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Pollination, Emasculation and hybridization methods in wheat: A review

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Abstract
Wheat is the most commonly adapted cereal and is produced all over the world. Wheat is utilised in a variety of applications, including food, feed, and industry. It is the world's second most important food crop, after rice, and feeds around one-third of the world's population. Wheat breeders have access to a huge gene pool with a high level of variety. Despite its greater performance, hybrid wheat is still only used in a small portion of commercial wheat production. With the recent development of practical hybrid seed production technologies, a shift from line to hybrid breeding in wheat appears to be feasible. We examine the implications for wheat breeding projects and offer suggestions for how quantitative genetic analysis can aid in the development of effective selection strategies.

Keywords: Gene pool, quantitative genetic analysis and hybrid wheat

Introduction

Wheat (*Triticum aestivum* L. Em. Thell.) is the most essential strategic cereal crop for the bulk of the world's people. It is the most important basic food for over two billion people (36 percent of the world population). Wheat accounts for about 55% of global carbs and 20% of global dietary calories (Breiman and Graur, 1995) [6]. It outnumbers every other grain crop in terms of acreage and production (including rice, maize, and so on) and is thus the world's most important cereal grain crop. It is cultivated under a wide range of climatic conditions, and understanding genetics and genome organisation using molecular markers is extremely valuable for genetic and plant breeding purposes.

Wheat is a self-pollinating crop, meaning that pollen from anthers germinates the receptive ovary in the same flower of a sole parent plant, resulting in seed production. The implantation of pollen grains from a flower's anther on the stigma from same flower or a different blossom in the same plant is referred to as self-pollination (Guptha *et al.* 2019). Hybrid wheat, which produces seed from two distinct parent plants, entails cross-pollination, which is uncommon in nature (usually 1%).

Wheat blossoms do not entice insect or animals for pollination because they lack bright petals, nectar, or appealing odours. They do, however, produce a huge amount of pollen grains. Wheat blooms feature lengthy stamens and are quite tiny.

Using scissors to emasculate wheat florets beneath anther tips several days before natural shedding proved to be an effective and successful new emasculation method. The trimmed florets retained their green anther bases. 3 days after scissor emasculation, bulk pollination resulted in excellent seed sets. Wheat pistils were too severely clipped following mass pollination due to emasculation by trimming the florets across the anther bases. Emasculation was not accomplished by clipping florets above the anthers.

Wheat hybridization is the crossbreeding of two genetically distinct kinds or species. And, like with cotton and corn, hybridising wheat is intended to boost the crop's strength, health, and ability to support a rapidly rising population.

In plant production, hybrid cultivars with improved yield and other beneficial agronomic features are commonly employed. In comparison to other cereals such as maize or rice, economic hybrid breeding and seed production for wheat, one of the most main staple crops, is still limited to a niche sector (Longin *et al*. 2012; Whitford *et al*. 2013) [30, 42]. Currently, only a few wheat hybrid genotypes based on chemical hybridized agents (CHAs, gametocytes) have been approved for the European market (Hybridwheat 2013) [20]. In China and India, hybrid wheat is grown using cytoplasmic male sterility (CMS) or photoperiodic sensitivity sterility (PPSS) systems (Longin *et al*. 2012) [30].

The main practical constraints to more widespread usage of hybrid wheat is seed production capacity and costs however work has been made to enhance hybrid wheat availability and economic competitiveness (Kempe and Gils 2011; Whitford *et al*. 2013) [23, 42]. Eventually, economically viable wide adoption of hybrid wheat will necessitate a combination of a feasible low-cost hybrid seed production system, excellent performance in qualities of concern such as grain yield and yield stability, and an efficient breeding strategy for further enhancement (Longin *et al*. 2012) [30].

The entire crop area in the country is around 29.8 million hectares. Wheat output in the country has risen dramatically, from 75.81 million Metric tonnes in 2006-07 to an all-time high of 94.88 million Metric tonnes in 2011-12. Wheat productivity grew from 2602 kg/hectare in 2004-05 to 3140 kg/hectare in 2011-12. Wheat productivity has increased significantly in the states of Haryana, Punjab, and Uttar Pradesh. MP has reported increased area coverage in recent years.

How Does Wheat Pollinate

1. Wheat is a prolific self-pollinating (autogamous) plant, with low outcrossing rates (only about1%) and most seed developed by selfing.
2. Autogamy is a plant reproductive method of ensuring seed formation in the absence of pollinators and potential mates. (Griffin, 1987; Martin, 1990; Hucl, 1996) [14, 33, 17]
3. Self-pollination has been further aided by cleistogamy, which in itself is closed pollination connected with flower shape and/or pollination behaviour.

