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Proposal of updated XYZ system for the production of hybrid wheat seed

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Abstract: The following updates have been proposed for the XYZ system for the production of hybrid wheat: The waxy characteristics of the grain were used as a classifying mark. The candidate pollen sterility gene *TIP2* was detected *in silico* based on similarity to known pollen sterility genes in rice. In order to maintain a sterile maternal component, the addition chromosome 7H was proposed, carrying wild-type alleles *Waxy-H* and *TIP2-H*. The concept of practical production of the commercial F_1 seed was designed.

Keywords: heterosis; male sterility; waxy

As one of the most important crops, wheat faces the challenge of dramatically increasing its yields by 2050 (Alexandratos & Bruinsma 2012). And yet the yield trends achieved using contemporary varieties and growing systems are insufficient to achieve this goal (Ray *et al.* 2013). One of the possible ways to increase the yield potential of wheat is to use hybrid varieties (Mette *et al.* 2015). A list of currently available systems of production of hybrid wheat seed is presented by Whitford *et al.* (2013). However, the main systems bring certain significant disadvantages. The problem with cytoplasmic male sterility (CMS) is the instability of the expression of sterility depending on external conditions, and the problematic restoration of fertility. At present, available varieties of hybrid wheat are created using the gametocide sintofen. And yet its disadvantages include the economic costs and the critical need for a precise time of application. The development of Seed Production Technology has brought about significant progress (Wu *et al.* 2016). And yet it has the disadvantage that the sterility maintainer component is a GMO, and another fundamental disadvantage is that it is patented and its use is conditioned on executing a licensing agreement with associated economic costs. It therefore seems necessary to continue to work on developing improved systems of production of hybrid wheat seed.

The principle of the XYZ system was published by Driscoll (1972). It uses an addition chromosome carrying a gene for pollen fertility and a gene for a phenotype marker – blue coloured aleurone layer. A disadvantage of the system is the weak coloration of grains bearing one dose of the gene for the blue aleurone, and therefore the insufficiently efficient sorting of grains (Dowell *et al.* 2009a). For this reason it has not entered common practice. In principle, however, it allows for the efficient production of hybrid seed. For this reason a study was performed to determine whether it would be possible to update this system such that it could be practically usable.

Another mark that could be used to sort the grain is the amylose content. So-called waxy wheat has almost zero amylose content caused by the presence of null *wx-A*, *wx-B*, and *wx-D* alleles. The amylose content of semi-waxy wheat, where the functional gene of subgenome D is retained in homozygote form, is 18–20%. For heterozygote assemblies where the donor of the wild-type (wt) allele is the mother, the amylose content is 13–15%; where the donor is the father, the amylose content is 7–8% (Sasaki 2005). The dosage effect of one gene is therefore 7%

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amylose. The individual genetic combinations create discrete classes, whereas effective sorting using single kernel near-infrared spectroscopy has been published (Dowell *et al.* 2009b).

Rice is a model plant for wheat and can serve for identifying orthologs of sensitive genes. From the RAP-DB database (Sakai *et al.* 2013), candidate genes were selected according to the key words "male-sterility", "tapetum", "pollen". On the basis of similarity their orthologs in the wheat genome were sought (Mayer *et al*. 2014) on the set of chromosome 7. To design a system of production of hybrid seed with the use of waxy markers, the ortholog of the gene *TIP2* may be used (RAP-DB locus *Os01g0293100*), which is the smallest distance from the locus of waxy, namely 33 669 kb for chromosome 7D. The *TIP2* gene has the role of primary switch in the early development of the anther. Its knockout causes recessive pollen sterility, whereas the heterozygote status is sufficient for renewal of pollen fertility (Fu *et al.* 2014).

For the option of the differentiating waxy grains carrying the pollen sterile gene *TIP2*, it is possible to use the line of wheat with the addition chromosomes of barley (Islam *et al.* 1981), specifically the addition chromosome 7H available in the JIC Seedstor database (Horler *et al.* 2018) under WPGS number #28375. This chromosome carries the fertile allele of the gene *HORVU7Hr1G024790.4* (BARLEX database, Colmsee *et al.* 2015), which is an ortholog of the *TIP2* gene and performs the same function. It also carries the wt allele of the *Waxy* gene (HORVU7Hr1G012380.5). The presence of the wt alleles of the *Ph2* locus (Sutton *et al.* 2003) ensures that the *Waxy* and *TIP2* genes will not recombine with the *waxy* and *tip2* of wheat. For the purposes of producing hybrid seeds of wheat, the following is created:

