Advances in hybrid wheat seed production system

Priyanka Jaiswal, Banshidhar and Satish Kumar Singh

Abstract
In hybrid seed production especially in self-pollinating crops, key role is played by precise control over pollen fertility. A number of techniques such as emasculation, male sterility, XYZ system and biotechnological approaches for pollen abortion have been proposed to prevent autogamy in female parental lines of wheat to facilitate hybrid seed production. However, use of these techniques are limited by restricted outcrossing behavior of wheat crop which can be improved by redesigning the floral architecture. In development of hybrid wheat, the novel approach of split gene system and redesigning the floral architecture could be a potent alternative. This manuscript provides an explicit information on the various techniques used in hybrid seed production in wheat and hopefully be a valuable resource in guiding future hybrid breeding programmes in wheat.

Keywords: Hybrid seed, emasculation, male sterility, XYZ system, split gene system, floral redesigning

Introduction

Wheat (Triticum aestivum L.) is one of the most important and staple food, covering nearly 20% of the world’s daily food supply (Reynolds et al., 2012) [40]. The yield of wheat has increased significantly from 1961 to 2012 (FAOSTAT, 2015) [12] however the increasing population, shrinking cultivated land, and the threat of environmental stresses are encumbrance to increase the productivity. To overcome the challenges posted by the biotic and abiotic factors and other factors, improved varieties with enhanced yield stability, improved abiotic and biotic stress resistance and other favorable agronomic traits are widely used in plant production (Schnable and Springer, 2013) [42]. In this context, hybrids varieties, produced by crossing of genetically diverse parent are one of the batter alternative. However, commercial exploitation of hybrid breeding and seed production in wheat is still limited as comparison to other cereals like rice or maize (Longin et al., 2012; Whitford et al., 2013) [29, 52]. Globally the area under hybrid wheat is less than 1% of total wheat sown area (Longin et al., 2012) [29]. In India, hybrid wheat is produced by utilizing CMS derived from T. timopheevii and CHA approach (Singh et al., 2010, Longin et al., 2012) [35, 29]. In Europe wheat hybrids are produced using chemical hybridization agents (CHAs), mostly Croislor100 is used for inducing male sterility in female parent. In China, photoperiod sensitivity and cytoplasmic male sterility (CMS) are commercially being exploited for hybrid seed production. However, potential hybrid vigor has not been fully exploited for development of hybrid in wheat. In wheat the hybridization techniques like emasculation and XYZ system is extremely labor-intensive, leading to the high cost of hybrid wheat seed (Singh et al., 2015) [46]. This increase in production cost has significantly reduced the benefits of hybrid breeding system. Therefore, there is a strong need to develop innovative and novel techniques that facilitate hybrid production. In development of hybrid wheat biotechnological approach viz. split gene system (Kempe et al., 2014) [26] and floral redesigning could be a potant alternative. The technique of split gene is based on functional interaction of complementary DNA fragments that confers male sterility. Floral redesigning is based on changing the architecture of flower to promote outcrossing. In this review we have discussed the development in hybrid seed production techniques in wheat and the paramount importance of split gene system and floral redesigning in hybrid seed production in wheat.

Hybrid breeding systems in wheat
Hybrid seed production requires an efficient mechanism that not only prevents self-pollination but also ensures for controlled pollination. A number of techniques viz. emasculation(Kadams et al., 1992) [21], XYZ system (Driscoll, 1972) [9] chemically induced (Parodhi and Gaju, 2009)
genetic male sterility (Zhou et al., 2008) [55], cytoplasmic male sterility (Liu et al., 2011) [38], and engineered male sterility (Kempe et al., 2009) [24] etc. have been proposed to prevent autogamy in the female parental lines of wheat and aids in hybridization.

**Emasculation**

The primitive technique of hybrid breeding i.e. emasculation is done by clipping of wheat florets, just above the anther tips with scissors, several days before anther dehiscence when anther are green in colour (Wells and Caffey., 1956) [53]. In hand emasculation, anthers along with the glumes are removed. However, it is observed that when glumes were left intact embryo formation occur at higher frequencies (Laurie., 1989) [27]. This technique is highly labor intensive and time-consuming process for large-scale production, due to the architecture of flower. Spike treatment with hot water (40–45°C for 4–6 min) is reported to be comparatively more effective method of emasculation in wheat (Mukasa et al., 2007; Otsuka et al., 2010) [34, 37].