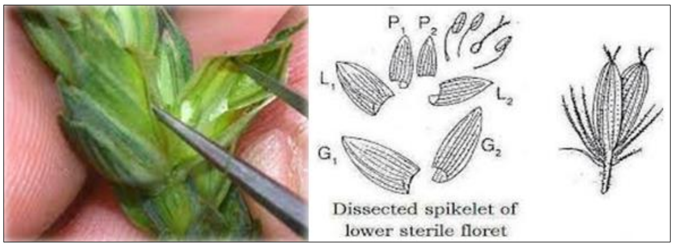

Fig 1: Floral Biology of Wheat

Emasculation in wheat

When the ear emerges, the upper third of the spikelet is clipped, and the inferior spikelets are also excised. Alternate spikelets on both sides of the shaft are removed from the remaining spikelets. The top spikelet is grasped with forceps and pushed downward upward to remove the spikelet's upper florets. The glumes are separated, and the exposed anthers are gently removed and wrapped with butter paper.

The crop is primarily self-pollinated, with the majority of pollen grains discharged within the floret. During the flowering process, the glumes typically open, the anthers protrude from the glumes, and a portion of the pollen grains is spilled outside the flowers. Foreign pollen entering the bloom may cause a limited amount of cross pollination, which is usually less than 1%.Wheat blooms can often be pollinated two to four days following emasculation. Whenever the spike was emasculated, the time varies depending on environment conditions and the stage of stigma growth. To take advantage of heterosis, methods for promoting natural hybridization of wheat have been devised. Breeders can employ such techniques to do bulk hybridizations required for recurrent selection, composite cross breeding, and synthetic manufacture.

Steps involved in emasulation of wheat (Singh *et al.* 2010) [41]

Here in this picture (A) indicates the spike is fully emerged from the flag leaf and would thus be ready for emasculation. Red arrows indicate spikelets that will be removed before proceeding. (B) Black arrows point out a green anther and the un-developed stigma versus (C) slightly yellow anthers and feathered stigma, which is a too advanced stage for emasculation. (D) Depending on the cultivar (here Paragon), flowering may already occur before the spike is fully emerged from the flag leaf. It is thus important to verify the anthers' developmental stage.

Why hybrid?

In terms of yield and other qualities, hybrid crops outperform their parents. The female parent of a hybrid does not generate viable pollen but is used as a seed plant. The male parent serves as a pollinator (Ibrahim *et al.*). They have the ability to unite and express hybrid vigour when combined. In the past, conventional breeding efforts enhanced hybrid vigour by approximately 10%, but in hybrids, they want to increase that amount to 15%-20% to make it more appealing to growers. "We anticipate hybrid wheat, which is far more climate tolerant than pure-line wheat, can help us achieve this goal," says the USDA (Batan *et al.*).

Hybridization Systems in Wheat

Hybrid seed generation necessitates the implementation of an excellent cross-pollination across wheat inbred lines, which overcomes wheat's naturally autogamous pollination mode. This is accomplished realistically by putting male-sterile mother plants with strong pollen receiving properties near paternal plants with strong pollen shedding properties. As a result, has very of the mother plants is a necessity that can be met by a variety of techniques.

Some significant efforts have been made to implement cytoplasmic male sterility (CMS) for hybrid wheat breeding. While CMS is useful in other key cereals like as rice and rye, it has shown to be difficult to establish, difficult to maintain, and only partially reliable in wheat. Although sterility-inducing cytoplasm's have been identified (e. g. from *Triticum timopheevii*), efficient restorer genes for wheat are not yet accessible (Angus 2001), and CMS systems are frequently sensitive to environmental conditions, particularly temperature and photoperiod (Kaul 1988; Murai *et al.* 2008) [22, 37]. As a result, no wheat CMS system with broader applicability than regional use is presently available for hybrid seed production.

