International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 3047-3051

Received: 13-09-2019
Accepted: 15-10-2019

Priyanka Jaiswal
Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Banshidhar
Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Satish Kumar Singh
Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Corresponding Author:
Banshidhar
Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

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) [45, 29]. In Europe wheat hybrids are produced using chemical hybridization agents (CHAs), mostly Croisor100 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 potent 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)

[38], genetic male sterility (Zhou *et al*., 2008) [55], cytoplasmic male sterility (Liu *et al*., 2011) [28], 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) [51]. 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 Devakumar, 2006) [7]. Use of hormone for inducing male sterility has also been investigated in wheat. Gibberellic acid (GA_3) 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 F_1 is backcrossed to cultivated wheat (Wilsonand Ross, 1962; Mukai and Tsunewaki, 1979; Martin *et al.*, 2008, Adugna *et al*. 2004) [53, 33, 1], 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) [30]. Use of CMS systems are often limited due to environmental sensitivity, especially temperature and photoperiod (Kaul, 1988) [22]. 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) [9]. 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 enginnered Barnase system was further enhance by novel innovation of split-transgene system that involves dividing Barnase gene into isoallelic 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 used currently, 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) [26]. Three introns (PSK7-i1 and PSK7-i3 from Petunia and UBQ10-i1 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 stiff glumes, 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 advancement which has greater potential as game changer in wheat hybrid seed production.

References

1. Adugna A, Nanda GS, Singh K, Bains NS. A comparison of cytoplasmic and chemically-induced male sterility systems for hybrid seed production in wheat (*Triticum aestivum* L.). Euphytica. 2004; 135(3):297-304.
2. Bahl PN, Maan SS. Chromosomal location of fertility-restoring genes in six lines of common wheat. Crop Science. 1973; 13:317-320.
3. Bentolila S, Alfonso AA, Hanson MR. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proceedings of the National Academy of Sciences, USA. 2002; 99:10887-10892.
4. Bisht Naveen, Jagannath Arun, Gupta Vibha, Burma Pradeep, Pental Deepak. A two gene - two promoter system for enhanced expression of a restorer gene (barstar) and development of improved fertility restorer lines for hybrid seed production in crop plants. Molecular Breeding. 2004; 14:129-144.
5. Block M, Debrouwer D, Moens T. The development of anuclear male sterility system in wheat. Expression of the barnase gene under the control of tapetum specific promoters. Theor Appl Genet. 1997; 95(2):125-131.
6. Burgess DG, Ralston EJ, Hanson WG, Heckert M, Ho M, Jenq T *et al*. A novel, two-component system for cell lethality and its use in engineering nuclear male-sterility in plants. Plant J. 2002; 31:113-125.
7. Chakraborty K, Devakumar C. Evaluation of chemical compounds for induction of male sterility in wheat (*Triticum aestivum* L.). Euphytica. 2006; 147(3):329-335.

