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NEW STRATEGY ON PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY

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Abstract – Biotechnology has become an important subject in our daily life as it implements new methods and technologies to be used in food quality with food quantity in food processing industry, medicine and agriculture. In agriculture, biotechnological tools was of great help for improving food crops, enhancing their nutritional quality, diseases and pest resistance; and mass production of improved cultivars which is able to feed the growing population demand worldwide. Continuous hard work and improvement in the skills of working in these areas is needed from the researchers and plant breeders to increase food production in our country. There are also other criteria which led to the decrease in food production such as climate change, adaptation by diseases and insects pests. Therefore, just an improved package of practices implemented in the field is not enough to bring more food or to increase the rate of production in the country. Crops which are able to withstand the biotic and abiotic stress should also be produce to the farming sector. Researchers have focussed on the use of biotechnology to enhance food crop production and also to bring quality food in our country. Our focus here is to learn the strategies implemented by biotechnologist such as tissue culture, marker assisted selection, genetic engineering technology, genome assisted breeding, genome sequencing and mapping and genome editing. The principles, methodologies, applications and constraints are discussed here in this review.

INTRODUCTION

Altering the genetic makeup of crop plants has been started since the beginning of agriculture eight to ten thousand years ago. Farmers started selecting the features such as faster growth, higher yields, pest and disease resistance, larger seeds, or sweeter fruits. In doing so a large number of germplasm has been created which is different from the wild ones. The variability or wide diversity of different cultivars is of great importance when selection is done. When the science of conventional plant breeding was further developed in the 20th century, plant breeders understood better how to select superior plants and breed them to create new and improved varieties of different crops. This has dramatically increased the productivity and quality of the plants we grow for food, feed and fibre. Conventional plant breeding methods was used to develop new varieties of crops for hundreds of years. However, conventional plant breeding can no longer sustain the global demand with the increasing population, decline in agricultural resources such as land and water, and the apparent plateauing of the yield curve of the staple crops. With increasing rate of population, the demand for increasing food production has been a major challenge for the research community.

Agricultural productivity has been progressively enhanced by constant innovations, including environment- specific improved crop varieties to increase production (Zepeda *et al*., 2008). Constant innovations including biotic and abiotic stress-specific improved crop varieties have been enhanced for boosting agricultural productivity.

Agricultural biotechnology is proving to be a

powerful complement to conventional methods for meeting worldwide demand for quality food. With the help of modern plant biotechnological tools, today we have access to massive gene pools that can be exploited to impart desirable traits in economically important crops. It is not so simple using conventional plant breeding methods for increasing food production as it normally take more than ten years for a plant breeder to develop a new plant variety which is homozygous and contain the desirable trait in it. Therefore, if someone ask why we need biotechnology when we have been making incredible achievements in our food supply using conventional methods. We have to realize that, today, over 800 million people face daily hunger; furthermore, a majority of the global population growth in the next 50 years will be in developing countries where malnutrition is already prevalent. About 40% of the world's land use for agriculture is already seriously degraded. In order to meet the nutritional needs of this growing population, cereal production alone will need to increase by 40% in the next 20 years. We simply cannot achieve the kinds of yield increases in a sustainable way using traditional methods of breeding. Biotechnological approaches have been developed to tackle the problems of conventional breeding reducing the time require to release a variety/hybrid. These approaches combined the techniques of tissue culture, genetic engineering, marker assisted breeding, genome sequencing and genome editing. Biotechnology is an important tool in addition to all of the other tools to produce a food supply that will be sustainable in the long run and will be able to meet these needs in the future (Young and Tanksley, 1989).

Through this paper we will discuss the strategy of plant molecular biology for crop improvement based on research and findings by different authors. We will discuss the methods, procedures and application of advanced biotechnology.

Crop improvement through tissue culture

In vitro tissue-culture techniques are aseptic growth of cells, tissues and organs. It consists of a large group of strategies and technologies, ranging through molecular genetics, recombinant DNA studies, genome characterization, and gene-transfer techniques. The different applications of tissue culture are discussed as follows.

