PLANT BREEDING AND GENETICS

[1]K. Malleswari, [2]D. Rama Brahma Reddy, [3]T. Praveen

[1] Associate professor,[2] principal,[3] student
Nalanda Institute of Pharmaceutical Sciences, Kantepudi (V),
Sattenapalli (M), Guntur (dist), 522438 India

Abstract:
Gene is a segment of nucleic acid that encodes a functional protein or RNA and is the unit of inheritance. The principal objective of plant biotechnology is to create new varieties of cultivated plants by manipulating DNA molecule. Plant transformation technology has become a versatile platform for cultivar improvement as well as for analysis of gene function in plants. This article discuses and summarizes important work in the literature regarding the gene transfer technologies in plants. The main techniques focused in this article are gene transfer by Agrobacterium tumefaciens, microprojectile bombardment, electroporation of protoplast, polyethylene glycol method, microinjection, silicon carbide mediated transformation, liposome mediated gene transfer and sonication assisted Agrobacterium-mediated transformation. Moreover, the application of gene transfer technologies related to the improvement of crops was also focused. This article will help the reader to have an idea on gene transfer technologies and also to the researcher working on plant genetic engineering.

Key Words: Gene; Gene transfer; Transformation technology; Crops improvement

INTRODUCTION

Plant breeding is a technology that deals with the evolution of crop varieties using the principles of various sciences and skills of the plant breeder gained over the years. Since its birth as a subject, systematic plant breeding has achieved two major landmarks i.e., pre and post (after 1965) green revolution[1]. The pre-green revolution era is marked by the nobalization of sugarcane, utilization of commercial heterosis and development of plant breeding and biometrical techniques. This resulted in the evolution of crop varieties more uniform in yield and growth. A major leap in yield was achieved with the onset of the green revolution era, specifically in the Indian subcontinent. After the green revolution, the per capita availability of cereals increased from 275 to 370 kg. The green revolution was achieved when dwarfing genes were exploited in plant species. The genes characteristically reduced plant height, induced early maturity, increased harvest index, stomatal conductance and defective plant growth regulators such as auxin and gibberellins[2-3]. Induction of dwarfing genes also helped to maintain high-density populations and a double cropping system. However, sustainability of high plant density and a double-cropping system was only possible after the introduction of inorganic chemicals in the form of fertilizers and pesticides. It has been estimated that plant breeding and better crop husbandry techniques have contributed in equal proportions to improve yield[4]. indicated that there was a 50% increase in yield over the past 50 years due to plant breeding showing a linear aual rate of 1% increase in yield.

Discovery of genome-doubling agents such as colchicine provided another exciting tool for plant breeders to set the foundation for polyploidy breeding. However, benefits of this technique were limited to evolution of a few cultivars meant for their vegetative parts or seedless fruits and vegetables[5]. In the 1950s micropropagation techniques evolved which helped to multiply and produce disease-free plant breeding stocks, to overcome the interspecific incompatibility and isolation of soma clones, etc.

Gene transfer technologies in plants

Presently, a number of methods exist for the genetic manipulation of plant cells. These procedures range from exploitation of the natural gene transfer system of Agrobacterium to the chemical treatment of isolated protoplasts by polyethylene glycol. It also includes physical procedures of DNA introduction, including electroporation of protoplasts and tissues, microinjection and silicon carbide fibre-mediated transformation. Moreover, microprojectile bombardment has also received much attention as a physical method of DNA transfer and in many laboratories, is now a routine and reliable technique for the production of transgenic plants. The importance of gene transfer technologies to plants are listed. A number of gene transfer technologies are discussed below.

Gene transfer by Agrobacterium tumefaciens

Agrobacterium tumefaciens has been extensively used to introduce gene into plant cells. This bacterium is responsible for crawn gall disease in a variety of dicotyledonous plants. A plsmid carried within this bacterium cause crown gall disease[6-7]. This plasmid is called tumor inducing plasmid (Ti). Ti plasmid is upto 200 bp large and carries genes that are required for infection. This plasmid has T-DNA that becomes integrated into plant genome at an apparently random position through non homologous recombination. The size of T-DNA is approximately 23 kbp and is responsible for the cancerous properties of the transformed cells. It also synthesizes opines. In the T plasmid, T-DNA is flanked by two 25 bp imperfect direct repeats. These sequences play roles in the integration of T-DNA into the plant genome [8]. Agrobacterium has proved to be an incredible useful tool for the integration of genes into plants[9].

