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Genomics-Guided Accelerated Improvement of Stress Tolerance in Grain Legume Crops

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Abstract

The current trajectory of population growth indicates that the world population is to cross 9 billion by 2050, and could likely reach 11 billion by the end of 21st century. This upward trend in turn necessitates a quantum leap in the global food production by 70% in order to meet the dietary demands of the global population. The increasing severity of the global crop looses accounted to the pest problems is reflected in the fact that the amount of yield lost to these attacks are adequate to feed almost one billion people worldwide. Pulses are generally grown on marginalized soil and risk prone environment where their production is subject to a number of yield-limiting constraints that include pests, diseases and various other agricultural adversities. Given growing concerns to agricultural sustainability and environmental safety, resistance breeding remains the most economically-viable and widely accepted strategy for developing improved crop cultivars showing markedly enhanced level of resistance to various biotic and abiotic constraints. In recent years, transformational developments in the area of legume genetics and genomics have resulted in the availability of modern genomic tools that are potentially underpinning various crop improvement schemes.

Keywords: Genomics, stress tolerance, grain legume crop

1. Background

The current trajectory of population growth indicates that the world population is to cross 9 billion by 2050, and could likely reach 11 billion by the end of 21st century (Godfray et al., 2010; http://science.nbcnews.com/_news/2013/06/14/18960165-worlds-population-could-hit-11-billion-by-2100?lite). This upward trend in turn necessitates a quantum leap in the global food production by 70% in order to meet the dietary demands of the global population (Tester and Langridge, 2010). Most importantly, the above-stated concern of food security is required to be addressed in the face of narrowing natural resource base, dramatically changing climatic conditions and shifting dietary patterns and land-use change (Pretty and Bharucha, 2014). Equally crucial is to protect our food crops with the rising incidents of biotic and abiotic stresses. The increasing severity of the global crop looses accounted to the pest problems is reflected in the fact that the amount of yield lost to these attacks are adequate to feed almost one billion people worldwide (Birch et al., 2011; see Pretty and Bharucha, 2014). In a similar way, abiotic stress encompassing a wide-array of constraints is also known to claim almost 50% of the production in case of major crops worldwide (see Reddy et al., 2012). Concerning food and nutritional security, grain-legumes along with their counterpart 'cereals' make the dominant portion of vegetarian diets in the less-developed nations (Bohra et al., 2014a).

Given growing concerns to agricultural sustainability and environmental safety, resistance breeding remains the most economically-viable and widely accepted strategy for developing improved crop cultivars showing markedly enhanced level of resistance to various biotic and abiotic constraints. In recent years, transformational developments in the area of legume genetics and genomics have resulted in the availability of modern genomic tools that are potentially underpinning various crop improvement schemes. Towards this end, public access to the whole genome sequences in several leguminous species including model as well as crop genomes represents one of the most noteworthy accomplishments achieved in the area of legume genetics and genomics (Bohra et al., 2014b). Among grain legumes, whole genome sequences have become available for soybean, chickpea, pigeonpea, narrow-leafed lupin and more recently in common bean and mung bean. In parallel, sequencing efforts aiming to decode the entire genomes are under way in other important pulse crops including lentil and adzuki bean. In parallel, next generation sequencing (NGS)-based rapid genome decoding of important pests like bruchid will also help in progressing towards pest management. For examples, Duan et al. (2014) reported the whole genome sequence of *Callosobruchus chinensis* L. using Illumina sequencing chemistry, and identified some valuable microsatellite markers.

2. Major Biotic and Abiotic Constraints Challenging Pulses' Productivity Worldwide

Pulses are generally grown on marginalized soil and risk prone environment where their production is subject to a number of yield-limiting constraints that include pests, diseases and various other agricultural adversities. The major diseases challenging pulses production worldwide are: Ascochyta blight, *Fusarium wilt* (FW), Botrytis gray mold, dry root rot (DRR) in chickpea (Varshney at al., 2013a), FW, phytophthora stem blight in pigeonpea (Varshney at al., 2013a; Bohra et al., 2014a), AB, FW, powdery mildew, rust in pea and AB, FW, DRR, anthracnose and blight in lentil (Bohra et al., 2014a) etc. Among insects of economic concerns, pod borer and storage pets such as bruchids cause substantial yield losses in these pulse crops particularly chickpea and pigeonpea. Apart from these fungal diseases like sterility mosaic disease (SMD) in pigeonpea, yellow mosaic (MYMV) and yellow India mosaic (MYMIV) in mung bean and crinkle virus in urd bean (Bohra et al., 2014b). With regard to abiotic constraints, stresses like drought, heat, cold, salinity and water logging are known to considerably affect the yield in pulse crops (Reddy et al., 2012; Varshney at al., 2013a; Bohra et al., 2014a; Jha et al. 2014).

