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DISTINCTNESS TESTING IN OILSEED RAPE–DIFFERENT MALE STERILITY SYSTEMS

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HYBRID PRODUCTION USING MALE STERILITY

The development of hybrid varities is one of the main topics in oilseed rape breeding. A stadily growing number of hybrid varieties is applied for plant breeders rights or enters VCU tests. The increasing total number of varieties in the collection and the use of parental lines each in more than one hybrid makes distinctness testing more complicate.

Prerequisite for the development of hybrid varieties is a working mechanism for pollination control. Rapeseed hybrid varieties which are under DUS tests today are developed using four different male sterility systems or self incompatibility. In the presented paper the known male sterility systems are discussed with reference to distinctness testing for restored hybrid varieties. Presently, the male sterility systems are used side by side and it is not possible to predict whether one system will dominate in future. Therefore, the question is whether the male sterility system may be used to facilitate the assessment of distinctness.

The following male sterility systems are used for the production of restored hybrids:

- CMS Polima (Fu, 1981): Originates from rapeseed; sterility is maintained by most rapeseed varieties; restorer genes are available; CMS Polima is only used in spring type; expression is temperature dependent.

- CMS Ogura (Pelletier et al., 1983, Delourme et al., 1991): Sterile cytoplasm was introduced from *Raphanus*; sterility is maintained by all rapeseed varieties, a restorer gene was also introduced from *Raphanus*, but it is linked to a gene for glucosinolates so far; currently used in male sterile hybrids (varietal associations), partly restored and restored hybrids.

- MSL (Male Sterility NPZ-Lembke): Originates from rapeseed; sterility is maintained by specific genotypes; restoration is possible with all rapeseed varieties.

- Seedlink™ (PGS-System, De Both 1995): Male sterility and fertility restoration are induced by transgenes; both genes are linked to herbizide resistance; without herbizide application the female parental line segregates in 50% sterile and 50% fertile plants.

DISTINCTNESS OF MALE STERILITY SYSTEMS

Assessment of all characteristics which are included in the table of characteristics in the Test Guideline for Rapeseed (TG/36/6) provides no general possibility to distinguish restored hybrids or male sterile lines which are developed with different male sterility systems. Faced to plants of any restored hybrid variety or male sterile line it is not possible to identify the male sterility system used.

Specific expression of morphological flower characteristic can be observed in some varieties. Sometimes, hybrid varieties or male sterile parental lines with CMS Polima exhibit different position of petals. The petals are arranged like the letter H. In the CMS Ogura system some varieties have crumpled or folded petals. The female parental lines in the MSL system are characterized by shedding of the lower flower buds at the main stem, a phenomenon which is reduced in new male sterile lines. Development of flower buds in the MSL hybrids is not disturbed.

The expression of the mentioned flower characteristics can easily and consistently be assessed. Nevertheless, they might be used as additional characteristics for establishing distinctness only in some pair-wise comparisons. The expressions mark the systems but occur only in some varieties of each system. Grouping of varieties according to the male sterility system is only possible on the basis of the general knowledge that the systems are genetically different. It is not possible to prove that assumption in the DUS test with the characteristics of the guideline. Grouping on that basis should therefore be rejected.

But, the four male sterility systems exhibit obvious genetical differences which include the following factors:

cytoplasm,
ms genes,
restorer genes.

Restoration of male sterility requires specific restorer genes in CMS Polima, CMS Ogura and Seedlink™. Therefore, it is possible to identify all mentioned male sterility systems in testcrosses between the male sterile parental lines and characterized pollinaters. The test pollinators have to be proved to bear only restoration ability for one male sterility system. A pollinator without restoration ability for Ogura, Polima or Seedlink™ has to restore the MSL system. The application of such a procedure in DUS testing would be connected with the following problems and can therefore not be recommended:

- Distinctness of the variety and therefore also the granted protection depends on the existance of another variety - the characterized pollinator with specific restoration ability.

