approach. Semagn (2002) suggested two reasons for low correlation between DNA markers and morphological as well as protein data: (a) DNA markers cover a larger proportion of the genome, including coding and non coding regions, than the morphological markers and (b) DNA markers are less subjected to artificial selection compared with morphological markers. In contrary to our results, Duarte et al. (1999), found a correlation of 0.89 between the genetic distances obtained with RAPD and Mahalonobis distances indicating that the markers provide similar estimates of genetic divergence to those obtained using morpho-agronomical data on bean cultivars. Beyene et al. (2006) report significant and positive relationship between morphological and AFLP-based distances in traditional Ethiopian highland maize accessions. Our results suggest that molecular approaches along with agro-morphological studies may be used to evaluate genetic diversity and assess the genetic relationships between faba bean populations with high accuracy. Therefore, the classification obtained for these faba bean populations, based on agro-morphological traits and molecular markers will be a useful tool to breeding and to plan crosses for positive agronomic characters by choosing genotypes with appropriate diversity. We think that correlation between faba bean populations could be improved if there was more morphological markers analyzed as was previously reported by other researchers (Martínez and Sancha 1997a; Martínez and Sancha 1997b) or more primer combination of SSAP were used. Agro-morphological and molecular markers could be used to characterize faba bean population. This will be of particular importance for future faba beans genetic improvement programs.

4. Conclusion

Agro-orphological traits and molecular markers could be used to characterize the Tunisian's faba bean populations. The relationship between agro-morphological and SSAP -based distances was very low. This correspondence between different methods might be improved by analysing more agro-morphological traits and DNA markers. Based on this study, distinctive genetic profiles and agro-morphological traits were identified. This information will be useful for further collections and conservation of the unique diversity included in the Tunisian's faba bean populations.

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