CONTRIBUTION OF MOLECULAR MARKERS TO FOOD SECURITY: INTROGRESSION AND PYRAMIDING

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Abstract

The development of sustainable tolerant/resistant lines with high precision in shortest possible time for agricultural sustainability has been accelerated by gene pyramids through Marker-Assisted Selection (MAS) and other procedures. Gene pyramiding involves the stacking of multiple genes that simultaneously exhibit more than one gene in a given species to develop a sustainable expression of resistance, thereby improving plant breeding efficiency leading to genetic resistance and the accurate development of broad spectrum resistance. Conventional crop-breeding technology alone cannot be adequate in modern day agriculture for the sustainable achievement of the growing population food demand. A number of environmental challenges, including drought, severe temperatures, excessive salt, parasite and insect pest infestation, etc, threaten sustainable agricultural output. In many nations, these difficult environmental circumstances can have negative consequences for future farming production. Therefore, advancement in molecular genetics to the selection of new plant species is promising instruments.

Keywords: *Gene pyramiding, Marker-Assisted Selection (MAS), broad spectrum resistance, food security, new plant species*

Introduction

In the world of increasing food demand scientists are trying to improve and produce more crop in an unfavorable environmental conditions by selecting for desired traits in most crop plants. However, biotic and abiotic stresses remain the greatest constraint to crop production which accounts to an annual yield loss globally (Suresh and Malathi, 2013). Biotic factors such as bacteria, viruses, fungi, nematodes, insect pests and weeds are considered to be that factors limiting crop production (Vincelli, 2016). A recent assessment documents how these stress factors collectively affect all of the components of food security from overall production to physical availability, distribution, economic access, stability of production, quality and nutritive value (Savary *et al*., 2017). For years, the damage caused by these stress factors is being controlled by the use of chemicals. But nowadays, interest in the use of chemicals against biotic stress is decreasing as a result of its various limitations such as there is need for more than one chemical application, an investment that is not affordable by most small-scale farmers (Brading *et al*., 2002). Aside this, application of chemicals may have adverse effects on human health and the environment, including beneficial organisms (Miedaner, *et al*., 2013). On the other hand, the use of resistant cultivars is currently seen as the best strategy, durable, economical, and environmentally friendly means of control (Ragimekula. *et al*., 2013, Klarquist, *et al*., 2016, Hansona, *et al*., 2016).

As regards to the above, molecular breeding efforts can be adopted to incorporate resistant gene into crop plants but care must be taken in doing so as most of the plants would eventually lead to resistance breakdown within a short period (Kottapalli. 2010). Hence, a phenomenon called gene pyramiding can be adopted to target cultivars that can withstand multiple stresses by assemblage of series of genes from different parents into a single genotype (Jain and Bar, 2010, Suresh and Malathi, 2013). This procedure is usually an end product of a genotype with all the target genes present (Guoyou and Kevin, 2008). The technique is very helpful for developing crops that confer broad spectrum resistance against different races of combination of these stress factors (Joshi and Nayak 2010).

This breeding approach involving a series of phenotyping and genotyping by screening of a large population to obtain desired variability and a high frequency of favorable genes (Kumar *et al*. 2014). But there is also a conventional breeding approach involving sequential selection of large segregating populations for biotic and abiotic stresses across generations which helps breeders to develop breeding lines that combines tolerance of both stresses. As a result, a superior line is promoted for release in terms of acceptable plant type, grain yield, quality attributes, and steady performance under various environmental situations (Kumar *et al*. 2014; Sandhu and Kumar 2017). Selection typically involves evaluating a breeding population for one or more traits in field or glasshouse trials. The goal of plant breeding is to develop more desirable combinations of genes in new varieties. (Collard and Mackill 2007).

Therefore, this review focuses on gene pyramiding approaches that have proven to be effective in modern agriculture for boosting crop tolerance to biotic and abiotic challenges and ensuring long-term crop improvement.

Conventional method

Traditional breeding for several years has been used to identify and incorporate multiple resistant genes into cultivars of interest to develop durable resistance to biotic and abiotic stresses (Ragimekula. *et al*., 2013). And this can be confirmed mostly by phenotyping at an individual level where the target genes are present. The breeder's art of recognizing desirable traits which is by physical identification should be good and, pollinating them to produce the next generation is very important. But, conventional phenotype screening approach to gene pyramiding is usually very difficult and sometimes not possible, because of the effects of epistasis of genes of disease resistance, dominance and also due to limitations associated to screening against the two diseases through the year (Sundaram et al., 2014).

