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Review

Marker-assisted selection in faba bean (*Vicia faba* L.)

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ABSTRACT

Among the multiple applications of DNA markers in breeding, the most promising for cultivar development is marker-assisted selection (MAS). Genetic improvement by MAS has been carried out with success in several legume crops such as soybean, common bean and pea, however, in other species such as faba bean it is still in its early stages. This article provides an overview of the genomic resources and molecular markers currently available in faba bean, with an emphasis on development and application of MAS for genetic improvement of the crop. In general, genetically simple traits have received more attention than genetically complex characters encoded by multiple genes. Research has mainly focussed on developing molecular markers for selecting resistance to a parasitic weed and other major diseases. As a result, molecular breeding for resistance to crenate broomrape, ascochyta blight, rust and chocolate spot is underway, and promising results have been obtained. Recently, markers linked to a gene controlling growth habit or to select against traits affecting the nutritional value of seeds (tannins, vicine and convicine content) have also been reported, which may facilitate a more efficient selection of new cultivars free of anti-nutritional compounds. In the near future, molecular markers should be developed for many other highly sought-after traits that are difficult to breed conventionally such as frost or drought tolerance. Comparative genomics and synteny analyses with closely related legumes, together with extensive mapping of resistance gene analogs (RGAs), will reveal new candidate genes and selectable markers for use in MAS. Finally genomic tools such as macro- and microarrays may eventually become available for use in faba crop improvement.

Contents

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1. Introduction

The potential benefit of using markers for indirect selection of traits of interest has been obvious for many decades (Sax, 1923). At present, different kinds of molecular markers are available, based on different methods of detection: hybridisation, polymerase chain reaction (PCR) and DNA sequencing. From the early, restriction fragment length polymorphism (RFLP), a new generation of DNA markers such as randomly amplified polymorphic DNA (RAPD) markers, amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs or microsatellites), sequence characterized amplified regions (SCARs), sequence tagged sites (STS), single strand conformational polymorphism (SSCP) and single nucleotide polymorphisms (SNPs), among others, have been introduced into modern plant breeding systems. The wide range of markers currently available have dramatically increased our knowledge of the genetic diversity within many plant species, and have greatly facilitated the mapping of regions of the genome that contribute to trait variation.

Among the multiple applications of DNA markers in breeding, the most promising for cultivar development is marker-assisted selection (MAS). This approach is based on the selection of markers in segregating populations to trace, and/or pyramid favourable alleles at target loci in a given genome. With MAS, markers associated with desirable traits are identified and used to assist phenotypic screening.

The fundamental advantages of MAS compared to conventional phenotypic selection are the simplicity and reliability of the screening process, which can be performed at early seedling stages, thus saving both time and resources. MAS can complement conventional field breeding by speeding up the selection of desirable traits (Morris et al., 2003; Peleman and van der Voort, 2003) and increasing selection efficiency (Dekkers and Hospital, 2002; Ragot and Lee, 2007). These advantages may translate into time and labour savings by replacing difficult and time-consuming field trials with DNA marker tests (field trials may need to be conducted at particular times of the year at specific geographic locations, or are technically challenging in case of traits that are difficult to score). Moreover, molecular markers are unaffected by the conditions in which the plants are grown. As a result, selection based on DNA markers can be much more reliable and cost effective than conventional screening for the target trait.

Applications of the MAS method have shown that its success depends upon several critical factors, including the genetic basis of the trait, distance between the flanking markers and the target gene, number of individuals that can be analysed, genetic background in which the target gene has to be transferred and the technical options available at the marker level (Francia et al., 2005). Accordingly, MAS has been mostly employed for simply inherited traits rather than for polygenic traits. For major gene traits such as many disease resistances, gene validation is fairly straightforward since the effect of genetic background is usually minimal and the relative ease of phenotyping makes mapping of the gene easier. However, in many cases, these markers may not be polymorphic in all breeding populations of interest, thus requiring the identification of alternative markers for new genetic materials.