Protoplast fusion

Protoplast fusion has often been suggested as a means of developing unique hybrid plants which cannot be produced by conventional sexual hybridization. However, while any two plant protoplasts can be fused by chemical or physical means, production of unique somatic hybrid plants is limited by the ability to regenerate the fused product and sterility in the inter-specific hybrids rather than the production of protoplasts. By protoplast fusion it is possible to transfer some useful genes such as disease resistance, nitrogen fixation, rapid growth rate, more product formation rate, protein quality, frost hardiness, drought resistance, herbicide resistance, heat and cold resistance from one species to another. Protoplast fusion an important tools in strain improvement for bringing genetic recombination. In protoplast technology, two genetically different protoplasts isolated from the somatic cells and are experimentally fused to obtain hybrid protoplasts. Protoplast fusion technology has been utilized in many crops to generate allotetraploid somatic hybrids, and sometimes autotetraploids as a by-product of the process. But the most important application of somatic hybridization is the creation of a new germplasm which will be the source of breeding parents for various types of conventional crosses. Successful somatic hybridization in citrus rootstock improvement has enabled rootstock breeding at the tetraploid level via sexual hybridization (Grosser and Gmitter, 2011).

Embryo culture

Embryo culture has been successful in overcoming the post-zygotic failure in distant wide hybridization as well as solving the problems of low seed set, seed dormancy, slow seed germination, inducing embryo growth in the absence of a symbiotic partner. This technique is now routinely used to produce rare hybrids which could not be produced by the conventional method of hybridization (Bhojwani and Dantu, 2013). Embryo culture techniques are also used to overcome dormancy of recalcitrant seeds.

Haploid plant production

Haploid plants originate from gametes that do not go through fertilization, but can still generate a viable individual. They contain only a single set of chromosome. After undergoing chromosome doubling the chromosome set of a haploid plant has been doubled spontaneously and become fertile. This is known as double haploid (DH) individual.

The two different methods for obtaining haploid plants can be classified into two categories i.e. in vitro techniques and in situ techniques. In vitro methods are based on the culture of haploid cells and their differentiation into haploid embryos and ultimately haploid plants. Secondly, in situ methods make use of particular pollination techniques using irradiated pollen, inter-specific crosses or so called 'inducer lines' which are commonly used in plant breeding for maize only (Gilles *et al.,* 2017). Microspore embryogenesis in anther/microspore cultures are the most commonly used methods to generate DHs (Maluszynski *et al.,* 2003). With many species, anther culture has proven to be more effective than isolated microspore culture (Ferrie and Caswell, 2011). In plant breeding, apart from maize, the production of DH plants requires at least an in vitro-based process which its process remains highly dependent on genotype.

Haploid plants are of interest to plant breeders because they allow the expression of simple recessive genetic traits or mutated recessive genes and because doubled haploids can be used immediately as homozygous breeding lines. Companies such as Pioneer and Syngenta are using DHs in their breeding programs to save time and money compared to conventional plant breeding. Haploid plant production allowed plant breeders to develop pure lines rapidly. Replicated trials with large DHs will respond to G × E interaction (Rajcan *et al.,* 2019). This method allows plant breeders to save time, money and help in developing profitable cultivars.

Somaclonal variation

The term somaclone was coined to refer to the plants derived from any form of cell culture, and the term 'somaclonal variation' was coined to refer to the genetic variation present among such plants. Usually variability occurs spontaneously and can be a result of temporary changes or permanent genetic changes in cells or tissue during in vitro culture. Somaclonal variation provides variability in the population which is important for plant breeders for their potential exploitation in crop improvement. Somaclonal variation has been most successful in crops with limited genetic systems (e.g., apomicts, vegetative reproducers) and/or narrow genetic bases. A number of cultivars have been developed through somaclonal variation in different horticultural crops for a range of useful traits.

Micro propagation

Micro propagation is the practice of rapidly multiplying plant material to produce sufficient no of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative production using modern plant tissue culture methods. Micro propagation is mainly used to multiply plants that have been genetically modified or bred through conventional plant breeding methods.

Case studies of micro propagation

Novel methods for recalcitrant plant propagation has been developed. Propagating *Paphiopedilum* orchidsin vitro has been very successful for producing large no of plantlets within a short span of times. A lot of orchid species has been produced in mass from protocorm like bodies' totipotent calli and direct shoot bud formation. This is done to save the large genus of orchids from extinction and to produce large no of flowers.

Synseed in propagation and preservation of some valuable ornamental plants in orchids has been reported through the encapsulation of PLBs of *Cymbidum* spp. Survival rate of these synseeds produce in vitro is 100% and regeneration capacity was also high. The plantlets derived from these synseeds had a survival rate of 100% after six months in green house. Cymbidium PLB was demonstrated to have good vitality for synseed production. Micro propagation has been applied mostly in flower species such as Anthurium, Chrysanthemum, Orchids, Lilium etc.

