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Application of Biotechnological Tools for Common Bean (*Phaseolus vulgaris L.*) Improvement

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Abstract

Beans comprise an important source of dietary protein for over half a billion people mainly in developing countries. Across farming systems, biotic and abiotic stresses continue to present the major constraints for increased bean production and high yields with bean diseases representing the major constraints to production by reducing yields and seed quality. The progress in genetic and breeding improvement of bean, applying classical methods reached to his limits in many attitudes. Due to a long process of breeding and selection, the bean genetic diversity has been very limited. Bean breeding programmes are well developed, but there are many limitations of the traditionally breading methods coming from the low recombination potential due to the selfing process, low heritability of some important characteristics (total yields and yield components), and embryo abortion of some inter specific hybrids, etc. Then, alongside the conventional breeding techniques, a biotechnological tool such as tissues culture, in vitro mutagenesis, Identification of quantitative trait loci (QTLs) with marker assisted breeding and genetic transformation has been made to obtain improved common bean varieties. Transgenic and Omics based technologies have been shown to be powerful tools holding a tremendous promise for the future.

Keywords: Transgenic; Omics; QTL; Marker-Assisted Selection; Tissues culture

Introduction

Common bean varieties can be classified into two major gene pools within the species based on their overall seed size and origin in terms of domestication and center of diversity [1] .Studies by means of molecular analyses in common bean strongly support the existence of two primary centers of origin known as the Mesoamerican (small to medium-sized) and Andean (large seed type) regions [2]. *Phaseolus* genus contains approximately 70 species and within this genus, common bean (*Phaseolus vulgaris L.)* is a diploid (2n = 2x = 22) and predominantly self-crossing species although 3% or more out crossing ratio has also been observed [3]. It is now widespread and cultivated as a major food crop in many tropical, subtropical and temperate areas ofthe Americas, Europe, Africa and Asia [4].

Worldwide statistics on common bean are difficult to collect, as the various *Phaseolus* and viggna species are often lumped together. According to FAO, dry beans production (theoretically only *Phaseolus species*) was about 23 million t in 2012, cultivated on 29 million ha. Myanmar, India, Brazil, China, USA, Mexico and Tanzania represented 2/3 of the world production of dry beans while China was the main producer of fresh beans (*Phaseolus and Vigna species*: 17 million t in 2011, 77% of the world production) [5]. According to other sources, 30% of common bean production comes from South America. Common bean is less known in Asia where other grain legumes are preferred [6]. However, production in China is important: estimated acreage in the 2010s was about 0.6 million ha [7]. Across all of Africa, a total of 4 million hectares are planted annually with a total production of around 2.8 million tons [8]. During the 2014/15 cropping year, Ethiopia produced 5137248.07 Quintals of common bean (White and Red) on 323327.27 ha of land and the farmers was achieved 18.45Quintal/ Hectare Yield, stood second after Faba bean [9].

Common bean is the most common food legume in tropical Latin America and eastern and southern Africa and is cultivated as both bush and climbing growth habits [10]. In Ethiopia, dry beans are grown by small scale famers. They are major source of proteins in the lowlands where they are consumed as Nifro, Wasa, Shirowat, soup and samosa [11]. It is one of the fast expanding legume crops that provide an essential part of the daily diet and foreign earnings for most Ethiopians [12]. The consumption of beans reduces of the risk of cancer, especially breast and colon cancer. This could be in part due to the significant amount of antioxidant activity found in the phenolic compounds in the seed coat of beans [13]. Beans are a rich source of folic acid which is especially important for women of child bearing age as low levels of folic acid during pregnancy can lead to neural tube defects in their infants [14]. Despite its importance, bean yields in developing countries are among the lowest in the world, with average of 0.5 tones ha-1 [15] compared to 1–2 tones ha-1 commonly reported in experimental fields. Across farming systems, biotic and abiotic stresses continue to present the major constraints for increased bean production and high yields with bean diseases representing the major constraints to production by reducing yields and seed quality. The progress in genetic and breeding improvement of bean, applying classical methods reached to his limits in many attitudes. Due to a long process of breeding and selection, the bean genetic diversity has been very limited. Bean breeding programmes are well developed, but there are many limitations of the traditionally breading methods coming from the low recombination potential due to the selfing process, low heritability of some important characteristics (total yields and yield components), and embryo abortion of some inter specific hybrids, etc. Then, alongside the conventional breeding techniques, a biotechnological tool such as tissues culture, in vitro mutagenesis, marker assisted breeding, and genetic transformation has been made to obtain improved varieties

Materials and methods

Tissue Culture in Common bean: Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. It includes techniques and methods used to research into many botanical disciplines and has several practical objectives [16]. The potential of plant tissue culture includes: 1) Rapid multiplication of select genotypes, 2) Production of disease-free plants, 3) Germplasm

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preservation and 4) Genetic manipulation. In the last decade, much progress has been made in common bean tissue culture.

