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Wide Hybridization in the Genus *Oryza*: Aspects and Prospects

Patu Khate Zeliang*, Arunava Pattanayak

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ABSTRACT

The cultivated species of rice has lost many valuable traits for stress tolerance in the process of domestication and selection, which resulted in uniformity in many agronomic traits. Although there are several instances of transfer of useful tolerant genes from the wild rice to the cultivated rice, it has now become essential to look at the newer breeding and selection methods that can be applied to wide hybridization in rice. The paper, discusses issues related to hybridization between different species of the genus *Oryza,* problems and difficulties encountered and the strategies for a successful breeding program.

Key words: Wild rice, Hybridization, Gene transfer

INTRODUCTION

The genus *Oryza* was first described by Linnaeus (1753), who recognized only one species, *O. sativa*. Today more than 100 species have been indicated in *Oryza* by different authors (Vaughan 1989). It consists of two cultivated species *viz*. *O. sativa* and *O. glaberrima* and twenty one wild species, which show a wide range of diversity. The wild *Oryza* species are known to have genes for resistance to various diseases and insects. These characteristics could be because the wild species have been subjected to natural selection pressures in their environments for a longer period than the cultigens, thus they have a rich source of genetic diversity for pest and diseases resistance as well as for adverse soil conditions (Vaughan 1994). In the case of cultivated rice, selection using the phenotypic characters has resulted in greater phenotypic diversity than that of the wild species with a low rate of seed shedding at maturity, a low degree of seed dormancy, synchronous heading, self pollination and high grain yield (Oka 1991) but in the process, many valuable traits of the landraces might have been lost. Therefore, it has become essential to create genetic variability and widen the genetic pool by obtaining useful genes from alien germplasm sources and wild relatives of rice. In crop improvement, though cross between varieties of the same species is the major form of gene transfer, in many cases, it may be desirable or even required to cross individuals belonging to two different species or genera of a wild type. Such type of crossing is known as wide/distant hybridization. In this paper, we discuss issues related to hybridization between the different species of the genus *Oryza,* problems and difficulties encountered and the strategies for a successful breeding program.

ORYZA GENOME TYPE

The 'genome' is defined as the minimum genetic set necessary for an organism to live with its own distinct characteristics and to propagate itself (Kurata 2008). The genus *Oryza* has been classified into nine genome types, and twenty three species have been identified based upon the method of 'genome analysis', which consists of the examination of chromosome pairing in meiosis of

Centre of Biotechnology, ICAR Research Complex for NER, Umiam-793103,India
*Corresponding author's Email : khatep@gmail.com

the F1 hybrid between the tester parent of known genome type and the parent with unknown genome (Katayama 1990; Agarwal et al. 1997; Kurata 2008). Chromosomes of both the cultivated species and closely related wild species are similar, and their genomes are designated as AA genomes. The chromosomes of other wild species, however, differ from those of cultivated rice, and they belong to genomes designated as BB, CC, EE, FF and GG. A few of the tetraploid species are reported to have BBCC, CCDD and HHJJ genomes. The AA-genome *Oryza* germplasm exhibits vast eco-geographical differentiation and are thus expected to have significant adaptive gene differences among accessions. Chromosome analysis was also carried out with various wild species of BB, CC, EE, FF and GG genomes, and it was found that the FF genome has the smaller chromosome complements.