Marker assisted selection

Selection for specific alleles (which affect a trait of interest) using genetic markers is referred to as marker assisted selection. It is an indirection selection of a specific trait using marker whether it's morphological, biochemical or molecular. DNA markers has been extensively used for several applications in crop genetics such as assessing genetic diversity and quantitative trait loci (QTL) mapping (Salem *et al.*, 2007).

Molecular marker-assisted breeding (MAB) is defined as the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant or animal traits on the basis of genotypic assays. This term is used to describe several modern breeding strategies, including

marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genome-wide selection (GWS) or genomic selection (GS) (Ribaut *et al.,* 2010). A novel method for generating plant DNA markers was developed based on data mining for short conserved amino acid sequences in proteins and designing polymerase chain reaction (PCR) primers based on the corresponding DNA sequence. This method uses single 15- to 19-mer primers for PCR and an annealing temperature of 50°C. PCR amplicons are resolved using standard agarose gel electrophoresis. This method as suggested by Collard and Mackill (2009) could be used in conjunction with or as a substitute to other technically simple dominant marker methods for applications such as targeted quantitative trait loci mapping, especially in laboratories with a preference for agarose gel electrophoresis.

Prerequisites for an efficient marker-assisted breeding program:

- Ease and low-cost of use and analysis DNA Marker;
- Small amount of DNA required;
- Marker which exhibit Co-dominance;
- Repeatability/reproducibility of results;
- High levels of polymorphism;
- Occurrence and even distribution genome wide;
- Close association with the target gene;
- Quick DNA extraction and high throughput marker detection;
- Estimation of Genetic linkage map;
- Knowledge of marker-trait association using QTL analysis, gene mapping etc;
- Quick and efficient data processing and management;

Marker assisted selection for qualitative traits

Characters that are controlled by few major genes are known as qualitative characters or traits. They are also known as oligogenic traits. This includes pests' resistance, colour, shape, male sterility etc. Transfer of such a gene to a specific line can lead to tremendous improvement of the trait in the cultivar under development. The marker loci which are tightly linked to major genes can be used for selection and are sometimes more efficient than direct selection for the target genes.

Marker assisted selection for quantitative traits

Characters that are governed by many minor genes are known as quantitative characters. Quantitative characters are also known as polygenic traits. Mostly all agronomic characters are polygenic or controlled by multiple QTLs. Usually these genes has a small effect on the phenotypic expression of the trait and expression is affected by environmental conditions.

Activities in marker assisted breeding

a) Select Parents which are diverse followed by crossing. If the trait of interest is qualitatively inherited, the donor line should have only the gene of interest and should not have any other genetic loci, which may influence the trait. Similarly, the recipient line should not have any gene controlling the trait of interest.
b) Selection of parents is followed by gene mapping. The choice of mapping population to be developed differs distinctly based on the target trait. If the trait of interest is qualitatively inherited, then early generation segregating population like F2, F3, and BC1F1 can be used. In autogamous species like rice, mapping studies frequently make use of F2 or backcross generations, because they are easiest and earliest to obtain. For mapping quantitatively inherited traits, advanced generation materials like Near Isogenic Lines (NILs), Recombinant inbred Lines (RILs) and Double Haploids (DHLs) are the most appropriate. Breeders/ geneticists also use a strategy called AB-QTL (Advanced backcross-QTL) strategy wherein through backcrossing the population is developed and simultaneously phenotype to identify co-segregating markers.
c) Molecular mapping of target genes.
d) Sampling plant tissues, usually at early stages of growth.
e) Extracting DNA from tissue sample of each individual or family in the populations, and preparing DNA samples for PCR and marker screening.
f) Running PCR or other amplifying operation for the molecular markers associated with or linked to the trait of interest.
g) Separating and scoring PCR/amplified products, by means of appropriate separation and detection techniques, e.g. PAGE, AGE, etc.
h) Identifying individuals/families carrying the desired marker alleles.
i) Selecting the best individuals/families with both desired marker alleles for target trait and desirable performance/phenotypes of other

traits, by jointly using marker results and other selection criteria.

j) Repeating the above activities for several generations, depending upon the association between the markers and the traits as well as the status of marker alleles (homozygous or heterozygous), and advancing the individuals selected in breeding program until stable superior or elite lines that have improved traits are developed.