Gene transfer by microprojectile bombardment

The concept of transfering DNA-coated particles directly into cells was first conceived by Sanford and co-workers in 1984 [10]. The first results using a gunpowder driven device to deliver tungsten microprojectiles coated with viral RNA into onion epidermal cells were published three years later [11]. In the same year, microprojectile mediated delivery of plasmid DNA resulted in the introduction of a foreign gene, also in onion cells[12]. Microprojectile bombardment has got much attention and attraction as a physical procedure of DNA transfer in many research laboratories during the past years. This method is routine and reliable way of producing transgenic plants. The method relies on a device which utilizes a propelling force, such as compressed gas or gunpowder, to accelerate inert (usually metal) particles (the microprojectiles), coated with DNA, into target cells. This technique is also referred to as particle bombardment, particle gun method, particle acceleration and Biolistics (Biological ballistics). A number of applications of this method in plant science have been listed.

Status of genetic diversity in various crop species:

Barley:

In the case of barley, Matus and Hayes showed low genetic diversity in elite breeding material compared with mapping populations. Similarly, genetic diversity was also found to be lower than wild cultivars. Contrasting reports are also available showing sufficient genetic diversity in cultivated germplasm compared to wild or land races of barley[13]. When temporal trends of barley genetic diversity were measured, showed non-significant changes Malysheva-Otto et al., reported to be low or raised[14]**.** Malysheva-Otto et al. showed that the impact of plant selection on diversity in barley was non-significant. The study was undertaken in 504 European barley cultivars released during the 20th century. Germplasm was categorized into four temporal groups (TG) i.e., 1900–1929 (TG1 with 19 cultivars), 1930–1949 (TG2 with 40 cultivars), 1950–1979 (237 cultivars as TG3), and 1980–2000 (TG4 consisting of 208 cultivars). TG4 was 84.3% similar to TG1 resulting in a loss of only 15.7% of alleles. TG4 contained 51 novel alleles that were absent in TG1. On the other hand, Condon et al. (2008) showed that plant breeder selection led to a reduction of genetic diversity and allelic losses at a few loci. A summary of some other reports is presented.

Beans:

Common bean (Phaseolus vulgaris) is one of the most widely grown crops in the world. Reduction of bean genetic diversity in various parts of the world both by the activities of plant breeders and during the pre-plant breeding era hasbeen reported. Plant breeder's selection for resistance or for yield-contributed traits led to a significant reduction in diversity[15]. On the other hand, losses during pre-plant breeding have also been reported such as during domestication of common beans. Furthermore, losses in genetic diversity during establishment of land races were also reported. In order to expand the genetic diversity of the cultivated germplasm, interspecific hybridization was recommended[16]. This interspecific hybridization occurred spontaneously through pollen contamination but in reverse order i.e., from cultivated to wild.

Maize and sunflower:

Maize (Zea mays L.) and sunflower (Helianthus annuus) are two crops in which heterosis is being exploited on a commercial basis and thereby several commercial hybrids are available for general cultivation. Exploitation of heterosis on a commercial basis requires the development of an inbred line through selection followed by inbreeding/self-pollination in open-pollinated populations. The focus of many studies carried out in sunflower and maize was to determine whether losses of genetic diversity occurred during the development of inbred lines or whether inbred lines were as diverse as an open-pollinated population. A summary of this research is presented, which indicates that allelic losses occurred in inbred lines, particularly in maize[17].

