Interspecific and Intergeneric Hybridization

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Contributor: Akila Wijerathna-Yapa, Vinita Ramtekey, Buddhini Ranawaka, Bhoja Raj Basnet

Interspecific hybridization occurs when crosses are made between different cultivated species belonging to the same genus. In contrast, the outcome of the combination of a distinct genus (cultivated species with their wild relatives) is known as intergeneric hybridization. These two approaches are the critical driving force in generating a different combination of hybrid lines, such as synthetic amphiploid lines, alloplasmic lines, and alien gene introgression lines, which act as a source of variation that leads to a broadening of the genetic variability and diversity of desired traits for crop improvement. However, the success rate of interspecific and intergeneric hybridization is comparatively low compared to intraspecific hybridization due to cross-incompatibilities mainly related to pre- and post-fertilization barriers. To overcome these challenges, in vitro techniques utilizing somatic hybridization or embryo rescue came into the picture and have proven to be the best alternative. Several embryo rescue techniques such as embryo culture, ovary culture, ovule culture, anther culture, and protoplast culture protect embryos from successful hybridization and from premature abortion. Due to the genomic shock, this successful hybridization induces genetic and epigenetic modification at the early stages (zygote formation and development) of hybrids and successive generations. Embryo rescue techniques such as immature embryo culture were used to develop an interspecific hybrid ACC between *B. napus* ‘Zhongshuang 9’ and *B. oleracea* ‘6m08.

Keywords: Intergeneric Hybridization ; Crop breeding ; Crossbreeding ; Interspecific Hybridization

1. Introduction

The microspore-derived plants from developed hybrids exhibited higher genetic diversity in microspore-derived plants compared to both the parents by generating individuals with euploid, aneuploid, and unreduced gametes. Somatic hybridization can be induced via protoplast fusion following polyploidization between *japonica* rice and bread wheat (intergeneric hybridization) to analyze the genetic and epigenetic changes at the level of chromosomal elimination and the DNA sequence [1]. In both cases, the *japonica* rice protoplast was used as the recipient and suggested that these genomic changes in symmetric and asymmetric somatic hybrids resulted from the genomic shock induced at the early stage of the somatic hybrid. Intergeneric hybrids, developed by crossing hexaploid and tetraploid wheat with *Ae. Cylindrica*, followed by embryo rescue, improve the salt tolerance capacity in developed amphidiploid progenies [2]. Wide cross/intergeneric hybridization is made between rye and maize by following ovary culture to produce haploid embryos in the rye [3]. Herein, it provides new possibilities for the introgression of genes through intergeneric hybridization in rye. However, a conventional embryo rescue method used to develop a haploid hybrid is problematic in auto-allogamous species.

A wheat–rice hybrid was created via an in vitro fertilization system, such as embryo culture, to overcome pre- and post-fertilization barriers in eliminating rice chromosomes at an early stage of zygote formation [4]. Herein, a different combination of alloplasmic zygote hybrids was observed. These wheat plants exhibited dwarf and infertile phenotypic behaviors. In the case of wheat, stripe and leaf rust is the most devastating disease to hamper its production. Wheat double haploid (DH) genetic stock was developed by the introgression of genes from *Imperata cylindrica* via embryo culture following colchicine treatment to produce a hybrid resistant to this fungal disease [5]. The developed DH lines exhibited better disease response and were more resistant against yellow and brown rust at seedling and adult plant stages than their parental lines. The identified resistant genes would be a valuable source of enrichment of the genetic base for resistance breeding and wheat improvement. Recently a suspension-derived protoplast fusion was performed to develop asymmetric somatic hybrids between bread wheat and *Agropyron elongatum* [6]. For plant regeneration in successive generations, ovary culture has been employed. The developed hybrids and progenies exhibited two different morphological characters: taller stems with large ears and grains.

In contrast, another type was short stems with strong tillering ability, and smaller ears and grains. Furthermore, GISH (genomic in situ hybridization) analysis showed the variation in somatic chromosome number ranges from 38 to 44, from which 70% of hybrid lines possess 2n = 42. Herein, it was suggested that asymmetric protoplast fusion could be a promising intergeneric hybridization tool. The chances of increasing genetic variation are much faster through interspecific and intergeneric hybridization than through intraspecific hybridization because the selected parental genotypes encounter diverse genetic backgrounds and regular interactions with enhanced sequence divergence [4].

2. Ph Locus

Another important discovery in wheat was the presence of the pairing homoeologous (Ph) locus, which enables the control of genetic recombination by suppressing meiotic pairing between wheat homeologs in interspecific and intergeneric hybridization [7]. Among all the identified Ph loci, Ph1 and Ph2 are the most important ones mapped on chromosome arm 5BL, 3DS, and 3AL, respectively, that effectively impose suppression of the homeolog pairing, which is not only restricted to wheat but its related species [8][9][10]. Looking into the importance of the Ph1 locus, a homoeologous pairing promoter gene, Hpp-5Mg (derived from *A. geniculata* with *T. aestivum*), promotes homoeologous recombination, and multiple crossovers between wheat and wild relative chromosomes led to an enrichment of the genetic base in pre-breeding materials [11]. However, suppression of the crossover in the hybrids between hexaploid wheat and wild relatives is possible when one of the parents carries a Ph1 deletion mutation. Furthermore, the screening of the Ph1 deletion mutant phenotype is cumbersome.