Construction of linkage map

Linkage maps are basically a kind of "road map" of the chromosomes drawn based on segregation pattern of markers. They indicate the position and relative genetic distances between markers along with chromosomes, which is quite analogous to signs or landmarks along a highway. This step is important for marker assisted selection after using the above information. A clear co-segregation data are required to draw linkage maps. Based on the segregation patterns, percentage of recombination is calculated for each pair of markers in terms of centiMorgans (cM), which is the unit for linkage distance. Preferably less than 5 cM genetic distance are reliable for a marker close to the target loci. Generally, two markers are used in order to reduce the chances of an error due to homologous recombination.

Development of linkage maps involves the following steps.

i. Development of a mapping population by crossing two diverse lines differing for the target trait.
ii. Identification of polymorphic markers.
iii. Evaluation of the mapping population for polymorphic markers
iv. Evaluation of mapping population for the trait of interest preferably in a replicated trial.
v. Linkage analysis using the data generated from genotyping and phenotyping.

QTL analysis

Quantitative traits governed by polygenes are influenced by environmental factors and shows continuous variation. Repeated field tests are required to accurately characterize the effects of the QTLs and to evaluate the stability across environments. The QTL × E interaction reduces the efficiency of MAS and epistasis can result in a skewed QTL effect on the trait. Their effect is minor but has a combined effect on the concerned traits. A quantitative trait locus (QTL) is a region in a chromosome which contains one or more polygenes involved in the determination of a quantitative trait. Although similar to a gene, a QTL merely indicates a region on the genome, and could be comprised of one or more functional genes. In a process called QTL-mapping association between observed trait values and presence/absence of alleles of markers that have been mapped onto a linkage map is analysed. When it is significantly clear that the correlation that is observed did not result from some random process, it is proclaimed that a QTL is detected. Also the size of the allelic effect of the detected QTL can be estimated. A breeder can analyse QTL occurrences and use this knowledge to his advantage, for instance by using indirect selection. When selection is (partly) based on genetic information retrieved through the application of molecular markers this is called marker-assisted selection. The detection of genes or QTLs controlling traits is possible due to genetic linkage analysis, which is based on the principle of genetic recombination during meiosis. This permits the construction of linkage maps composed of genetic markers for a specific population. Segregating populations such as F2, F3 or backcross (BC) populations are frequently used. Advanced backcross QTL analysis (AB-QTL) was proposed by Tanksley and Nelson (1996) for transferring the QTLs of agronomical important traits from a wild species into a crop variety. This is done by backcrossing a wild species with a superior cultivar with selection of domesticated traits followed by evaluation of segregating BC2F2 or BC2F3 population for traits of interest and genotyped with polymorphic molecular markers. These data are then used for QTL analysis, potentially resulting in the identification of QTLs while transferring these QTLs into adapted genetic backgrounds.

Using statistical methods such as single-marker analysis or interval mapping to detect associations between DNA markers and phenotypic data, genes or QTLs can be detected in relation to a linkage map. The identification of QTLs using DNA markers was a major breakthrough in the characterization of quantitative traits.

Molecular marker maps have been constructed for a wide range of crop species. Information on major plant projects (such as the sequencing of the entire rice genome) can be found at www.ncbi.nlm.nih.gov/genomes/PLANTS/ PlantList.html.

Application of marker assisted selection in agriculture

The advantages described above may have a profound impact on plant breeding in the future and may alter the plant breeding paradigm. The main uses of DNA markers in plant breeding, with an emphasis on important MAS schemes, have been classified into five broad areas:

a. marker-assisted evaluation of breeding material;
b. marker-assisted backcrossing (MAB);
c. marker assisted gene pyramiding;
d. genomic selection;
e. and combined MAS,

(A) Marker assisted evaluation of breeding material

Prior to crossing (hybridisation) and line development, there are several applications in which DNA marker data may be useful for breeding, such as cultivar identity, assessment of genetic diversity and parent selection, identification of genomic regions under selection and confirmation of hybrids. Traditionally, these tasks have been done based on visual selection and analysing data based on morphological characteristics.