3. Omics Advances to Tackle Yield-Limiting Biotic and Abiotic Factors

An array of omics technologies has become accessible over the last few years that are indeed helpful in refining the genetic landscape of important complex traits.

3.1. Mapping genetic loci conferring resistance to stress

Resolving the genetic architecture of important traits or component traits that collectively contribute towards stress tolerance or resistance is of foremost importance while addressing the problem of yield-limiting factors. The genetic architecture of traits is dissected by adopting following strategies:

3.1.1. Locating candidate loci using biparental mapping populations

Different types of genetic populations were generated in these pulse crops to construct the genetic linkage map and to discover the responsible genetic factors that reside within genome. In the absence of a linkage map, bulked segregant analysis (BSA) directly delivers a DNA marker that is associated with the trait-of-interest *via* pooling of phenotypic extremes (Collard et al., 2005). If entire population is genotyped, QTL analysis can be performed that connects phenotypic data with the segregation data. In pulse crops, a number of robust markers have been identified which are tightly associated with various abiotic and biotic stress relevant traits (Table 1a b).

Table 1a: Some studies concerning mapping of resistance to biotic stresses

Constraint	Causal organism	Crop	Associated DNA marker	References
Rust	Uromyces fabae	Pea	SSR	Rai et al. (2011)
Ascochyta blight	Ascochyta lentis	Lentil	RAPD	Chowdhury et al. (2001)
	Ascochyta rabiei	Chickpea	SSR	Udupa and Baum (2003); Iruela et al. 2006, 2007; Sabbavarapu et al. (2013)
Fusarium wilt	Fusarium oxysporum f.sp. Ciceri	Chickpea	SSR	Gowda et al. (2009); Sabbavarapu et al. (2013)
Sterility mosaic disease	Pigeonpea sterility mosaic virus	Pigeonpe	SSR	Gnanesh et al. (2012)
Bruchid	Zabrotes subfasciatus	Common bean	SSR	Blair et al. (2010)
	Callosobruchus maculatus	Urd bean	SSR	Souframanien et al. (2010)
	Callosobruchus clanensis	Mung bean	RAPD or SSR	Sun et al. (2008)

Table 1b: Mapping of resistance to abiotic stresses

Constraint	Crop	Associated DNA Marker	References
Drought	Cowpea	AFLP	Muchero et al. (2009)
	Chickpea	SSR, SNP	Varshney et al. 2014; Jaganathan et al. 2014
Heat tolerance	Cowpea	SNP	Lucas et al. (2013)

3.2.2. Detecting gene-trait relationships across natural populations via GWAS

Alternatively, the high-resolution genetic maps and high-density genotyping assays coupled with easy availability of historical phenotypic data are opening up avenues to perform whole genome scans in order to detect the causative genetic loci. For instance, genomes of 300 chickpea accessions were interrogated to discover the candidate genomic regions for drought and heat stresses using 1,872 markers (DArTs, SSRs and SNPs) across multiple locations and seasons. As a result of association analysis, over 300 noteworthy marker-trait associations were found, majority of which contributed to 100-seed weight (Thudi et al., 2014).

3.2. Marker assisted selection (MAS) to enhance the efficacy of breeding schemes

Once a significant QTL is detected, faster transfer of this QTL or gene to diverse genetic backgrounds is an important step in developing the desirable genotype (see Bohra, 2013). Recently, a QTL hot spot region detected originally in two bi-parental populations was transferred from ICC 4958 to a popular variety JG 11 using marker assisted back cross (MABC) method (Varshney et al., 2013b). With the increasing number of published reports on QTL discovery, more instances of MAS are likely to become available in near future.