Further studies showed the *ZIP4 (TaZIP4-B2)* homologue in the Ph1 locus, exhibiting a high homoeologous chromosome level when crossed with wild relatives [12]. This suggested that the utilization of Tazip4-B2 mutants rather than complete Ph1 locus deletions would be much easier for alien gene introgression. The ph1b deletion line has been used to introgress powdery mildew resistance genes from *A. triuncialis* (5U) to bread wheat (5A) by inducing homoeologous meiotic pairing [13]. In addition to Ph1, a Ph2 locus encodes repair protein MSH7-3D, which plays a crucial role in the genomic and meiotic stabilization of allopolyploidy and paves the way for alien gene introgression from distantly related species of wheat to enrich the genetic diversity for substantial crop improvement [14]. This genetic control of meiotic homoeologous recombination has widely been used as a novel strategy in chromosome engineering technology to induce genetic variation and the introgression of desirable traits across wheat species [11].

3. Synthetic Wheat

At the dawn of the 20th century, to increase alien genetic variation, CIMMYT was emphasized on a wide crossing program by the utilization of synthetic hexaploid wheat as one of the parents in a distant hybridization or wide crossing program and with the utilization of translocation stock as well. The most significant breakthrough in wheat breeding came after developing the cultivar Veery ‘S’ from CIMMYT, which possesses the 1BL.1RS translocation from bread wheat and rye [15]. Later on, extensive efforts were made by CIMMYT to produce synthetic hexaploid wheat that combines the genomes of tetraploid and diploid distantly related wheat (*Ae. Tauschii*). A newly synthesized interspecific hybrid (*T. dicoccoides x Ae. Squarrosa* and their hybrid with *T. spelta and T. vulgure*) was developed through a union of unreduced gametes by artificial self-pollination and embryo culture followed by colchicine treatment for doubling the chromosome [16]. The developed synthetic hybrid was physiologically and phenotypically different from its parents and exhibited a wide range of essential and desirable variations in the segregating generation (F2, F3, and their offspring) in chromosome pairing and minor characteristic variation for agronomical traits, viz., plant height, ear length, awn, and many more. Similarly, crossing between elite *T. turgidum L*. s. lat. cultivars and *Ae. tauschii* accessions produced 1014 synthetic hexaploid combinations ($2n = 6x = 42$) through artificial hybridization, embryo rescue, and chromosome doubling of F1 hybrids [17]. This developed synthetic diversity by acting as a valuable source of beneficial alleles that are effectively being utilized in pre-breeding programs for alien gene transfer to broaden the genetic variability of the wheat gene pool at the DNA level through homology-directed introgression as well as the introduction of transcriptome shock that induces variation at RNA level [7][18]. Later on, the proportion of utilization of synthetic hexaploid wheat as a bridge species increased from 8–46% for the transfer of alien genes, and the addition and substitution of alien chromosomes linked to desirable traits from several wild relatives to bread wheat have enormously increased the genetic variability in the cultivated genotypes of wheat [19]. They have previously developed intergeneric hybrids among different alien genera of *Triticeae* (*Aegilops, Agropyron, Elymus, Haynaldia, Hordeum, Secale,* and *Triticum*) through controlled pollination, embryo rescue, embryo differentiation, and doubling of the chromosome [20]. The authors detected the involvement of 1B, 6B, and 5D satellited wheat chromosomes in the developed hybrids by somatic cytological investigation. Later, chromosome engineering transferred multiple alien segments into cultivated wheat that carry 7AL, 3BS, and 1AS from *Thinopyrum ponticum*, *Ae. longissimi* and *Triticum aestivum*, respectively [21]. The developed F1 hybrids and their translocated recombinant lines possess several valuable genes that enhance the genotypes’ genetic base and broaden genetic diversity. Similar results have observed the enhanced efficiency of alien gene transfer from *A. tauschii* segments to common wheat with the help of synthetic octoploid wheat by using the in vitro technique of embryo culture followed by chromosome doubling [22]. However, the wheat–rye 4R chromosome disomic addition line (WR35), through wide hybridization and embryo culture followed by chromosome doubling, exhibited resistance to multiple diseases [23]. The higher success rate of alien gene transfer followed by embryo culture or embryo rescue has overcome the limitation of reduced genetic variability [24]. Such synthetic interspecific and intergeneric hybridization paved the way for the introgression and incorporation of alien genes from distantly related genera.

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