(B) Marker assisted back crossing

Marker-assisted backcrossing (MAB) strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgression across the rest of the genome. This is the simplest form of marker-assisted selection, and at the present it is the most widely and successfully used Molecular Markers and Marker-Assisted Breeding in practical molecular breeding. The use of molecular markers, which permit the genetic dissection of the progeny at each generation, increases the speed of the selection process, thus increasing genetic gain per unit time (Tanksley *et al*., 1989; Hospital, 2003). In a MABC program, the population to be analysed should contain at least one genotype that has all favourable alleles for a particular QTL. Foreground and background selection are two separate procedures to follow while selecting marker alleles from both donor and recurrent parent. Later, the number of QTLs may be increased progressively, but not beyond six QTLs in most cases because of prohibitive difficulty in handling all QTLs (Hospital, 2003). In addition, the more QTLs/genes are transferred, the larger the proportion of unwanted genes would be due to linkage drag. In general, most of the unwanted genes are located on non-target chromosomes in early BC generations, and are rapidly removed in subsequent BC generations. Generally, several markers are involved and MABC should be performed over two or more generations.

The general procedure of MABC is as follow, regardless of dominant or recessive nature of the target trait in inheritance:

a. Select parents which are genetically dissimilar and make the cross; one parent should be agronomically superior performance and serves as recurrent parent (RP), and the other one used as donor parent (DP) should possess the desired trait and the DNA markers allele(s) associated with or linked to the gene for the trait.

b. Plant F_1 population and detect the presence of the marker allele(s) at early stages of growth to eliminate false hybrids, and cross the true F1 plants back to the recurrent parent.

c. Plant BCF_1 population, screen individuals for the marker(s) at early growth stages, and cross the individuals carrying the desired marker allele(s) (in heterozygous status) back to the recurrent parent. Repeat this step in subsequent seasons for two to four generations, depending upon the practical requirements as it is very unlikely that the selection objective can be realized in a single BC generation.

d. Plant the final backcrossing population (e.g. BC4F1), and screen individual plants with the marker(s) for the target trait and discard the individuals carrying homozygous markers alleles from the RP. Have the individuals with required marker allele(s) self and harvest them.

e. Plant the progenies of backcrossing-selfing (e.g. BC4F2), detect the markers and harvest individuals carrying homozygous DP marker allele(s) of target trait for further evaluation and release.

The efficiency of MABC depends upon several factors, such as the population size for each generation of backcrossing, marker-gene association or the distance of markers from the target locus, number of markers used for target trait and RP background, and undesirable linkage drag. Based on simulations of 1000 replicates, Hospital (2003) presented the expected results of a typical MABC program, in which heterozygotes were selected at the target locus in each generation, and RP alleles were selected for two flanking markers on target chromosome each located 2 cM apart from the target locus and for three markers on non-target

chromosomes. As shown in Table 2, a faster recovery of the RP genome could be achieved by MABC with combined foreground and background selection, compared to traditional backcrossing. Therefore, using markers can lead to considerable time savings compared to conventional backcrossing (Frisch *et al.* 1999; Collard *et al.* 2005).

Among the molecular breeding methods, MABC has been most widely and successfully used in plant breeding up to date. It has been applied to different types of traits (e.g. disease/pest resistance, drought tolerance and quality) in many species, e.g. rice, wheat, maize, barley, pear millet, soybean, tomato, etc. (Collard *et al*., 2005; Dwivedi *et al*., 2007; Xu, 2010).

The main advantages of MAB are:

i. Efficient foreground selection for the target locus,
ii. Efficient background selection for the recurrent parent genome,
iii. Minimization of linkage drag surrounding the locus being introgressed, and
iv. Rapid breeding of new genotypes with favourable traits.

The effectiveness of MAB depends on the availability of closely linked markers and/or flanking markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch and Melchinger, 2005). A variety of maize with high lysine opaque2 gene was incorporated using MABC.

In a study conducted by Neeraja *et al.* (2007) using Marker assisted backcrossing approach to develop submergence-tolerant rice cultivars that are widely grown in the region. The results showed that the mega variety Swarna could be efficiently converted to a submergence tolerant variety in three backcross generations, involving a time of two to three years. Polymorphic markers for foreground and recombinant selection were identified for four other mega varieties to develop a wider range of submergence tolerant varieties to meet the needs of farmers in the flood-prone regions. This approach demonstrates the effective use of marker assisted selection for a major QTL in a molecular breeding program.