- a maternal sterile component (M) carrying the homozygote genes *wx-A*, *wx-B*, *wx-D*. The donors of these genes are described by Graybosch (1998); molecular markers for their selection are also available (Saito *et al.* 2010). It also carries the homozygote knockout genes *tip2-A*, *tip2-B*, *tip2-D*. *TIP2* genes may be effectively knocked out using the CRISPR-Cas9 technique (Shan *et al.* 2014).
- the line of the maintainer of the sterile maternal line (N) which is isogenic to line M whereas it also contains the monosomic addition of chromosome 7H with the *wt* allele of the *Waxy* gene (*Wx-H*) and the *wt* allele of the *TIP2* gene (*TIP2-H*). A line created in this manner is pollen fertile and produces a grain of a semi-waxy character with amylose content of approx. 7%.
- the paternal line (O) is created by a line of wheat with the *wt* alleles of the gene *Wx-A*, *Wx-B*, *Wx-D*,

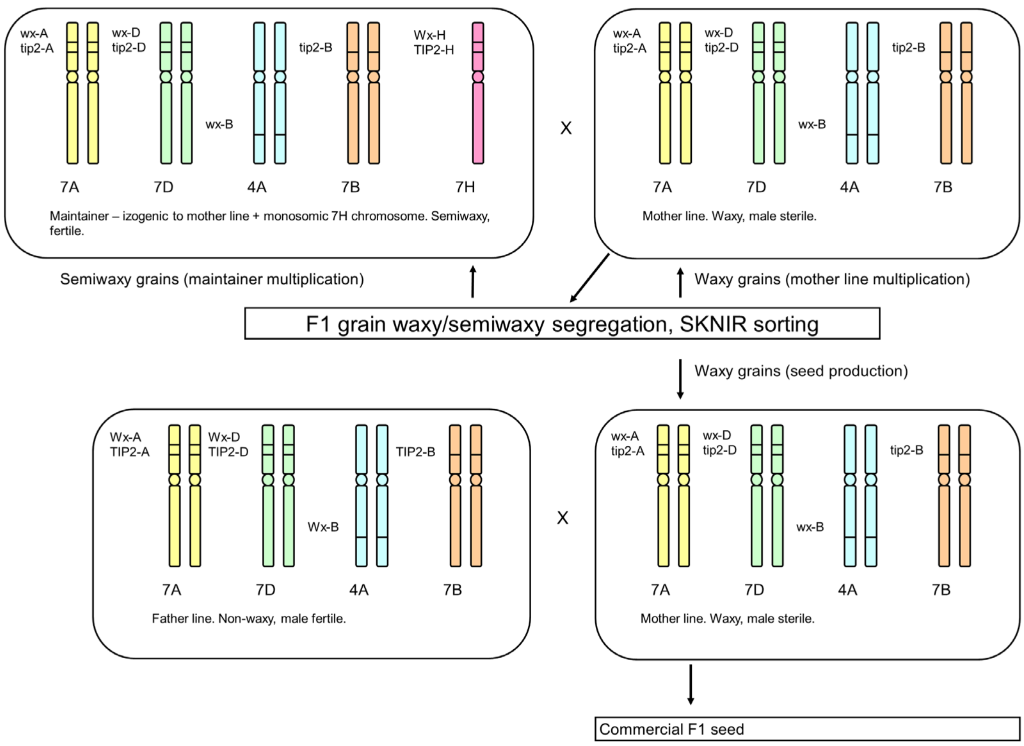

Figure 1. Schematic proposal of updated XYZ system for the production of hybrid wheat seed

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TIP2-A, *TIP2-B*, and *TIP2-D*. For the purposes of producing hybrid seed it seems appropriate when the paternal line anthesis is several days later than the line M, it is higher and has an anther extruding character.

Production of hybrid seed takes place in the following manner:

(A) Multiplication of components. Line M is sown in parallel rows with line N. During anthesis, fertile N pollinates the sterile M. The monosomic addition of 7H is spread only to part of the pollen grains. The harvested grain of line M is therefore a blend of lines M and N. The mixture is separated using SKNIR into waxy (M) and semi-waxy (N) parts. The sorted grain forms seed for further multiplication of components and production of commercial hybrid seed. The paternal line is multiplied in the conventional manner.

(B) Production of hybrid seed. Line M is planted in parallel rows with line O. During anthesis, fertile O pollinates sterile M. The harvested grain of line M forms the commercial hybrid seed. An overview of the design is given by Figure 1.

In terms of costs and labour requirements of hybrid components development relative to XYZ, proposed system is more difficult. Driscoll's XYZ system was based on a single locus for sterility as well as a single seed colour locus. When backcrossed to desired mother line, required sterility gene and seed colour gene will be presented in 25% of pedigree. Proposed system uses three recessive male sterility genes, three seed marker genes and 7H chromosome addition, distributed in five linkage groups. When backcrossed to mother line, required sterility genes, seed marker genes and 7H chromosome addition will be presented in less than 3% of pedigree. This would require more extensive backcrossing and exact tracking of required genes using molecular markers. But when developed, hybrid seed production will be comparable to XYZ.

To distinguish presented proposal from the XYZ system, we suggest designating this as the "MNO" system. The design is freely available to interested parties for further development and commercial use.

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