8. Colombo N, Favret EA. The effect of gibberellic acid on male fertility in bread wheat. Euphytica. 1996; 91(3):297-303.
9. Driscoll CJ. XYZ system of producing hybrid wheat. Crop Science. 1972; 12:516-517.
10. Driscoll CJ. Modified XYZ System of Producing Hybrid Wheat1. Crop Sci. 1985; 25:1115-1116. DOI: 10.2135/cropsci1985.
11. Fan P, Cui D, Fan HW. Studies on the male sterility induced by CHA-SC 2053 in common wheat. Acta Agric Univ Henanensis. 1998; 32(2):149-153.
12. FAOSTAT. Available online: http://www.fao.org/home/en/ (accessed on 22 October 2015)
13. Gao QR, Sun L, Liu B. Induced male sterility and its effects on growth and development of winter wheat. J Shandong Agric Univ. 1996; 27:241-248.
14. Gils M, Rubtsova M, Kempe K. Split-Transgene Expression in Wheat. In: Dunwell J., Wetten A. (eds) Transgenic Plants. Methods in Molecular Biology (Methods and Protocols), vol 847. Humana Press, 2012.
15. Gils M, Marillonnet S, Werner S, Grutzner R, Giritch A, Engler C *et al*. A novel hybrid seed system for plants. Plant Biotechnol J. 2008; 6:226-235.
16. Gleba Y, Marillonnet S, Klimyuk V. Engineering viral expression vectors for plants: the full virus and the de-constructedvirus strategies. Curr Opin Plant Bio. 2004; l7:182-188.
17. Hanson MR, Bentolila S. Interaction of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell. 2004; 16:S154-S169.
18. Hoagland AR, Elliott FC, Rasmussen LW. Some histological and morphological effects of maleic hydrazide on a spring wheat. Agron J. 1953; 45(10):468-472.
19. Horn R. Recombination: cytoplasmic male sterility and fertility restoration in higher plants. In: Esser K, Lüttge U, Beyschlag W, Murata J, eds. Progress in Botany. 2006; 67, Berlin: Springer, 31-52.
20. Jiang ML, Wang DQ, Zhang A, Huang C. Male sterile effect of a new pyridazine compound 9403 on wheat. J China Agric Univ. 1998; 3(5):39-44.
21. Kadams AM, Fautrier AG, Scott V. Controlled pollination in hexaploid triticale (Triticosecale × Wittmack), New Zealand Journal of Crop and Horticultural Science. 1992; 19(1):15-18.
22. Kaul MLH. Male sterility in higher plants. In: Frankel R, Grossman M, Maliga P, eds. Monographs. Theoretical and applied genetics, 10. Berlin, Heidelberg, New York: Springer, 1988, 775-797.
23. Kempe K, Gils M. Pollination control technologies for hybrid breeding. Plant Breed. 2011; 27:417-437.
24. Kempe K, Rubtsova M, Gils M. Intein- mediated protein assembly in transgenic wheat: production of active barnase and acetolactate synthase from split genes. Plant biotechnology journal. 2009; 7(3):283-297.
25. Kempe K, Rubtsova M, Riewe D, Gils M. The production of male-sterile wheat plants through split barnase expression is promoted by the insertion of introns and flexible peptide linkers. Transgenic Res. 2013; 22(6):1089-1105.
26. Kempe K, Rubtsova M, Riewe D, Gils M. Split-gene system for hybrid wheat seed production. PNAS. 2014; 111:9097-9102.
27. Laurie DA. Factors affecting fertilization frequency in crosses of *Triticum aestivum* cv. Highbury 9 *Zea mays* cv. Seneca 60. Plant Breed. 1989; 103(2):133-140.
28. Liu H, Cui P, Zhan K, Lin Q, Zhuo G, Guo X *et al*. Comparative analysis of mitochondrial genomes between a wheat K-type cytoplasmic male sterility (CMS) line and its maintainer line. BMC Genom. 2011; 12:163.
29. Longin CFH, Mühleisen J, Maurer HP *et al*. Hybrid breeding in autogamous cereals. Theor Appl Genet. 2012; 125:1087-1096.
30. Ma ZQ, Sorrells ME. Genetic analysis of fertility restoration in wheat using restriction fragment length polymorphisms. Crop Science. 1995; 35:1137-1143.
31. Mariani C, Beuckeleer MD, Truettner J, Leemans J, Goldberg RB. Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature. 1990; 347(6295):737-741.
32. Martín AC, Atienza S, Ramírez M, Barro F, Martín A. Malefertility restoration of wheat in *Hordeum chilense* cytoplasm is associated with 6HchS chromosome addition. Australian Journal of Agricultural Research. 2008; 59:206-213.
33. Mukai Y, Tsunewaki K. Basic studies on hybrid wheat breeding. VIII. A new male sterility-fertility restoration system in common wheat utilizing the cytoplasm of *Aegilops kotschyi* and *Ae. variabilis*. Theoretical and Applied Genetics. 1979; 54:153-160.
34. Mukasa Y, Suzuki T, Honda Y. Emasculation of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) using hot water. Euphytica. 2007; 156(3):319-326.
35. Murai K, Tsutui I, Kawanishi Y, Ikeguchi S, Yanaka M, Ishikawa N. Development of photoperiod sensitive cytoplasmic male sterile (PCMS) wheat lines showing high male sterility under long-day conditions and high seed fertility under short-day conditions. Euphytica. 2008; 159(3):315-323.
36. Ohtsuka I, Konzak CF. Cytoplasmic male sterility-based system for hybrid wheat plant and seed production, 2002.
37. Otsuka J, Yamaguchi S, Chigira O, Kato K. Application of hot water emasculation to *Acacia auriculiformis* for controlled pollination. J Forest Res. 2010; 15(3):210-216.
38. Parodi PC, Gaju MDLA. Male sterility induced by the chemical hybridizing agent clofencet on wheat, *Triticum aestivum* and *T. turgidum* var. durum. Cien Inv Agr. 2009; 36(2):267-276.
39. Perler FB. Protein splicing of inteins and hedgehog autoproteolysis: Structure, function, and evolution. Cell. 1998; 92(1):1-4.
40. Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E *et al*. Achieving yield gains in wheat. Plant Cell Environ. 2012; 35:1799-1823.
41. Saleh L, Perler FB. Protein splicing in cis and in trans. Chem Rec. 2006; 6(4):183-193.
42. Schnable PS, Springer NM. Progress toward understanding heterosis in crop plants. Annu Rev Plant Biol. 2013; 64:71-88.
43. Sedlacek T, Horcicka P. Proposal of updated XYZ system for the production of hybrid wheat seed. Czech J Genet. Plant Breed. 2019; 55:35-38.
44. Simons KJ, Fellers JP, Trick HN, Zhang Z, Tai YS, Gill BS *et al*. Molecular characterization of the major wheat domestication gene Q. Genetics. 2006; 172(1):547-555.
45. Singh SK, Chatrath R, Mishra B. Perspective of hybrid wheat research: a review. Indian J Agric Sci, 2010, 8.