RICE IMPROVEMENT THROUGH WIDE CROSSES

Rice has been domesticated from AA genome. Wild *Oryza* and the present *Oryza* cultivars have been bred to bear many of the agronomic characteristics inherent to wild rice such as tolerance to biotic and abiotic stresses, some of which have been lost to domestication and breeding. Wild *Oryza* species are known to have genes for resistance to various diseases and insects such as blast, bacterial blight, viral diseases, brown plant hopper, white backed plant hopper, green leafhopper, whorl maggot and stem borer (Heinrich et al. 1985). Traits from the wild rice populations have made rapid progress possible in rice improvement programs throughout the world. A major dominant gene for resistance to the grassy stunt virus was found from *O. nivara* (Khush et al. 1997). Traits for conferring cytoplasmic male sterility was also first transferred from the wild rice *O. rufipogon* (Katsuo and Mizushima 1958) and later from *O. sativa* L.f. *spontanea* (Lin and Yuan 1980), *O. perinnis* (Dalmacio et al. 1995), *O. glumaepatula* (Dalmacio et al. 1996) into cultivated rice. Alleles from *O. rufipogon* increased grain weight in Hwaseongbyeo a Korean cultivar (Xie et al. 2006) and QTLs for yield and yield components and other agronomic characters were identified from *O. rufipogon* (Moncada et al. 2001; Septiningsih et al. 2003) and transferred to elite varieties of *O. sativa* through wide crosses. Moreover, genes for resistance to brown planthopper (BPH) (Ishii et al. 1994), bacterial blight (Ikeda et al. 1990; Song et al. 1995), blast (Amante-Bordeos et al. 1992), tungro, acid sulfate soils, and iron toxicity have been introgressed from AA, BBCC, CC, CCDD, EE, and FF genomes into rice. Genes introgressed from wild species (*Bph10*, *Bph18*, *Xa21*, *Pi-9*) have been mapped and also used in marker-assisted selection. The rice bacterial blight disease–resistant gene *Xa21* from *O. longistaminata* has been transferred to *O. sativa* (Khush et al. 1991), and the resulting progenies have been widely used for breeding new varieties resistant to *Xanthomonas* infection (Singh et al. 2001). Blast resistance and insect resistances have been successfully transferred from wild species to cultivated rice (Amante-Bordeos et al. 2004). Gene transfers to *O. sativa* from species other than the AA genome has also been possible through embryo rescue technique (Multani et al. 2004). Hybrids have been successfully produced between cultivated rice and most of the species in the genus *Oryza* through a series of interspecific hybrids, alien introgression lines, monosomic alien addition lines (MAALs) (Yasui and Iwata 1991), and chromosome segmental substitution lines (CSSLs) (Kubo et al. 2002; Ebitani et al. 2005). In CSSLs, a particular chromosomal segment from a donor line is substituted into the genetic background of the recurrent line. CSSLs can be used in a genetic analysis to associate QTLs with distinct chromosomal regions and to quickly develop NILs of target regions containing QTLs of interest (Yamamoto et al. 2008). With the availability of information from the entire rice genome sequence (Anonymous 2005), many new tools for the genetic study have been designed with a paradigm change in plant breeding and improvement of rice. Many phenotypic traits of economic interest are controlled by multiple genes and often show complex and quantitative inheritance. With progress in rice genomics, these traits have been identified into single genetic factors or quantitative trait loci (QTLs). Such genetic factors can subsequently be identified at the molecular level by map-based strategies (Yano 2001). These natural variations and QTLs can be exploited with the help of molecular cloning and marker assisted selection (MAS) for the biological study and breeding of rice. Some important characteristics of agronomic value that

have been mapped with the help of the map based cloning include i) heading date (Yano et al. 2001; Takahashi et al. 2001; Kojima et al. 2002), (ii)Submergence tolerance (Xu et al. 2006), (iii) Salt tolerance (Ren et al. 2005), (iv) Seed shattering (Konishi et al. 2006), (v) regeneration ability (Nishimura et al. 2005).

GENETICS OF HYBRIDIZATION

Hybridization is not always successful where development of young zygote may be arrested by hybrid breakdown, hybrid sterility and hybrid non-viability. In wide hybridization, though seeds may be obtained, the F_1 plants may sometime show sterile and semi-sterile characteristics. This may be attributed to the abortion of female and male gametes by respective allelic interactions (Kitamuara 1961, 1962) since seed development is regulated by the balance of maternal and paternal genomes in the endosperm in plants. For example, in one of our experiments, in the cross between *O. sativa* and *O. rufipogon* and reciprocals, F_1 plants from *O. sativa* x *O. rufipogon* were fertile but F_1 plants of the reciprocal were all sterile (unpublished data). Sometimes even when F_1 hybrids are vigorous and fertile, their progenies may have severe infertility or a high rate of mortality. This phenomenon is known as the hybrid breakdown. Hybrid sterility can be genic, chromosomal, or cytoplasmic (Grant 1981).