(C) Marker-assisted gene pyramiding

Gene pyramiding has been proposed and applied to enhance resistance to disease and insects by selecting two or more genes at a time. Marker-assisted gene pyramiding (MAGP) is one of the most important applications of DNA markers to plant breeding. For example in rice such pyramids have been developed against bacterial blight and blast (Huang *et al.,* 1997; Singh *et al.,* 2001; Luo *et al.,* 2012). Castro *et al*. (2003) reported a success in pyramiding qualitative gene and QTLs for resistance to stripe rust in barley. The advantage of using markers in this case allows selecting for QTL-allele-linked markers that have the same phenotypic effect. To enhance or improve a quantitatively inherited trait in plant breeding, pyramiding of multiple genes or QTLs is recommended as a potential strategy Richardson *et al.* (2006). Pyramiding of multiple genes/QTLs may be achieved through different approaches: multiple-parent crossing or complex crossing, backcrossing, and recurrent selection. A suitable breeding scheme for MAGP depends on the number of genes/QTLs required for improvement of traits, the number of parents that contain the required genes/QTLs, the heritability of traits of interest, and other factors (e.g. marker-gene association, expected duration to complete the plan and relative cost). Assuming three or four desired genes/QTLs exist separately in three or four lines, pyramiding of them can be realized by three-way, four-way or double crossing. They may also be integrated by convergent backcrossing or stepwise backcrossing. However, if there are more than four genes/QTLs to be pyramided complex or multiple crossing and/or recurrent selection may be often preferred. The advantage is that gene pyramiding is more precise and easier to implement as it involves only one gene/QTL at one time and thus the population size and genotyping amount will be small. The improved recurrent parent may be released before the final step as long as the integrated genes/QTLs (e.g. two or three) meet the requirement at that time. The only disadvantage of gene pyramiding is time consuming. Theoretical issues and efficiency of MABC for gene pyramiding have been investigated through computer simulations (Ribaut *et al.* 2002; Servin *et al.* 2004; Ye and Smith, 2008). Practical application of MABC to gene pyramiding has been reported in many crops, including rice, wheat, barley, cotton, soybean, common bean and pea, especially for developing durable resistance to stresses in crops.

(D) Genomic selection

Genomic selection (GS) or genome-wide selection (GWS) is a form of marker-based selection, which

was defined by Meuwissen (2007) as the simultaneous selection for many (tens or hundreds of thousands of) markers, which cover the entire genome in a dense manner so that all genes are expected to be in linkage disequilibrium with at least some of the markers. In GS genotypic data (genetic markers) across the whole genome are used to predict complex traits with accuracy sufficient to allow selection on that prediction alone. Selection of desirable individuals is based on genomic estimated breeding value (GEBV) Nakaya and Isobe (2012), which is a predicted breeding value calculated using an innovative method based on genome-wide dense DNA markers (Meuwissen *et al*., 2001). GS does not need significant testing and identifying a subset of markers associated with the trait Meuwissen *et al.* (2001). In other words, QTL mapping with populations derived from specific crosses can be avoided in GS. However, it does first need to develop GS models, i.e. the formulae for GEBV prediction (Nakaya and Isobe, 2012). In this process (training phase), phenotypes and genome-wide genotypes are investigated in the training population (a subset of a population) to predict significant relationships between phenotypes and genotypes using statistical approaches. Subsequently, GEBVs are used for the selection of desirable individuals in the breeding phase, instead of the genotypes of markers used in traditional MAS. For accuracy of GEBV and GS, genome-wide genotype data is necessary and require high marker density in which all quantitative trait loci (QTLs) are in linkage disequilibrium with at least one marker. GS can be possible only when high-throughput marker technologies, high-performance computing and appropriate new statistical methods become available. This approach has be come feasible due to the discovery and development of large number of single nucleotide polymorphisms (SNPs) by genome sequencing and new methods to efficiently genotype large number of SNP markers. As suggested by Goddard and Hayes (2007), the ideal method to estimate the breeding value from genomic data is to calculate the conditional mean of the breeding value given the genotype at each QTL. This conditional mean can only be calculated by using a prior distribution of QTL effects, and thus this should be part of the research to implement GS. In practice, this method of estimating breeding values is approximated by using the marker genotypes instead of the QTL genotypes, but the ideal method is likely to be approached more closely as more sequence and SNP data are obtained (Goddard and Hayes, 2007). Since the application of GS was proposed by Meuwissen *et al*. (2001) to breeding populations, theoretical, simulation and empirical studies have been conducted, mostly in animals (Goddard and Hayes, 2007; Jannink *et al*., 2010). Relatively speaking, GS in plants was less studied and large-scale empirical studies are not available in public sectors for plant breeding Jannink *et al.* (2010), but it has attracted more and more attention in recent years (Bernardo, 2010; Bernardo and Yu, 2007; Guo *et al.* 2011; Heffner *et al.* 2010, 2011; Lorenzana and Bernardo, 2009; Wong and Bernardo, 2008; Zhong *et al.* 2009). Studies indicated that in all cases, accuracies provided by GS were greater than might be achieved on the basis of pedigree information alone (Jannink *et al*., 2010). In oil palm, for a realistic yet relatively small population, GS was superior to MARS and PS in terms of gain per unit cost and time (Wong and Bernardo, 2008). The studies have demonstrated the advantages of GS, suggesting that GS would be a potential method for plant breeding and it could be performed with realistic sizes of populations and markers when the populations used are carefully chosen (Nakaya and Isobe, 2012). GS has been highlighted as a new approach for MAS in recent years and is regarded as a powerful, attractive and valuable tool for plant breeding. However, GS has not become a popular methodology in plant breeding, and there might be a far way to go before the extensive use of GS in plant breeding programs. The major reason might be the unavailability of sufficient knowledge of GS for practical use (Nakaya and Isobe, 2012). Statistics and simulation discussed in terms of formulae in GS studies are most likely too specific and hard for plant breeders to understand and to use in practical breeding programs. From a plant breeder's point of view, GS can be practicable for a few breeding populations with a specific purpose, but may be impractical for a whole breeding program dealing with hundreds and thousands of crosses/populations at the same time. Therefore, GS must shift from theory to practice, and its accuracy and cost effectiveness must be evaluated in practical breeding programs to provide convincing empirical evidence and warrant a practicable addition of GS to a plant breeder's toolbox (Heffner *et al*., 2009). Development of easily understandable formulae for GEBVs and user-friendly software packages for GS analysis is helpful in facilitating and enhancing the application of GS in