Rice (Oryza sativa):

Details of genetic studies carried out in rice are providedestablished the benefits of using genetically diverse germplasm. When a mixture of blast disease-susceptible and -resistant varieties were grown, disease incidence was reduced by 94 and 89% increase in yield was noted in susceptible varieties. In order to determine the status of genetic diversity in rice, cultivated rice germplasm was compared with wild germplasm[18]. The former had lower diversity than the latter and allelic losses were also observed. These losses were attributed to human and natural selection in the course of evolution of modern rice. Temporal changes in rice genetic diversity were also observed by Mantegazza et al.

Sugarcane and potato:

In case of vegetatively propagated species such as sugarcane and potato, there are many studies indicating low genetic diversity in cultivated germplasm. However, only a few studies have shown significant genetic diversity in wild germplasm. estimated high genetic diversity in S. spontanum, but lower in S. officinarium. Similarly, [19] also indicated high genetic diversity in 79 cultivars produced by interspecific crosses. Therefore, in order to expand genetic diversity of vegetatively propagated material, interspecific crossing should be carried out.

MAJOR MATING DESIGNS IN PLANT BREEDING AND GENETICS:

Mating design refers to the procedure of producing the progenies, in plant breeding, plant breeders and geneticists, theoretically and practically, they use different form of mating designs and arrangements for targeted purpose. However, the choice of a mating design for estimating genetic variances should be dictated by the objectives of the study, time, space, cost and other biological limitations. Thus, several studies [20] described and contrasted different mating designs and six types of mating designs have been described so far: (1) bi-parental progenies (BIP), polycross, topcross, North Carolina (I, III, III), Diallels (I, II, III, IV) and Line X tester design. In all mating designs, the individuals are taken randomly and crossed to produce progenies which are related to each

other as half-sibs or full-sibs. A form of multivariate analysis or the analysis of variance can be adopted to estimate the components of variances.

BI-PARENTAL MATING:

The bi-parental design is also called paired crossing design and it is reported to be the simplest mating design. In this design, the breeder selects a large number of plants (n) at random and cross them in pairs to produce 1/2n full-sib families. Their progeny is tested and the observed variation partitioned by straightforward analysis of variance into between and within families[21]. The simplicity of this design is counterbalanced by its inability to yield sufficient information to estimate all parameters required by the model[22]. Only two statistics are available for estimating VA, VD, VEW and VEC. This is because the progeny from this design is either full sibs or unrelated; no other relationship exists among them. The estimates of the parameters can only be obtained either by simplifying assumptions, or if extra statistics become available (Hill et al., 1998). If dominance is assumed to be absent (VD=0), and there is no common environment (VEW=0), that is individuals from the same family do not share the same environment. Consequently, if these assumptions are unjustified, it will lead to an overestimate of the genetic component relative to the environmental component. These difficulties can be circumvented to a limited extent in practice. Family plots can be dispensed by randomizing individual plants over the whole experiment. In this way VEC becomes zero. For biometrical geneticist individual plant randomization is a useful device for increasing the precision for estimates, but for a plant breeder it may be an unaffordable luxury. Nevertheless, for shrubs and trees, single tree plot designs are frequently used. Usually, however, it may be simpler for the breeder to estimate VEC directly from the families X replicates interaction mean square in properly replicated experiment. The breeder must make unjustifiable assumptions in order to estimate the genetic and environmental variance.

POLYCROSS:

This design is for intermating a group of cultivars by natural crossing in isolated block. Term polycross was coined by Tysdal, Kiesselbach and Westover in 1942, to indicate progeny from seed of a line that was subject to outcrossing with other selected lines growing in the same nursery (Hill et al., 1998). It is most suited to species that are obligate cross-pollinaters (e.g., forage grasses and legumes, sugarcane, sweet potato), but especially to those that can be vegetatively propagated crops such as sugarcane, cassava and sweet potato. The design provides equal opportunity for each and every clone or parent to naturally cross with each other in the block such that self-pollination is prevented. However, to achieve this objective, a proper design in the polycross block is critical. It provides an equal opportunity for each entry to be crossed with every other entry. It is critical that the entries be equally represented and randomly arranged in the crossing block [23].

