



Research review paper

# Genomics and molecular breeding in lesser explored pulse crops: Current trends and future opportunities



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## ABSTRACT

Pulses are multipurpose crops for providing income, employment and food security in the underprivileged regions, notably the FAO-defined low-income food-deficit countries. Owing to their intrinsic ability to endure environmental adversities and the least input/management requirements, these crops remain central to subsistence farming. Given their pivotal role in rain-fed agriculture, substantial research has been invested to boost the productivity of these pulse crops. To this end, genomic tools and technologies have appeared as the compelling supplement to the conventional breeding. However, the progress in minor pulse crops including dry beans (*Vigna* spp.), lupins, lablab, lathyrus and vetches has remained unsatisfactory, hence these crops are often labeled as low profile or lesser researched. Nevertheless, recent scientific and technological breakthroughs particularly the next generation sequencing (NGS) are radically transforming the scenario of genomics and molecular breeding in these minor crops. NGS techniques have allowed *de novo* assembly of whole genomes in these orphan crops. Moreover, the availability of a reference genome sequence would promote re-sequencing of diverse genotypes to unlock allelic diversity at a genome-wide scale. In parallel, NGS has offered high-resolution genetic maps or more precisely, a robust genetic framework to implement whole-genome strategies for crop improvement. As has already been demonstrated in lupin, sequencing-based genotyping of the representative sample provided access to a number of functionally-relevant markers that could be deployed straight away in crop breeding programs. This article attempts to outline the recent progress made in genomics of these lesser explored pulse crops, and examines the prospects of genomics assisted integrated breeding to enhance and stabilize crop yields.

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**Abbreviations:**  $\alpha$ AI,  $\alpha$ -amylase inhibitor; AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; BES, BAC-end sequence; BSA, bulked segregant analysis; CAPS, cleaved amplified polymorphic sequence; cDNA, complementary DNA; CmYLCV, *Cestrum* yellow leaf curling virus; DAiT, diversity arrays technology; ELISA, enzyme-linked immunosorbent assay; EST, expressed sequence tag; FISH, Fluorescence *in situ* hybridization; *gfp*, green fluorescent protein; *GPX-PDE*, glycerophosphodiester phosphodiesterase; GS, genomic selection; GUS,  $\beta$ -glucuronidase; GWAS, genome-wide association study; *hpt*, hygromycin phosphotransferase; Indel, insertion–deletion; ISSR, inter simple sequence repeat; *LaMATE*, *Lupinus albus* multidrug and toxin efflux; *LaPEPC*, *L. albus* phosphoenolpyruvate carboxylase; *LaSCR1*, *L. albus* Scarecrow1; MAS, marker assisted selection; MFLP, microsatellite-anchored fragment length polymorphism; MYMV, mungbean yellow mosaic virus; NGS, next generation sequencing; *npt*, neomycin phosphotransferase; PIC, polymorphism information content; QTL, quantitative trait loci; RADSeq, restriction-site associated DNA sequencing; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; RNAi, RNA interference; RNA-seq, RNA sequencing; SCAR, sequence characterized amplified region; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence tagged sites; TILLING, targeting induced local lesions in genomes; WGP, whole genome profiling.

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## Introduction

Grain-legumes are one of the key resources to generate income for the small-scale farmers practicing subsistence agriculture in the developing world (Varshney et al., 2012). The intrinsic abilities such as symbiotic fixation of atmospheric nitrogen and enduring agricultural adversities (drought, winter, less fertile soil etc.) justify their suitability in sustainable ecosystems and low-input farming systems (Graham and Vance, 2003). Further, by virtue of their high nutritional value (lysine-rich protein content) and better digestibility, grain-legumes proportionately balance the carbohydrate-rich and lysine-poor cereal diets that are predominant in the Third World. Besides satisfying human dietary needs, legumes serve as valuable cover crops, green manure and silage/stock feed (Firincioglu et al., 2010).