Rather, modern commercial hybrid wheat cultivation is mostly reliant on the use of CHA in the field to inhibit the generation of fertile pollen on the maternal crossing partner (Cisar and Cooper 2002) [7]. On a production site, the desired mother line is grown in strips that alternate with strips of the targeted pollen donor lines, and maternal plants are drenched with CHA while paternal plants are not treated. Hybrid seeds are then extracted from pollinated mother plants, producing F 1 progeny that exhibit the heterosis (hybrid vigour) effect (Kempe and Gils 2011) [23]. Croisor®100 (Sintofen, erstwhile Dupont-Hybrinova, Saaten Union Recherche, France) is the only CHA for wheat now registered for commercial production in Europe (Hybridwheat 2013) [20]. Despite being modern, Although CHAs are functional for a wide range of genotypes and have low phytotoxicity in wheat, they do have limitations such as reduced seed set on treated plants (Adugna *et al*. 2004) [1] or change in field-efficiency dependent on meteorological conditions at the time of application.

Alternatively, the use of transgenic technologies could be beneficial in the construction of hybrid wheat production technologies (Kempe and Gils 2011; Whitford *et al*. 2013) [23, 42]. As an example, a recessive split-gene transgenic system that uses complementary barnase fragments to increase male-sterility in maternal plants while keeping pollen fertility and consequently grain yield in the subsequent F 1 hybrids been proposed (Gils *et al*. 2008; Kempe *et al*. 2009) [24]. Functional barnase synthesis in the tapetum layer of anthers, as well as associated male-sterility, should be limited to heterozygous plants, which can subsequently function as mother plants in a hybrid cross. Because both barnase gene segments are positioned on allelic chromosomes and hence "connected in repulsion," hybrid F 1 plants acquire only one of them barnase fragments and, as a result, remain fully fertile (Kempe and Gils 2011) [23].

Furthermore, modifying the naturally enclosed inflorescence morphology of wheat adapted to self-pollination to a more open structure permitting more rapid pollen reception as well as shedding will be an essential breeding goal (Whitford *et al*. 2013) [42]. With these recent breakthroughs in hybrid seed production in sight, it appears that it is now time to consider how to produce optimal parental combos for best yield and quality increases in wheat hybrid breeding.

Advantages of Hybrids in Comparison to Lines

Hybrid variants have already entirely supplanted population varieties in some best possible output crop plant species (Coors and Pandey 1999) [9]. The main advantages of hybrids over population types include increased yields due to optimal heterosis exploitation, enhanced biotic and abiotic stress resistance, and improved yield stability (Hallauer *et al*. 1988) [16]. In compared to outcrossing cereal species such as maize, the quantity of midparent heterosis for grain production is less prominent in wheat as an autogamous species, and commercial heterosis, the difference across hybrids and the best widely viable line variety, is lower (Longin *et al*. 2012) [30]. Concerning yield stability, Léon (1994) [27] observed that for autogamous crops, results in hybrids vs lines differ, ranging from stronger significantly of hybrids (Borghi *et al*.) to lower yield stability of lines (Borghi *et al*.) 1988; Oury *et al*. 2000; Oettler *et al*. 2005; Gowda *et al*. 2010) [5, 39, 38] to no differences (Borghi and Perenzin 1990; Peterson *et al*. 1997; Bruns and Peterson 1998; Koemel *et al*. 2004) [4, 26].

However, recent large-scale phenotyping studies involving vast collections of inbred lines and hybrids indicated a positive commercial heterosis for grain production in hybrid winter wheat acclimated to Central Europe (Gowda *et al*. 2012; Longin *et al*. 2013) [12, 28]. As a result, switching from line to hybrid types has the chance of breaking through yield restrictions in wheat breeding. Mühleisen *et al*. (2013) [28] s basically grains yield consistency in wheat, triticale, and barley hybrids in contrast to lines using a large data set from multilocation field trials. In all three autogamous crops, the authors found that hybrids had consistently superior production stability. Furthermore, the lack of consistency in prior studies on yield stability of hybrids vs lines is most likely due to an unbalanced definition of the environmental index created by using solely on data from inbred lines rather than hybrids. In conclusion, current experimental research findings show the benefits of hybrids over inbred lines in terms of increased production and yield stability. Because of the predicted climate change with increasingly harsh weather conditions, enhanced performance resilience will be even more critical in the future.