- The test pollinator has to fulfill the requirements of DUS inclusive of fertility restoration ability. If a variety used as test pollinator is withdrawn a new suitable pollinator has to be defined.

- The DUS test is very work- and time-consuming. Test crosses and test of progenies have to be carried out.

CHARACTERISTICS FOR DISTINCTNESS OF HYBRID VARIETIES BASED ON MALE STERILITY

New characteristics have to be identified to allow discrimination between male sterility systems in hybrid varieties and inbred lines. Biochemical and molecular characteristics are discussed very much for their usefulness in variety testing. If they fulfill the requirements of

uniformity and stability, biochemical and molecular characteristics additionally to morphological characteristics can be powerful for the establishment of distinctness and variety description. Concerning male sterility systems specific markers have already been developed for various selection purposes in breeding procedures:

- Ogura cytoplasm: A polymerase chain reaction (PCR)-based molecular marker was developed to detect the sterile ogura cytoplasm (Tinchant et al., 1997). With a specific primer a region of the male fertile (normal) mitochondrial DNA is amplified. There is no amplification in the sterile ogura cytoplasm because of the insert from *Raphanus* in the mitochondrial DNA. The test was developed for the estimation of contamination of seed lots. The marker should be checked for possible use in variety testing. It may be possible to group all varieties bearing the CMS ogura cytoplasm or not with such a marker.

- Ogura restorer: The gene for fertility restoration of ogura male sterility was shown to be tightly linked to a *Raphanus* PGI-2 allele (Delourme et al., 1995). The suitability of the isozyme marker for variety grouping has to be checked. All restorer lines and restored CMS Ogura hybrids should express the *Raphanus* PGI-2 allele. The marker can only be used for distinctness if it is uniform in the candidate and the reference. In partly restored hybrid varieties the marker is expected to segregate in linkage with pollen production.

- Seedlink™: The gene for male sterility and the restorer gene are transgenes. Therefore, it is possible to prove the presence of the genes by gene specific molecular markers. In the hybris all plants have the restorer gene and 50% of the plants have the gene for male sterility. The restorer lines should be homozygous and uniform for the restorer gene. After herbizide application all plants of the female parental line express the gene for male sterility.

The mentioned characteristics can be introduced into DUS testing in different ways:

1. DUS test is carried out without variety grouping for the male sterility system. In a case where two varieties are not distinct with the characteristics of the Test Guideline TG/36/6 the mentioned additional characteristics may be used to establish distinctness. Uniformity and stability has to be checked only for the two varieties.

2. Varieties are grouped for additional characteristics with which the sterility systems can be identified. For example three groups could be established: CMS Ogura, Seedlink™, others. Further distinctness testing is done only within the groups. However, grouping can only be carried out after checking all varieties for the addressed characteristics, i.e. all varieties belonging to the third group have to be checked for the grouping characteristics, too (inbred lines and hybrids with Polima and MSL, all varieties other than hybrids).

CONCLUSIONS

The table of characteristics in the Test Guideline TG/36/6 does not allow distinction between different male sterility systems in parental lines or hybrid varieties. Furthermore, there are no additional morphological characteristics common for any type of male sterility. Therefore, distinction of different male sterility systems can not be considered *per se* as distinct. The definition of at least one characteristic is required which can be assessed in all varieties giving clearly different states of expression in different male sterility systems.

Some biochemical and molecular markers are already known which are suitable for identification of different factors of specific male sterility systems. The objective should be to test these markers for their suitability in variety testing, including the aspects of uniformity and stability has to be addressed. Identification of marker characteristics for the CMS Polima and the MSL system would enable to discriminate between all male sterility systems.

Assessment of the mentioned markers for the whole collection require very high effort. Therefore, they should not be used for grouping of varieties. However, if the methods are adapted to the conditions of DUS testing they could be applied in individual comparisons to establish distinctness.

A large-scale assessment of biochemical or molecular characteristics for every variety could be highly efficient if the detected set of markers allows not only distinction between varieties with different male sterility systems but provides high levels of discrimination between varieties in the whole collection.

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