In Vitro Propagation of Common bean: Reliable and efficient in vitro culture systems that result in efficient differentiation shoot development and whole plant regeneration is an essential requirement for improvement of common bean through genetic transformation or mutagenesis. Direct or indirect Somatic embryogenesis and organogenesis realized Process of in vitro regeneration in common bean. Different study has been conducted to establish the protocol for in vitro regeneration of common bean (*Phaseolus vulgaris L.)* reported In vitro plant regeneration of *Phaseolus* by organogenesis whereas, also reported regeneration through *somatic embryogenes* is also achieve platelets of common bean by Direct Organogenesis.

Production of Disease-Free Plants: Meristem-tip culture is the excision of the organized apex of the shoot from a selected donor plant for subsequent in vitro culture. It could be used in mass propagation, elimination of viral pathogens (including seed borne viral infections in legumes) and germplasm preservation. The conditions of culture are regulated to allow only for organized outgrowth of the apex directly in to a shoot, without the intervention of any adventitious organ.

Genetic Transformation in Common bean: Gene transfer is a transfer of a gene from one DNA molecule to another DNA molecule. Genetic transformation is a powerful tool and an important technique for the study of plant functional genomics, i.e., gene discovery, new insights into gene function, and investigation of genetically controlled characteristics. It enables the introduction of foreign genes into crop plants, expeditiously creating new genetically modified organisms. Gene transfer represents a relatively new possibility for the treatment of rare genetic disorders and common multi factorial diseases by changing the expression of a person's genes (**Figure 1**).

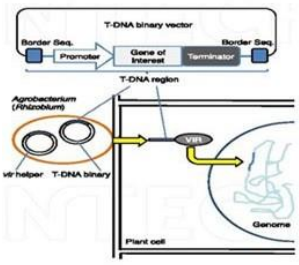

Figure 1: The Agrobacterium-mediated transformation process.

Results and discussions

Marker assisted Selection in Common bean: Traits that is difficult to manage through conventional phenotypic selection because they are expensive or time-consuming to measure, or have low penetrance or complex inheritance. Traits whose selection depends on specific environments or developmental stages can influence the expression of the target phenotypes. Maintenance of recessive alleles during backcrossing or for speeding up backcross breeding in general Pyramiding multiple monogenic traits (such as pest and disease resistances or qualitative traits) or several QTL for a single target trait with complex inheritance (such as drought tolerance or other adaptive traits). Introgression and pyramiding of multiple genes affecting the same trait is a great challenge to breeding programs (**Figure 2**).

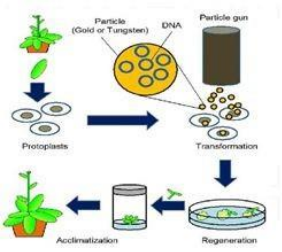

Figure 2: Plant transformation process using particle bombardment.

Molecular Markers: Molecular markers are based on naturally occurring polymorphisms in DNA sequences (i.e. base pair deletion, substitutions, additions or patterns). They are superior to both morphological and biochemical markers because they are relatively, abundant through the genome even in a highly inbred cultivars, completely independent of environmental conditions and can be detected at virtually any stage of plant development. DNA based markers provide very effective and reliable tool for measuring genetic diversity in crop germplasm and studying evolutionary relationships than other available techniques for assessing the genetic variability and relatedness among crop germplasm . They can be applied in the identification of cultivars and clones, genetic mapping, marker assisted selection (MAS), population genetics, molecular systematics and etc. According to molecular markers are 'land marks' which can be identified on the genome and, therefore, offer the best possible means of identifying individuals from biological samples. In recent years, different marker systems such as RFLP, RAPD, ISSR, STS, AFLP, SSR, SNPs, and others have been developed and applied to a range of crops.