Hybridization results in genetic recombination between chromosomes of cultivated and wild species. These can take place through the reciprocal replacement of alleles of the wild type with that of the improved cultivar rather than through substitution of a complete or an arm of chromosomes of wild species (Brar and Khush 1997). Sometimes due to the genomic interactions of cultivated and wild species or activation of transposable elements, some novel genes may also arise. Hybridization may also result in genomic change, including alteration to gene expression, chromosomal structure and genome size. Changes such as gene loss, gene silencing, gene expression and tissue-specific expression of copies of some loci from the two genomes may occur after hybridization. Sometimes the phenomenon of hybridization is also accompanied by chromosome doubling, which may lead to speciation and adaptation (Baack and Riesberg 2007). Chromosomal rearrangements frequently cause sterility in hybrids as indicated by abnormal chromosomal pairing, formation of multivalent and other abnormalities at meiosis. Cryptic differences in chromosomes are regarded as a major cause of hybrid sterility in plants. Hybrid sterility is also caused by cytoplasmic difference, as in the case of *O. rufipogon* where the cytoplasm frequently induced male sterility (Shinjo 1988). Genes causing hybrid sterility are either gametophytic or sporophytic in action, which could affect the development of gametes produced by male or female or both. Findings by several workers (Olsen et al. 2006; Li et al. 2006; Sweeny et al. 2006; Konishi et al. 2006) suggest that modest changes in single genes could induce dramatic changes in phenotype during and after domestication. Conventionally, alien chromosome is identified by studying their morphology and karyotyping, which may not be practical. Genomic in situ hybridization (GISH) is used to detect alien introgression. Studies on QTLs have shown that not only QTLS with major effects but also those with minor effects generate natural variations among cultivars in traits with agronomic importance. Therefore, to understand the complex traits controlled by these minor QTLs, artificial mutations may be applied to verify the function of target QTLs in conjunction with map based cloning. Several candidate genes are subjected to functional validation, where mutants of candidate genes will provide evidence for the molecular identification of QTLs. Mutant panels in rice have been generated by Tos17 or T-DNA insertion, exposure to chemical and gamma irradiation (Wu et al. 2005) and the mutants of target genes screened by using Tos17 sequences and T-DNA by Tilling (Raghavan et al. 2007). Once the particular mutant of interest is obtained, morphological and physiological analyses can be done for functional validation of the candidate genes. Sometimes genes from the wild or unadapted material while enhancing the performance of elite cultivars may disrupt favorable gene complexes. Thus, knowing what other genes are likely to be affected by an allele substitution at one locus can prove to be an invaluable tool to a plant breeder regarding the phenotypic consequences of a particular genetic change.

MECHANISM OF WIDE HYBRIDIZATION: ADVANTAGES AND DISADVANTAGES

The advantages of hybridization include disease resistance, wider adaptation, heterosis, better quality, higher yield, development and utilization as new crop varieties. The disadvantages are incompatible crosses, F_1 sterility, problems in creating new species, undesirable linkages, dormancy and problems in using improved varieties since wild relatives cross more easily with land races than with highly improved varieties.

Hybridization-introgression representing gene flow between cultivated and wild rice has been widely observed in nature (Vaughan et al. 2008). The phenomenon of gene flow exists between the cultivated and weedy rice species (Chen et al. 2004), and though pollen competition between wild and cultivated rice has caused a low rate of crop-to-wild gene flow, but it does not completely prevent gene flow from the crop (Song et al. 2005). Thus, it may alter the genetic structure of natural populations and eventually lead to its genetic erosion. Many of the wild relatives of rice in their natural habitats at present may be hybrid derivatives of wild types and domesticates. On the other hand, due to the great genomic differences among different genomes of the genus *Oryza*, reproduction barriers, such as low crossability and hybrid non-viability, are common problems encountered in wide hybridization between cultivated rice with the AA genome and distantly related wild species with a non-AA genome. Isolating barriers can be classified into (i) pre-mating barriers like divergence in spatial and ecological habitats, flowering time, floral organs, and reproductive modes (autogamy and apomixes) and (ii) post-mating barriers, which include cross-incompatibility, hybrid inviability, hybrid sterility and hybrid breakdown. Several incompatibility barriers such as low crossability and limited recombination between homologous chromosomes of wild and cultivated species limit the transfer of useful genes. Sometimes the F_1 embryos and endosperms begin to deteriorate a few days after fertilization or the embryos may fail, which could be due to the presence of a barrier controlled by a set of complementary dominant lethal genes. Failure of the embryo and seed maturation could be the consequence of some disturbances occurring during endosperm development. Moreover, to use genes from wild germplasm requires repeated backcrossing to the female cultivated parents to eliminate undesirable traits from the wild germplasm. Therefore, a carefully planned program or 'prebreeding' is necessary to transfer useful genes from many wild species to an improved plant type before the breeder can use the germplasm. In a conventional breeding program, the breeder has to first identify a useful character, capture its genetic diversity and put those genes into a usable form. These can be carried in different ways viz. by using non-adapted (exotic) land race or germplasm and by dynamic management of gene pools thereby broadening the genetic base of new cultivars. Cai et al. (2008) also stated that prolonged selfing decreases inbreeding depression by purging deleterious genes. Challenges for a breeder include raising of the segregating generations (F_2 and later generations), which consists of several thousand plants. Raising of F_2 requires money, labour, land and other facilities, and handling of the segregating generations also become tedious.