However, conventional method of crop improvement has been criticized for being slow, inflexible, labor-intensive and expensive (Wieczorek, 2003; Choudhary *et al*., 2008). With conventional breeding approach, breeder's capability to track the presence or absence of the target genes is very slow and limited. This limits the number of genes to be stacked into elite cultivars at many times (Malav, 2016). Therefore, an intervention that can reduce the cost and time is necessary to be developed so as to release new cultivars with durable resistance

Molecular markers

Recently, Molecular Marker, a biotechnological tool is a widely used tool crop improvement for efficient and rapid accumulation of desirable genes from various sources into a single background to produce broad spectrum/durable resistance (Malav, 2016, Ragimekula. *et al*., Suresh and Malathi, 2013). This has made it easier to identify, map and efficiently pyramid desirable genes/QTLs into crop plants (SH 2003). Hence, identification of genes/QTLs with closely linked DNA-markers is useful for successful transfer of the gene/QTLs into improved cultivars via Marker-Assisted Selection MAS (Campbell *et al*., 2003).

Most of the time in plant breeding program, the primary goal is to find the favorable genes for a desirable traits (Yan and Rajcan 2002, Dehghani *et al*., 2008). Usually, selections of parents used in crosses are from advanced and developed lines. While after crossing the new cultivars should possess superior combinations of characters and parents should serve as excellent donors of one or more traits being targeted by the breeding program (Yan and FrégeauReid 2008). Hence, identifying and utilizing genotype that possesses these targeted characters is of most importance in producing a population for developing a new variety (Samonte *et al*., 2013). However, to target, identify and utilize this approach, Marker Assisted Selection (MAS) needs to be adopted. This approach is applied extensively in numerous ways in agriculture in breeding a new cultivar (Mackill, 2007).

Marker assisted selection

Marker Assisted Selection is used in the pyramiding process for selecting the progenies that possess the desired traits and taking them to the next cross. In such way, the time for selection in the field can be decreased and the efficiency can be enhanced; ultimately creating a broad-spectrum pyramid genotype (Manoj Sapkota 2018). Most times it is difficult to select the multiple traits based only on the plant phenotype since they might be expressed due to epistasis. MAS are often used for selecting the resistance against the stress factor by the application of genetic markers to identify and select the specific genes of interest governing the resistance (Shi, *et al*., 2008). We should not forget also that the desired gene should also be effective and must provide the desired trait and should not have any negative effects on other desired traits.

The target genotypes can be more effectively selected which may enable certain traits to be 'fast-tracked', resulting in faster line development and variety release. Markers are also used as a replacement for phenotyping, which allows selection in off-season nurseries making it more cost-effective to grow more generations per year (Ribaut& Hoisington 1998). Another advantage of MAS is that it can reduce the number of lines that need to be examined. Since many lines can be discarded after MAS early in a breeding scheme, this permits more efficient use of glasshouse and/or field space which is often limited because only important breeding material is maintained.

Furthermore, aids to selection can be carried out at the seedling stage. This may be useful for many traits, but especially for traits that are expressed at later developmental stages. Therefore, undesirable plant genotypes can be quickly eliminated. This may have tremendous benefits in plant breeding because typical plant production practices involve sowing pre-germinated seeds and transplanting seedlings as the case may arise, making it easy to transplant only selected seedlings to the main field. There are several instances when phenotypic screening can be strategically combined with MAS. In the first instance, 'combined MAS' (Moreau et al., 2004) may have advantages over phenotypic screening or MAS alone in order to maximize genetic gain (Lande & Thompson 1990). When there are still unidentified QTLs influencing a characteristic or when a large number of QTLs need to be altered, this strategy could be adopted. This strategy is more efficient than phenotypic screening alone, according to simulation studies, especially when large population sizes are used and trait heritability is low (Hospital et al., 1997). Bohn et al., (2001) investigated into the possibility of using MAS to improve insect resistance in tropical maize and discovered that it was less effective than traditional phenotypic selection. When MAS and phenotypic screening were combined, however, relative efficiency increased slightly. For an example in wheat, MAS combined with phenotypic screening was more effective than phenotypic screening alone for a major QTL on chromosome 3BS for Fusarium head blight resistance (Zhou et al., 2003b). The advantages of MAS over conventional breeding must be fully explored for MAS to attain its full potential for crop improvement.

Pyramiding of multiple traits

With the development of gene identification technologies, gene pyramiding technique targeted for introgression has been successfully demonstrated and typically used in improving biotic and abiotic factors. This strategy has been used as an advanced approach incorporating durable and multiple pathogens for resistance in many crops. (Joshi and Nayak 2010). The most common use of this technique is to integrate one or several highly inherited features into an adapted or elite variety. These different resistance genes often confer resistance to different isolates, races or biotypes. In most cases, for instance, the elite variety used for crossing possesses a large number of desired traits but only has a few features while the other parent, known as the donor parent, has one or more genes that control an important feature that is not present in the elite variety (Hansan et al., 2016).