**Chemically induced male sterility**

Use of Chemical hybridizing agents (CHAs) or gametocides for inducing male sterility is a primitive technique in hybrid wheat breeding program. These chemicals selectively effect pollen (male gamete) and restrict pollen development, pollen shedding, and/or pollen viability. Firstly, Maleic hydrazide was used for slowing down pollen development in wheat (Hoagland et al., 1953) [18]. Consequently, remarkable progresses have been made in the development of CHAs. A number of CHAs viz. ethrel, DPX-3778 (Parodi and Gaju., 2009) [38], BAV-2 (Gao et al., 1996) [13], Pyridazine-9403 (Jiang et al., 1998) [20], SC2053 (Fan et al., 1998) [11] were found promising in causing pollen abortion in wheat. Additionally, eleven CHAs have been reported to effectively trigger more than 98 % male sterility at the pre-meiotic stage of wheat (Chakraborty and Devvakumar, 2006) [7]. Use of hormone for inducing male sterility has also been investigated in wheat. Gibberellic acid (GA₃) can effectively induce male sterility in wheat (Colombo and Favret., 1996) [8]. However, biosafety issues, variable effect, cost effectiveness and optimum dosages recommendation limits the use of chemicals for achieving complete male sterility (Ohtsuka and Konzak., 2002; Murai et al., 2008) [36, 35]. This is further complicated by poor performance and poor seed germination or reduced seedling vigor of hybrid developed using CHAs (Adugna et al., 2004) [1].

**Cytoplasmic Male sterility (CMS)**

In plants CMS is a consequence of chimeric genes in mitochondrial DNA, which prevents development of viable pollen (Hanson and Bentolila, 2004; Horn, 2006) [17, 19]. CMS can be due to spontaneously and induced mutagenesis, or the result of wide crosses viz. interspecific, intraspecific, and intergeneric crosses (Kaul, 1988) [22]. In cultivated wheat CMS line can be developed by crossing cultivated wheat as the male parent and wild wheat (e.g. *Triticum timopheevii Zhuk.*) or related species such as *Aegilops, Hordeum,* and *Secale* as female parent, and then the F₁ is backcrossed to cultivated wheat (Wilsonand Ross, 1962; Mukai and Tsunewaki, 1979; Martin et al., 2008, Adugna et al. 2004) [33, 34]. The restorer genes (RF genes) are grouped as sporophytic or gametophytic depending on the target tissues (Bentolila et al., 2002) [3]. In wheat two or three major restorer loci are required for complete fertility restoration (Bahl and Maan, 1973) [2]. The universal expression of as many RF genes as possible seems to be beneficial for obtaining stable and high fertility restoration (Ma and Sorrells, 1995) [39]. Use of CMS systems are often limited due to environmental sensitivity, especially temperature and photoperiod (Kaul, 1988) [52]. Thus, no wheat CMS system with more than regional application is currently available for hybrid seed production.

**Genetic male sterility (GMS)**

Negative alloplasmic and cytoplasmic effects on yield as well as problems associated with complete fertility restoration can be effectively overcome by using GMS. Nuclear (NMS) or genic (GMS) male sterility (spontaneous or induced) have a greater potential to provide options in selecting parental lines for male sterility based hybrid breeding programmes. Trottet et al., (2010) [50] first identified, partial male sterility due to monogenic recessive mutation, in wheat cultivar Moulin. The two-line system of hybrid breeding viz. photo or thermo or photo-thermo or thermo-photo male sterility systems) is considered as an alternative to overcome the problems associated with the three-line breeding methods (Zhou et al. 2011) [56]. The male sterility genes may be dominant (Ms2, Ms3) or recessive (ms1, ms5), and can be classified as being conditional or non-conditional, depending on whether environmental factors revert fertility (Whitford et al., 2013) [52]. The GMS plants do not show any detectable phenotypic difference (except non-viable pollen), from their male-fertile counterpart. Hence, remarkable advances are required for identifying the fertile plants and their rouging in female lines.

**XYZ system in wheat**

The XYZ system uses an addition alien chromosome with a gene for pollen fertility and a gene for blue coloured aleurone layer as a phenotypic marker (Sedlacek and Horcicka, 2019) [43]. This system involves three lines namely Z-line(GMS), a Y-line similar to the Z-line but containing an additional alien chromosome that bears a compensating gene for male fertility and an X-line similar to the Y-line but containing 2 alien chromosomes (Driscoll, 1972) [19]. In the maintenance plot, X-line is used as male and the Z-line is used as female to produce a Y-line, which in turn used as male to pollinate the Z-line to produce an enlarged Z-line. In the hybrid seed production plot, the Z-line is used as the female parent and normal wheat is used as the male parent. However, this system involves too many steps which demands for considerable time and effort. Thus, some modifications were suggested to improve the efficiency of this system which involves replacement of the Y-line by a selfed Y-line, removing the need for an X-line (Driscoll, 1985) [10].

**Transgenic Male Sterility**

The exploitation of transgene technologies holds better promise in establishment of hybrid wheat production system (Kempe and Gils 2011, Whitford et al., 2013) [52]. The first transgenic male sterility system was introduced by Mariani et al., (1990) [31]. However, in wheat it takes almost a decade to exploit engineered nuclear male sterility using Barnase gene under the control of tapetum specific promoters from corn and rice (Block et al., 1997) [5]. Barnase, a cytotoxic gene (from *Bacillus amyloliquefaciens*) expressed under regulation of tapetum specific promoter, TA 29 results in pollen abortion (Bisht et al., 2004) [4]. The efficiency of engineered Barnase system was further enhance by novel innovation of split-transgene system that involves dividing Barnase gene into
isolallelic positions on two chromosomes thus the transgene fragments complement their expression. This technique has addition advantage of reducing chances of gene flow to off target plants (Gils et al., 2012)[14].