Aside from increased grain production and yield consistency, hybrid wheat surpasses inbred lines in resistance to abiotic and biotic stress. Longin *et al*. (2013) [28] discovered a positive midparent (albeit negative better parent) heterosis in hybrid winter wheat for frost tolerance as well as resistance to leaf rust, stripe rust, septoria tritici blotch, and powdery mildew. Zhao *et al*. (2013a) [45] and Gowda *et al*. (2013) [13] evaluated the degrees of dominance of hypothesized QTL underlying the abiotic and biotic stress resistances described above in two companion genome-wide mapping analyses. The level of dominance was in the area of partial dominance for most QTL with a considerable effect on genotypic variation. Thus, if specific features are of interest, superior genotypes should have resistance QTL in the homozygous condition. If numerous important QTLs are to be pyramided, hybrid breeding will provide significantly greater flexibility in combining advantageous conditionally dominant alleles arising from either maternal or paternal inbred lines. This advantage of hybrids is demonstrated by computing a stress susceptibility index based on data on cold tolerance and resistance to leaf rust, stripe rust, septoria tritici blotch, and powdery mildew for a large collection of winter wheat inbred lines and hybrids developed from them. Hybrids have generally lower stress susceptibility than similar inbred lines, which may contribute to the higher yield stability seen for hybrids (Mühleisen *et al*. 2013) [28].

Apart from the benefits of hybrid vs line varieties in lower yield, yield stability, and biotic and abiotic stress resistance, it is critical to note that the recurrence shortlisting gain in hybrid breeding is at least as effective as in line breeding. The projected recurrent selection gain is affected by quantitative genetic characteristics such as the additive variance ratio, which can be used in combination versus line breeding (Hermsen *et al*. (2017) [18]. In addition to quantitative genetic characteristics, the economic framework, such as return on investment, is critical in determining the selective benefit of line against hybrid breeding. Longin *et al*. (2012) [30] conducted the first rough theoretical evaluation of projected selection benefit for line vs hybrid breeding using the simplifying assumption of an equal budget. According to the model calculations, if the dominance impact is significant, hybrid breeding can result in a recruiting and selection gain over line breeding (Longin *et al*. 2012, 2014) [29]. These theoretical arguments are supported by a study that compared hybrid vs line breeding utilizing experimental data from official variety tests in hard winter wheat over a 20-year period in the United States (Koemel *et al*. 2004) [26]. When comparing hybrids to line breeding, the scientists found that hybrids had a larger selection gain over time. These findings are highly encouraging, but more experimental data are required to support the long-term selected gain for hybrid against line breeding in autogamous cereals.

Prediction of Hybrid Wheat Performance

Every year, numerous thousand inbred lines are created in a typical wheat breeding programme. Multi-stage selection techniques are used to efficiently identify superior genotypes. The huge quantity of viable single-cross combinations among accessible elite parental lines, on the other hand, afflicts the selection of superior hybrids (Zhao *et al*. 2013) [35]. As a result, inspection of all probable hybrid combinations is impractical, resulting in a high necessity for hybrid prediction methodologies.

Midparent productivity is only marginally related with hybrid performance for complicated variables such as grain yield (Longin *et al*. 2012) [30]. Predicting hybrid performance based on their parents' general combining ability (GCA) effects is accurate in instances when variance due to GCA (2 GCA) predominates over variance due to specific combining ability (SCA) effects (2 SCA). A recent large scale experimental investigation in wheat found that 2 SCA is fairly significant for grain yield in wheat (Longin *et al*. 2013) [28], implying that field testing of single-cross combinations is required, at least in the last phases of a selection programme. As a result, credible methodologies for predicting hybrid wheat performances are urgently needed.

The ability of genomic selection to estimate hybrid wheat performance for grain production was examined using a modest factorial mating design consisting of 90 hybrids fingerprinted with a 9k SNP array (Zhao *et al*. 2013) [35]. The findings of this validation data study indicate that genetic selection has a great potential for predicting hybrid wheat performance. This finding was supported by additional research anticipating hybrid wheat performance with genetic analysis concentrating on numerous agronomic variables (Miedaner *et al*. 2013; Zhao *et al*. 2013, 2014) [35-36]. However, additional empirical data analyses are required to fully assess the possibilities of genomic modelling approaches for forecasting hybrid wheat performance.

Upcoming Challenges for Wheat Hybrid Breeding

Wheat yields a cereal grain that is extensively consumed as a food staple. Wheat is pollinated by wind since it is a grass. Wheat flowers have many modifications to allow wind pollination. Finally, the most pressing challenges for wheat hybrid breeding will be I the development of a sustainable hybridization system to lower the amount of hybrid seed production particularly in comparison to the use of CHA, (ii) the identification of the genetic background of pollination ability for knowledge-based improvement of cross-pollination among elite lines, and (iii) the optimal design of hybrid wheat breeding programmes, including dimensioning of multi-stage selection programmes including genome based selection.

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