In addition, since the advent of molecular markers, marker assisted selection has been successfully used in gene pyramiding program in targeting, transferring and pyramiding resistance loci to create more durability and broad-spectrum resistance in various crops (Joshi and Nayak 2010). The use of these molecular markers, permits completes gene identification of the progeny at each generation, thereby increasing the speed of pyramiding process. Thus, this technique permits resistance genes to be accumulated into a single genotype, thereby making use of major genes, different or the same alleles of one gene (Tan et al., 2010). However, gene pyramiding scheme can be distinguished into two parts. The first part is known as a pedigree, and it aims to combine all target genes into a single genotype known as the root genotype, while the second part is known as the fixation step, and it aims to fix the target genes into a homozygous state, i.e. to derive the ideal genotype from a single genotype.

Marker-assisted gene pyraniding in developing crop varieties

Gene stacking, also known as pyramiding, is a useful technique for transferring multiple desired genes or QTLs from different parents into a single genotype in the shortest time possible (two to three generations), as opposed to conventional breeding, which takes at least six generations to recover 99.2 percent of the recurrent parent genome [Suresh et al., 2013, Hasan et al., 2015]. Plant scientists have successfully utilized this technique to pyramid resistant genes or QTLs into crops to increase its durability through the help of closely associated markers against biotic stresses.

For instance, a study carried out by Kumar et al. 2019 used marker-assisted backcrossing and phenotypic selection to pyramid three tomato leaf curl virus resistance genes (Ty-1, Ty-2, and Ty-3), two late blight resistance genes (Ph-2 and Ph-3), and one root knot nematodes resistance gene (Mi1.2) from distinct donor parents. From the study, it was deduced that the introgression of the various genes remarkably enhanced resistance against leaf curl, late blight and root knot nematodes diseases. However, it was concluded that these enhanced lines of resistant multiple diseases can play a significant role in future. Moreover, another study was investigated by pyramiding more than one resistance genes in sweet Charleston pepper line Y-CAR. Molecular markers and biological assay was used to carry out this study. The study successfully transferred resistance genes to these viruses on molecular markers and biological tests to the superior sweet Charleston pepper line Y-CAR. A new line has therefore been developed that is resistant to PVY, TSWV and PMMoV. The results also show that a pyramid strategy for the reproduction of multiple resistance viruses is applicable in pepper.

Furthermore, marker assisted gene pyramiding was successfully carried out to incorporate Phg-2 R of the angular leaf spot resistance gene and two CBB main quantitative feature loci (RQTLs) into the background of the susceptible and most common bean cultivar REDWOLAITA, or RW in Ethiopia. It was deduced that the lines developed were highly resistant to the CBB and ALS strains under screening conditions (Rezene *et al.,* 2019). In a high yielding Malaysian Putra 1 with a genetic background of 3 blasting resistance genes (piz, Pi2 and Pi9) two dominant (Xa4 and Xa21) and two recessive (xa5 and Xa13) Xoo resistance genes were introgressed. The discovery from this investigation through Marker-assisted background selection revealed a high percentage of about 95.9% recurrent parent genome recovery. The incorporation in the newly developed lines of four bacterial leaf blight and three blast-resistant genes (Xa4 + xa5 + xa13 + Xa21; Pi9 + Pi2 + Piz) would provide for wide spectrum and lasting resistance to two important diseases investigated. It is worth noting that blast is caused by *Magnaporthe oryzae* and bacterial leaf blight is caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) are major diseases responsible for significant yield loss in rice production across all rice growing regions (Chukwu *et al.,* 2020).

The technique of Marker-Assisted Selection (MAS) was used to develop ten new lines with blast, bacterial blight (BB) and BPH resistance genes. A higher line was obtained with blast resistor genes, BB and BPH. In general, information about the development of the restorers is useful for rice resistance breeding through the pyramiding various resistance genes (Zhi-juan *et al*., 2016). Arunakumari *et al*., 2016 was able to pyramid bacterial leaf blight (BB) resistance genes and blast resistance gene into an Indian rice variety MTU1010 through Marker-Assisted Selection and it was observed that the improved lines were highly resistant to the disease. Yanchang and Zhongchao 2013 also recorded a success in pyramiding multiple traits of semi-dwarf gene, submergence tolerance gene, blast resistance gene and bacterial blight resistance genes in rice by Marker-Assisted Selection and the improved line was able to carry the designated multiple traits.