Following domestication of grain-legumes, intensive breeding efforts have been dedicated so far to improve their yield. However, the increase in productivity of these “slow runners” lagged far behind than the key dietary staples, in particular the cereals (Borlaug, 1973; Graham and Vance, 2003). A number of factors like cultivation in risk-prone environments, erratic rainfall and prolonged dry spell limit pulse production (Varshney et al., 2011). Equally exacerbating has been their extreme vulnerability to a variety of pests, diseases and abiotic stresses (Table 1). Productive breeding strategies were adopted to overcome these barriers; however, the rate in gains is not large enough to meet the rising protein-calorie demands of the global population and to supply the adequate quality-feed for livestock (Edgerton, 2007; Godfray et al., 2010). To this end, marker assisted selection (MAS)-enabled breeding has recently emerged as a promising approach to answer the questions relating to stagnated crop productivity (Varshney et al., 2012).

Among the various grain-legumes categorized as pulses by the Food and Agriculture Organization (FAO), the *Phaseolus/Vigna* beans including common bean, urdbean, mungbean, limabean, azukibean, ricebean and mothbean are collectively termed as ‘dry beans’ (<http://www.fao.org/waicent/faoinfo/economic/faodef/fdef04e.htm#4.02>). Except common bean which constitutes the major fraction of global dry bean, the rest of the beans are of regional importance and hence, have attracted meager research investments. Similarly, due to cultivation in limited area and restricted market/trade (Naylor et al., 2004), other minor pulse crops like lupins, lablab, lathyrus and vetches rarely catch the attention of the global scientific community. Given prolonged scientific negligence, these crops are often labeled as the forgotten, neglected or underutilized crops (Maass et al., 2010; Vaz Patto and Rubiales, 2014).

In general, pulses have long been overlooked concerning the biotechnological investments, and the research advances in these orphan crops were guided largely by the overflow of the research invested in major crops mainly cereals and model legumes (Naylor et al., 2004).

However, the last decade has witnessed notable achievements related to genomics tools and technologies not only in the major crops but also in lesser explored pulse crops (Gupta and Gopalakrishna, 2013; Maass et al., 2010; Moe et al., 2012; Somta and Srinives, 2007; Vaz Patto et al., 2006; Vaz Patto and Rubiales, 2014). One must thank rapidly decreasing cost and massively increasing throughput of NGS and high throughput genotyping technologies. These NGS-driven advances have facilitated high-confidence detection of large-scale DNA markers, rapid and accurate DNA typing and the development of saturated genetic maps in these crops. Moreover, application of genome-wide strategies like restriction-site associated DNA sequencing (RADSeq) is creating exciting opportunities for marker development, trait mapping and molecular breeding in these crops (Yang et al., 2012, 2013a,b).

Availability of sequenced genomes in orphan legumes represents one of the greatest accomplishments in the area of crop genomics. Further, increasing access to genome sequence data opens up new prospects for applying reverse genetics techniques that determine biological functions of gene(s)/known DNA sequences by suppressing or modifying these candidates (Parry et al., 2009). Likewise, tremendous scope has been generated for forward genetics approaches like QTL mapping that intend to unravel the gene(s)/QTLs underlying a particular phenotype. The advances in genomics and molecular breeding of major pulse crops were comprehensively reviewed by various researchers (Bohra et al., 2014a,b; Kumar et al., 2012; Varshney et al., 2013a, in press), however, only a limited amount of information is available about the trends emerging in genomics of minor pulse crops. Taking into account the immense socio-economic importance of these less-researched crops in some of the poorest regions of the world (Naylor et al., 2004), this article offers an in-depth summary of the latest developments in genomics and molecular breeding of these crops along with a brief discussion on challenges and scope of applying these newly developed tools in predictive crop improvement.

## Available genomic tools and their applications

In recent years, the technological and scientific breakthroughs have allowed the generation of a wide range of genomic tools for these resource-poor crops. These tools include genomic libraries, DNA markers, purposefully built experimental populations, genetic maps, QTLs and the NGS-derived transcriptome and genome assemblies.

### Wide-coverage genomic libraries

The genomic or DNA-insert libraries with extensive genome coverage are the fundamental resource for genomics and cytogenetics research, especially for generating locus-specific or large-scale DNA markers and genome wide as well as chromosome specific-physical

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