TOP CROSS DESIGN:

Topcross refers to a mating between a selection, line, clone and a common pollen parent which may be a variety, inbred line or single cross. The selected plants are crossed with a common tester(s) of known performance, generally in open pollination. The design was proposed by Jenkins and Brunsen in 1932 for testing inbred lines of maize in cross-bred combinations and later renamed topcross by Tysdal and Grandall in 1948. The tester parent should have well known genetic background; either narrow- or broad-based testers [24]. The purpose of using top is to increase the chance of obtaining a desirable gene or genes from exotic or difficult materials. Exotic refers to lines from other countries which are generally poorly adapted to local conditions. Difficult material refers to varieties or lines which are tall, poor combiners, or dominant susceptible, etc. i.e., lines which have given poor results (progeny) from single crosses in previous crossing cycles. In making top crosses, only single cross F1's are utilized because they are uniform. The top cross F1's will be segregating and it is impossible to identify superior plants at crossing; therefore, they are not used.

Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances:

Significance of Genetic Conservation of Crop Plants:

The growing population pressure and urbanization of agricultural lands and rapid modernization in every field of our day-to-day activities that create biodiversity are getting too eroded in direct and indirect way. For instance, land degradation, deforestation, urbanization, coastal development, and environmental stress are collectively leading to large-scale extinction of plant species especially agriculturally important food crops. On the other hand, system driven famine such as, Irish potato famine and Southern corn leaf blight epidemic in USA are the two instances of food crises caused by large-scale cultivation of genetically homogenous varieties of potato and corn, respectively. Even after these historical events, the importance of PGR had only got popular recognition when the spread of green revolution across cultivated crops threatened the conservation of land races[25]. Green revolution technologies introduced improved crop varieties that have higher yields, and it was hoped that they would increase farmers' income. Consequently, the Consultative Group of International Agricultural Researches (CIGAR) initiated gene banks and research centers of domestication for conserving PGR in most of the stable food crops around the world. Center for domestication: maize (Mexico), wheat and barley. The Food and Agriculture Organization (FAO) supported the International Treaty on Plant Genetic Resources (ITPGR) and UN supported the Convention on Biological Diversity (CBD) which are the international agreements that recognize the important role of genetic diversity conservation. Such treaty still plays in current and future food production as one of the major supremos [26].

Erosion of Genetic Diversity due to Population Size: A Bottleneck Concept It is well known that inbreeding is the most common phenomena in cross-pollinated crops, and in small outcross populations it has resulted in deleterious effects and loss of fitness of the population due to recombination between undesirable genes (recessive identical alleles). In natural population too,

severe reductions in population size, the so-called genetic bottleneck, leads to loss of genetic diversity and increased susceptibility to infectious pests and diseases that supervene increased chances of extinction of an individual crop in question. Genetic models that predict the proportion of initial heterozygosity retained per generation is $[1 - (1/2Ne)]$ where Ne is the effective population size, usually less than N, the actual population size. Thus, a population of $Ne = 10$ individuals loses 5% of its heterozygosity per generation. This indicates that severe bottlenecks degrade heterozygosity and genetic diversity [27]. Therefore, plant breeders have been advised to maintain the optimum population size for any trait conservation for specific purpose and its utilization for crop improvement. Thus, before quantifying the genetic diversity, it is essential to know the optimum population size and its representatives to ensure no biasness in diversity assessment that leads to wrong prediction of its value.

Climate Change and Its Impact on Plant Genetic Resources:

The most profound and direct impacts of climate change over previous decade and the next few decades will surely be on agriculture and food security. The effects of climate change will also depend on current production conditions. The area where already being obstructed by other stresses, such as pollution and will likely to have more adverse impact by changing climate. Food production systems rely on highly selected cultivars under better endowed environments but it might be increasingly vulnerable to climate change impacts such as pest and disease spread. If food production levels decrease over the year, there will be huge pressure to cultivate the crops under marginal lands or implement unsustainable practices that, over the long-term, degrade lands and resources and adversely impact biodiversity on and near agricultural areas. In fact, such situations have already been experienced by most of the developing countries. These changes have been seen to cause a decrease in the variability of those genetic loci (alleles of a gene) controlling physical and phenotypic responses to changing climate[28]. Therefore, genetic variation holds the key to the ability of populations and species to persist over evolutionary period of time through changing environments[29]. If this persists, neither any organism can predict its future (and evolutionary theory does not require them to) nor can any of those organisms be optimally adapted for all environmental conditions. Nonetheless, the current genetic composition of a crop species influences how well its members will adapt to future physical and bioticenvironments.