LINKAGE DRAG: HOW IMPORTANT IS IT?

During an introgression breeding program, a wild plant with a favorable trait is crossed with a high-quality cultivar. The wild plant, however, passes on not only its genes of interest to the progeny but also deleterious genes sometimes. When the gene of interest is tightly linked with the deleterious gene and inherited together, it is known as linkage drag. The extent of linkage drag depends on numerous variables- such as population size; the number of meiotic generations before selection is applied and the genomic location of the locus of interest. To reduce linkage drag, plant breeders carry out successive generations of recurrent backcrossing with the cultivated plant and simultaneous selection for the trait to generate a genotype in which the gene of interest is no longer linked to any undesired genes. This results in a long breeding period for developing an improved variety. With the help of molecular markers, recombinant individuals that retain only a very small piece of wild chromosome can be selected. The region of the genome associated with specific components of a phenotype and the donor parent of the favorable allele at a particular locus can also be determined.

The genes underlying the QTL are then cloned for developing markers, which provides the basis for understanding how these genes interact in biochemical and regulatory pathways (McCouch et al. 2007). Fine mapping also provides an opportunity to identify key recombination events that break linkage drag, separating favorable from the deleterious alleles along a chromosome and to deliver high-quality NILs for application in plant breeding. Crosses between *O. sativa* landraces might contain fewer deleterious 'wild alleles' and thus generate fewer linkage drags than in crosses with a wild variety, but the progenies will be more similar.

STRATEGIES FOR A SUCCESSFUL BREEDING PROGRAM

Three main strategies that can be used include the following:

1) Identify gene(s) controlling homologous chromosome pairing in *Oryza* and thereby enhance recombination events between wild and cultivated species -Traditional introgression breeding of cross-fertilizing plants does not allow the introduction of genes from wild germplasm without mixing up the combination of alleles in the existing heterozygote elite recipient genotype. In addition, the donor sequence is inserted into the genome in an unknown position which might affect DNA methylation and other factors that can in turn influence gene expression, since the fragments can contain hundreds of genes.
2) Embryo culture through tissue culture could be used to save alien introgressed lines from aborting after chromosomal exchange between genomes of cultivated and wild species.
3) Further in-depth study of the cytogenetic properties at the different meiotic levels will also increase our knowledge about the size/length of the introgressed segments of the F1 hybrids.

FUTURE PROSPECTS

Discovering useful genes and traits hidden in the plant genome and applying these findings to crop breeding are the ultimate aim of wide hybridization programs. Crosses between *O. sativa* and wild relatives could lead to the discovery of useful QTLs from a range of allelic variations much wider than that present in cultivated lines (Ashikari and Sakamoto 2008). Genes coding important agronomic traits can be identified with the growing infrastructure of plant genomics. These scientific discoveries and tools will lead to more effective and practical breeding programs. New technologies that will assist in enhancing and directing the natural process of meiotic recombination will also facilitate the introgression of favourable alleles (McCouch et al. 2007) and minimize the current requirement of screening large populations. Strategies in the future containing a broad knowledge of and access to modern technology, which will combine the application of new tools and techniques with traditional and efficient plant breeding methods may allow populations to regain traits that have been lost. And also possibly to replace damaged alleles with functional copies from related species through the process of wide hybridization.

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