Split gene system in wheat
Split-inteins can be used to form an agriculturally important trait (male sterility) in wheat plants. Contrary to, hybrid seed production techniques that are being currently used, the technology involves genetic modification of just one parent, and is therefore much simpler to develop and use. The intein-mediated assembly of a cytotoxic barnase gene in anther tissue can induce male sterility in wheat for hybrid seed production (Gils et al., 2008)[15]. It is a useful tool for gene-flow control systems (Burgess et al., 2002; Gleba et al., 2004; Kempe and Gils, 2011) [6, 16, 23] entirely based on the functional interaction of two isoloci on homologous chromosomes, achieved through intein-based trans-splicing of two pairs of complementary protein fragments that provide for male sterility and herbicide resistance, expressed in heterozygous plants only. The genetic basis of “Split-gene” system relies in the expression of a barnase gene, encoded by two non-overlapping sequences at complementary genetic loci (Gils et al., 2008) [15]. These overlapping sequences get assembled into an active phytotoxic enzyme after translation through autocatalysis of intein-mediated trans-splicing (Perler, 1998; Saleh and Perler 2006) [39, 41]. The phytotoxic activity of barnase resulted in tapetum degradation, thus leads to formation of inviable pollen. These barnase gene fragments functionally mimic a pair of recessive alleles as a plant will produce active barnase and exhibit a male-sterile phenotype only as a “heterozygote” (Kempe et al., 2014) [26]. Male sterility and herbicide resistance in wheat (Triticum aestivum) has been established through complementation of inactive precursor of protein fragments through a split intein system. The N- and C-terminal fragments of a barnase gene were fused with intein sequences from the Synechocystis sp. gene DnaB. Both barnase fragments expressed under the control of a tapetum-specific promoter. The fusion product is then delivered into the wheat genome for transformation. Upon translation, both barnase fragments are assembled by an autocatalytic intein-mediated trans-splicing reaction, thus forming a cytotoxic enzyme. Kempe et al., (2009)[24] reported that under this system up to 51% of primary transformed plants produced sterile pollen and the expression of the cytotoxic barnase in the tapetum remained stable under increased temperatures. This system keep focus on growth and maintenance of male-sterile maternal lines, whereas the hybrids are fertile. The technology does not depend on fertility restorers and is relies on the genetic modification of the maternal line (Kempe, et al., 2014) [28]. Three introns (PSK7-1i and PSK7-13 from Petunia and UBQ10-1i from Arabidopsis) were tested for their ability to enhance the tapetum-specific expression of a split barnase trans-gene. Furthermore, the average barnase copy number in the plants displaying male sterility could be reduced. The metabolic profiles generated from leaf tissues of male-sterile transgenic plants and non-transgenic plants were compared using gas chromatography mass spectrometry displayed no differences, thus established the anther specificity of barnase expression (Kempe, et al., 2013)[25].

Redesigning of floral architecture
The split-gene system proposed by Kempe et al., is a time honoured advancement in hybrid wheat production. However, it is yet not commercially exploited on ground of being a GM crop. Redesigning of floral architecture involves insight from flower development model where organ identities are determined by a specific class or a combination of classes of genes (Theissen, 2001) [48]. The floral traits like stigms, lemmas, and paleas are responsible for autogamy in wheat (Zhang et al., 2009) [54] and generally oligogenic in nature. Thus, redesigning of these genes will be easy for efficient production of hybrid seed (Thompson and Hake, 2009)[49]. A large number of genes, such as various MADS box genes are reported to be involved in determination of fate of floral organs and differentiation of the glume, lemma, and lodicule (Sreenivasulu and Schnurbusch, 2012) [47] which are potential targets for manipulating wheat’s floral architecture, e.g. TaQ gene in wheat is determine glume shape, lodicule size, and other floral traits in wheat (Simons et al., 2006)[44].

Conclusion
Hybrid seed production requires efficient pollination control system which could promote cross-pollination between wheat genotypes by overcoming the bars of naturally occuring self-pollination system of wheat. The available methods for hybrid seed in wheat traverse from labour and time intensive emasculation technique and XYZ system to efficient genic-based techniques viz. CMS, GMS and TrGMS and use of CHAs for hybrid seed production. The split-transgene system which relies on the expression of a barnase gene that is encoded by two non-overlapping sequences at complementary genetic loci and use of floral redesigning for development of hybrid seeds are some of the recent advancements which has greater potential as gene changer in wheat hybrid seed production.

References


36. Ohtsuka I, Konzak CF. Cytoplasmic male sterility-based system for hybrid wheat plant and seed production, 2002.