plant breeding. Kumpatla *et al*. (2012) recently presented an overall review on the GS for plant breeding.

(E) Combined marker-assisted selection

There are several instances when phenotypic screening can be strategically combined with MAS. In the first instance, 'combined MAS' may have advantages over phenotypic screening or MAS alone in order to maximize genetic gain. In some (possibly many) situations, there is a low level of recombination between a marker and QTL, unless markers flanking the QTL are used. In other words, a marker assay may not predict phenotype with 100% reliability. However, plant selection using such markers may still be useful for breeders in order to select a subset of plants using the markers to reduce the number of plants that need to be phenotypically evaluated. This may be particularly advantageous when the cost of marker genotyping is cheaper than phenotypic screening, such as for quality traits.

Marker assisted recurrent selection versus genome wide selection

Genetic marker-assisted recurrent selection scheme with genotyping associated cost considering two different kinds of markers. A specific form of marker-assisted selection in maize (*Zea mays* L.) is marker-assisted recurrent selection (MARS) in which (i) one generation of phenotypic selection in the target environment is conducted, (ii) markers with significant effects are used to predict the performance of individual plants, and (iii) several generations of marker-only selection are performed in a year-round nursery or greenhouse. In MARS, the breeders take advantage of QTL information generated on their populations of interest to develop superior lines with an optimum combination of favourable alleles originating from both parents. QTL alleles impacting the major traits of interest to the breeders are identified within breeding populations and accumulated through successive inter-crossing using only genotypic selection. Recombined lines are then subjected to a final phenotypic screen to select the best varieties to release and the selection index adopted the strategy and numbers considered at the different recombination steps will have to be adjusted accordingly.

Genome wide selection (GWS) is marker assisted selection without identifying markers with significant effects. Genome wide selection is based on molecular markers distributed over the entire genome irrespective of whether or not they are linked to a QTL affecting the target trait. It is based on two separate populations: (i) a training population and (ii) a breeding population. Genome wide selection (GWS), also called genomic selection Meuwissen *et al.* (2001) does not involve tests of significance and uses all available markers to predict performance.

In a study conducted by Massman *et al*. (2013) on Genome wide Selection versus Marker-assisted Recurrent Selection to Improve Grain Yield and Stover-quality Traits for Cellulosic Ethanol in Maize it is found that using all available markers for predicting genotypic value leads to greater gain than using a subset of markers with significant effects.

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