Challenges

In some circumstances, there is inadequate linkage between the marker and the gene, resulting in recombination between the marker and the gene/QTL (Sharp et al., 2001). In that wise marker validation is essential to determine a marker's ability to predict phenotype, and this highlights the benefits of employing flanking markers. Furthermore, breeding material has a restricted number of markers and polymorphism of markers. Ideally, markers would be able to detect traits in a wide spectrum of breeding stock. In other words, markers should be able to distinguish between varieties that display the feature and those that do not. Even in some crops with a greater number of accessible markers, there may be certain chromosome regions harboring an important gene or QTL for which polymorphic markers are difficult to discover. Meanwhile, another problem with pyramiding is the influence of genetic background. It has been discovered that QTLs found in one mapping population may not be effective in other populations (Liao *et al.,* 2001). For instance, Steele et al. (2006) discovered in rice that just one of four root-length QTLs was effective when backcrossed into a new variety of rice. In certain circumstances, this is owing to the small effect of an allele transferred into elite varieties (Charcosset & Moreau 2004).

Parents representing the extreme ends of the trait phenotype are often selected for QTL mapping experiments. This increases the likelihood of discovering these QTLs because QTL mapping is based on statistically distinct group of markers. However, the fundamental disadvantage of this method is that one (or both) parents may have QTL alleles that are similar, if not identical, to the elite germplasm employed in breeding programs (Holland 2001).

Moreover, high Marker-Assisted Selection costs are another problem. The cost of using MAS can vary significantly compared to conventional phenotypes, although this is covered by only a relatively small number of studies. The cost-benefit ratio of MAS has been shown to depend on various factors such as the inheritance of the trait, method of phenotypic assessment, the cost of field and glasshouse experiments as well as the cost of labor (Dreher *et al.,* (2003) and Morris *et al*,. (2003). It should also be noted that large initial investments in capital are needed in equipment purchases and that regular maintenance costs are incurred. The costs of MAS could also be affected by intellectual property rights, such as patent granting costs (Brennan *et al*., 2005). One approach to this challenge is to contract out the Marker-Assisted Selection program to sophisticated laboratories that can help in saving cost by the use of high-performance equipment. Generally speaking, the cost of MAS remains a major barrier to its use. It should be noted, depending on the number of samples or the number of marker assays, that MAS cost estimates may change. Dreher *et al*., (2003) study indicated that cost may decrease as the number of samples and/or marker tests increases because many components of MAS are economical in scale and lack divisibility. A current trend is the establishment of marker genotyping firms, which will make it possible to outsourced marker genotyping. In the event that genotyping outsource costs are cheaper and logistical problems are not created or minimized, the breeding programs can offer more MAS opportunities.

Finally, the knowledge gap between plant breeders, molecular biologists, and other disciplines. In recent decades, rapid developments have been observed with DNA marker technology, QTL theory and statistical methodology for the QTL analysis. These concepts used by molecular biologists are not clearly understood by plant breeders and other plant research scientists (Collard *et al*., 2005). Moreover, wide ranges of highly specialized equipment are based on advanced molecular-genotyping techniques. Similarly, molecular biologists may not understand fundamental concepts in plant breeding. This reduces the integration level between conventional and molecular breeding and eventually influences the evolution of new breeding lines.

Conclusion

The breeding of plants has made significant progress in improving crops and it is crucial that this continues. In other words, MAS could be very helpful in reaching this objective, although to date there has been a minimal impact on varietal development. To achieve the MAS potential, greater integration with breeding programs, and the development of current barriers and solutions are imperative. The use of the benefits of MAS in relation to conventional breeding could have a significant effect on crop improvement.

The high cost of MAS will remain a major obstacle for adoption in near future in the developing countries, as a result s budgets may need to be adjusted. Nonetheless new technology for markers would significantly reduce the cost of MAS. When validating the efficiency of the new methods and easily getting equipment, this should allow MAS be more wide-scaled for crop breeding programs.

Future prospects

In this coordinated effort to increase food production, plant breeding will continue to play a crucial role. In the light of current yield trends, projected population growth and environmental pressures, yield stability and sustainability characteristics should be a key focus of efforts in plant breeding. These include resistance to durable diseases, abiotic stress tolerance, and efficiency in nutrient and water use (Mackill *et al*., 1999; Slafer *et al*., 2005; Trethowan *et al*., 2005). Moreover, cultivation of varieties in marginal areas, especially in developing countries, need to be developed (Naylor *et al*., 2004).

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