Identification of QTL: A QTL (Quantitative Trait Locus) is a chromosomal region supposed to contain a gene or genes that contribute to a quantitative trait. In QTL mapping experiments the genetic basis of quantitative traits is dissected into their single components. Many traits of agricultural importance are quantitative, i.e. based on polygenes. As environmental influences can have a considerable effect on the expression of these traits, DNA markers can have a great impact in breeding for such traits, because selection for quantitative traits normally requires large scale testing in various environments.

Association mapping: The classical method of QTL identification is conducted by a bi-parental QTL mapping approach. Association analysis which does not require development of a bi-parental mapping population is becoming a common method of QTL mapping mainly due to its high resolution, broader allele coverage and cost effectiveness. In this method, diverse lines or cultivars can be used for obtaining information on marker-trait associations. It has the potential to identify QTL associated with a desired trait and even to detect the causal polymorphisms within a gene that are responsible for the difference in two alternative phenotypes.

Genome Wide Association Mapping: Genome-wide association mapping is becoming a widespread method to identify quantitative trait loci (QTL). Its benefit over traditional bi-parental mapping approaches depends on the extent of linkage disequilibrium (LD) in the mapping population and dense marker coverage across the genome.

Candidate Genes association mapping: Candidate-gene association mapping is a hypothesis driven approach to complex trait dissection, with biologically relevant candidates selected and ranked based on the

evaluation of available results from genetic, biochemical, or physiology studies in model and non-model plant species. Because SNPs offer the highest resolution for mapping QTL and are potentially in LD with the causative polymorphism they are the preferential candidate-gene variant to genotype in association studies.

Marker-assisted backcrossing (MABC): Backcrossing is used in plant breeding to transfer (introgress) favorable traits from a donor plant into an elite genotype (recurrent parent). In repeated crossings the original cross is backcrossed with the recurrent parent until most of the genes stemming from the donor are eliminated. However, the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations. In order to minimize this linkage, drag, marker assays can be of advantage.

Omics Technologies: The new "global" methods of measuring families of cellular molecules, such as RNA, proteins, and intermediary metabolites have been termed "-omic" technologies, based on their ability to characterize all, or most, members of a family of molecules in a single analysis. With these new tools, we can now obtain complete assessments of the functional activity of biochemical pathways, and of the structural genetic (sequence) differences among individuals and species, that were previously unattainable. The terms 'Ome' and 'Omics' are derivations of the suffix -ome, which has been appended to a variety of previously existing biological terms to create names for fields of endeavor like genome, proteome, transcriptome and metabolome that are either speculative or have some tangible meaning in particular contexts (**Figure 3**).

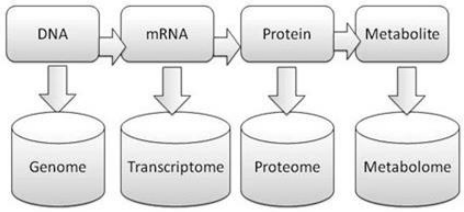

Figure 3: The Central Dogma and the interacting "ome" includes the study of genome, proteome, transcriptome and metabolome source

Conclusion

Common bean (*Phaseolus vulgaris L.*) improvement objectives have been successful using conventional breeding methods to accomplish a wide breeding strategy. Success include the identification of stable genotype, the development of cultivars with resistance to biotic and aboitic stress. Bean breeding programmes are well developed, but there are many limitations of the traditionally breading methods coming from the low recombination potential due to the selfing process, low heritability of some important characteristics (total yields and yield components), and embryo abortion of some inter specific hybrids, etc. Then, alongside the conventional breeding techniques, a biotechnological tool such as tissues culture, in vitro mutagenesis, Identification of quantitative trait loci (QTLs) with marker assisted breeding and genetic transformation has been made to obtain improved common bean varieties. Many bean improvement programs use molecular markers to facilitate cultivar development. Several recent germplasm releases have used molecular markers to introgress and or pyramid major genes and QTL for disease resistance. Genetic mapping in common bean for different traits is facilitated by highly polymorphic and co-dominant microsatellite-based markers and studied from different researchers. Marker assisted selection (MAS) also a good progress and identified a superior targeting of required genes especially for biotic andF abiotic stress.

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