Assessment of Genetic Diversity in Crop Plants:

The assessment of genetic diversity within and between plant populations is routinely performed using various techniques such as (i) morphological, (ii) biochemical characterization/evaluation (allozyme), in the pregenomic era, and (iii) DNA (or molecular) marker analysis especially single nucleotide polymorphism (SNPs) in postgenomic era. Markers can exhibit similar modes of inheritance, as we observe for any other traits, that is, dominant/recessive or codominant. If the genetic pattern of homozygotes can be distinguished from that of heterozygotes, then a marker is said to be codominant. Generally codominant markers are more informative than the dominant markers.

Analysis of Genetic Diversity from Molecular Data:

It is essential to know the different ways that the data generated by molecular techniques can be analyzed before their application to diversity studies. Two main types of analysis are generally followed: (i) analysis of genetic relationships among samples and (ii) calculation of population genetics parameters (in particular diversity and its partitioning at different levels). The analysis of genetic relationships among samples starts with the construction of a matrix, sample × sample pair-wise genetic distance (or similarities). The advent and explorations of molecular genetics led to a better definition of Euclidean distance to mean a quantitative measure of genetic difference calculated between individuals, populations, or species at DNA sequence level or allele frequency level. Genetic distance and/or similarity between two genotypes, populations, or individuals may be calculated by various statistical measures depending on the data set. The commonly used measures of genetic distance (GD) or genetic similarity (GS) are (i) Nei and Li's coefficient (GDNL), (ii) Jaccard's coefficient (GDJ), (iii) simple matching coefficient (GDSM)[30], and (iv) modified Rogers' distance (GDMR). Genetic distance determined by the above measures can be estimated as follows:

$$\text{GDNL} = 1 = [\ 2N11\ (2N11 + N10 + N01)\ ]$$
$$\text{GDJ} = 1 = [N11\ (N11 + N10 + N01)\ ]$$
$$\text{GDSM} = 1 = [\ (N11 + N00)\ (N11 + N10 + N01 + N00)\ ]$$
$$\text{GDMR} = [(N10 + N01)\ 2N\ ]\ 0.5$$

(1) where $N11$ is the number of bands/alleles present in both individuals; $N00$ is number of bands/alleles absent in both individuals; $N10$ is the number of bands/alleles present only in the individual i; $N01$ is the number of bands/alleles present only in the individual j; and N represents the total number of bands/alleles. Readers are requested to read Mohammadi and Prasanna [31] review paper for more details about different GD measures.

CONCLUSIONS:

After examining large number of scientific reports, it may be generalized that losses in plant material occurred in a specific order i.e. the highest in elite open pollinated cultivars or inbred lines. Losses of genetic diversity occurred at each step of germplasm transformation. Among various categories of germplasm, wild germplasm and land races showed the highest genetic diversity and thus can contribute toward the broadening of genetic base of cultivated germplasm and or inbred line/hybrids (Fig. 3). Among evaluated plant breeding methods, plant introduction was found to add up the genetic diversity when local germplasm was partially substituted or supplemented by the introduced germplasm. Therefore, genetic diversity in many parts of the world was found high due to time to time plant introduction. However, introduction of a new species in an area was also found to cause the genetic pollution. Plant breeder's selection was found to enhance the genetic differentiation at the expense of genetic diversity.

Losses in the genetic diversity were observed when plant populations were subjected to various type of plant selection i.e., domestication during the pre-systematic plant breeding era and half or full sib pedigree selection during systematic plant breeding era. The systematic effect of various plant breeding selection methods on genetic diversity in descending order is: participatory plant selection > mass selection recurrent selection > bulk selection > pedigree selection. Among various plant selection schemes participatory Systematic losses in genetic diversity in various form of germplasm where > means superior in genetic diversity.

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