In case of polygenic traits, there are much more factors that influence the accuracy of quantitative trait loci (QTL) mapping, such as population size and type, level of replication of phenotypic data, environmental effects and genotyping errors. These factors are particularly important for more complex quantitative traits with many QTLs each of which has relatively small effects (e.g. some types of disease resistance, drought tolerance or yield). Therefore, it has become widely accepted that QTL confirmation, validation and/or additional marker testing steps may be required after QTL mapping and prior to MAS. These steps include: (1) test the accuracy of results from the primary QTL mapping study in different environments, (2) verification that a QTL is effective in different genetic backgrounds and (3) marker conversion of the most tightly linked markers (e.g. SCARs) to make genotyping technically simpler for MAS or to improve its reliability.

With the availability of molecular markers and genetic maps, MAS has now become feasible both for traits controlled by major genes as well as for QTLs. To date, however, MAS has proven to be more effective for simple traits related with the control of pest and diseases. Selected examples in cereals, roots and tubers were reviewed by Dwivedi et al. (2007). By contrast, there are very few reports of successful single gene transfer by MAS in legumes, and these are mostly limited to three crops soybean, common bean and pea. Thus, MAS schemes for resistance to bacterial blight in common bean (Yu et al., 2000) and to cyst nematode in soybean (Concibido et al., 1996) have allowed the transfer of desired genes into improved breeding lines.

For more complex traits, MAS has been less effective, although different reports of successful applications exist in crops such as maize, rice and barley (Collard et al., 2005; Francia et al., 2005; Dwivedi et al., 2007). In legumes, working examples of gene pyramiding combining QTLs for resistance with monogenic traits have been carried out by Walker et al. (2002, 2004) in soybean and Faleiro et al. (2004) in common bean. This situation clearly reflects the stage of development of genomics and the number of completed trait mapping studies in these legume crops.

Faba bean is a relatively small crop compared to soybean, the fourth most important crop worldwide. By cultivation area, it ranks fourth among the cool season food legumes (2.6 million hectares per year), behind pea, chickpea and lentil (http://faostat.fao.org). Traditionally grown in the Mediterranean basin, the Nile valley, Ethiopia, Central and East Asia, Latin America, northern Europe, North America and Australia, more than 80% of the cultivation has been traditionally performed in developing countries where research funding and expertise in novel molecular breeding approaches is limited. However, significant advances have been made towards understanding the faba bean genome ($2n = 12$; $x = 6$), one of the largest among legumes (1C = 13.33 pg $\approx$ 13.000 Mbp, Bennett and Smith, 1976; Johnston et al., 1999). Thus, the use of molecular markers and the development of suitable F_2 and advanced inbred populations have allowed significant progress in mapping to enhance breeding strategies in the species. This article provides an overview of the genomic resources currently available in faba bean, with an emphasis on development and application of MAS for genetic improvement of the crop.

2. Progress towards molecular breeding in faba bean

From the preliminary faba bean linkage maps based on RFLPs, RAPDs and morphological traits reported by van de Ven et al. (1991) and Ramsay et al. (1995), additional F_2 populations derived from trisomic plants were developed at the IFAPA in Córdoba, allowing the assignment of loci and linkage groups to their respective chromosomes (Torres et al., 1993, 1995; Satovic et al., 1996). Subsequent isozyme loci assignment (Torres et al., 1998) and mapping efforts used additional F_2 families to detect, for the first time in the species, putative QTLs for seed weight (Vaz Patto et al., 1999).

In order to map QTLs controlling crenate broomrape, Roman et al. (2002) developed a linkage map using a F_2 population derived from the cross Vf6 $\times$ Vf136, that was also used to detect QTLs controlling ascochyta blight (Roman et al., 2003). Finally, a composite map of the *V. faba* genome was constructed incorporating data from 11 F_2 families as published by Satovic et al. (1996), Vaz Patto et al. (1999) and Roman et al. (2002), all sharing the common female parent Vf6. The composite map was enriched by