3.3. Genomic selection (GS)

Encouraged from the simulation as well as empirical results obtained in major crops such as rice, maize, wheat and barley (see Nakaya and Isobe, 2012), GS method is increasingly extended to grain-legume crops (Bohra et al., 2014a; Varshney et al., 2015). Taking the full advantage of modern high-density genotyping assays, GS acts on whole genome marker information recorded on individuals from both *test* and *breeding* populations. Phenotypic evaluation, however, is performed only for *test* or *training* set that basically aims to calibrate the prediction models (Jarquín et al., 2014). Jarquín et al. (2014) have demonstrated the utility of genome-wide prediction models in soybean. Genotyping-by-sequencing (GBS) approach was adopted to generate the high-density DNA marker data, and the analysis achieved satisfactory level of prediction accuracies.

3.4. Phenomics platforms enabling high-throughput phenotypic screens

Recording accurate phenotypic observation on experimental or natural populations still represents a gargantuan task (Hamblin et al., 2011), hence imposes a key restraint to the regular implementation of genomics-assisted breeding for crop improvement. Nevertheless, rapid advances are being made towards establishment of automated and interactive screening of large sets of mapping individuals (see Cobb et al., 2013 for further details). For instance, imaging platforms coupled with softwares like RootReader3D enabled the three dimensional measurement on various root traits (Clark et al., 2011). Such platforms would be crucial in dissecting traits that potentially contribute to stress tolerance in important crop plants.

3.5. Genetic engineering of grain-legumes for incorporating tolerance to biotic and abiotic stress

Genetic engineering is one of the fastest adopted technologies, benefitting 18 million farmers in 27 countries worldwide. The global hectarage has increased 100 fold (~ 175 m h) since last 18 years, testifying in terms of resilience and benefits it delivers to farmers and consumers (ISAAA 2013; http://www.isaaa.org/resources/publications/briefs/46/executivesummary/). Legumes are important source of human nutrition, however yield stagnation, pose a serious threat in face of increasing demand, changing climatic scenarios and emergence of pests and diseases. The potential of this technology in integrated management practices has been demonstrated in the legume soybean, engineered for the traits like insect resistance and herbicide tolerance, occupying highest area of cultivation worldwide. Recently, transgenic common bean immune to the bean golden mosaic virus has been approved for commercial cultivation in Brazil. The time is ripe to harness the potential of this technology for our indigenous legumes or pulses like chickpea, pigeonpea, mung bean, urd bean, peas etc. The important traits to engineer are insect (pod borer, storage pest), virus (yellow mosaic) and fungus (*Fusarium*) resistance, enhanced drought tolerance and herbicide tolerance.

Efforts are on at Indian Institute of Pulses Research, Kanpur in collaboration with national and International Institutes, for development of engineered pulses. Advanced lines of transgenic chickpea and pigeonpea for the *in built* pod borer resistance has already been developed. Engineering chickpea for enhanced drought tolerance, root-knot nematode resistance in fieldpea using *RNAi* technology and control of mosaic viruses by *ribozyme* technology has been demonstrated. Though there are plenty of reports of transgenic development (Table 2), the need of the hour is

Table 2: List of reports of transgenic development in various pulse crops

Crops	Trait	Reference
Chickpea	Gram pod borer resistance	Singh et al. (2009), Sharma et al. (2007), Acharjee et al. (2011), Sanyal et al. (2003), Kar et al. (1997), Mehrotra et al. (2011)
	Callosobruchus spp	Sarmah et al. (2004)
	Aphids	Chakroborti et al. (2009)
	Enhanced drought tolerance	Bhatnagar-Mathur et al. (2009, 2014); Das et al. (2014)
Pigeonpea	Gram pod borer resistance	Lawrence and Koundal (2001), Sharma et al. (2006); Das et al. (2013)
Mungbean	Callosobruchus spp	Sonia et al. (2007)

Crops	Trait	Reference
Black gram	Enhanced drought tolerance	Bhomkar et al. (2008)
Pea	Callosobruchus spp	Schroeder et al. (1993)
	Nematode (RKN) resistance	Das et al. (2011)
Common bean	Golden mosaic virus	Bonfim et al. (2007)

translational research: bringing the materials generated in the laboratories to the field. Apprehensions regarding biosafety of genetically engineered food crops needs to be addressed, before it can be accepted by farmers and consumers.

4. Conclusion

To ensure food security to over nine billion people by 2050, radical transformation is needed in crop breeding technologies. In the context, the rising trend depicting the remarkable technological and scientific advancements in the area of plant genomics are truly noteworthy. We expect that with the help of currently available and rapidly evolving state-of-the-art tools and technologies, designer and climate-smart genotypes endowed with tolerance to various biotic and abiotic stresses could be bred with greater efficiency and improved pace.

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