46. Singh Sudhir, Srivastava Rakesh, Kumar Jitendra. Male sterility systems in wheat and opportunities for hybrid wheat development. Acta Physiologiae Plantarum. 2015; 37:1713. 10.1007/s11738-014-1713-7.
47. Sreenivasulu N, Schnurbusch T. A genetic playground for enhancing grain number in cereals. Trends in plant science. 2012; 17(2):91-101.
48. Theissen G. Development of floral organ identity: stories from the MADS house. Current Opinion in Plant Biology. 2001; 4:75-85.
49. Thompson BE, Hake S. Translational biology: from Arabidopsis flowers to grass inflorescence architecture. Plant Physiology. 2009; 149:38-45.
50. Trottet M, Deffains D, Jahier J. A novel partial male sterility resource in bread wheat. Breed Sci. 2010; 60(4):454-457.
51. Wells DG, Caffey HR. Scissor emasculation of wheat and barley. Agron J. 1956; 48(11):496-499.
52. Whitford R, Fleury D, Jochen C, Reif MG, Okada T, Langridge P. Hybrid breeding in wheat: technologies to improve hybrid wheat seed production, Journal of Experimental Botany. 2013; 64(18):5411-5428.
53. Wilson JA, Ross WM. Male sterility interaction of the *Triticum aestivum* nucleus and *Triticum timopheevii* cytoplasm. Wheat Information Service (Kyoto). 1962; 14:29-30.
54. Zhang G, Mergoum M, Kianian S, Meyer DW, Simsek S, Singh PK. Genetic relationship and QTL association between kernel shattering and agronomic traits in wheat. Crop Science. 2009; 49:451-458.
55. Zhou K, Wang S, Feng Y, Ji W, Wang G. A new male sterile mutant LZ in wheat (*Triticum aestivum* L.). Euphytica. 2008; 159(3):403-410.
56. Zhou L, Song G, He B, Hu YG. A ras GTPase activating protein-binding protein, TaG3BP, associated with the modulation of male fertility in a thermo-sensitive cytoplasmic male sterile wheat line. Mol Genet Genomics. 2011; 286(5-6):417-431.