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DUS PROBLEMS ASSOCIATED WITH NUCLEAR AND GENOCYTOPLASMIC STERLITY IN OILSEED RAPE VARIETIES

Document prepared by experts from France

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The following considerations apply to oilseed rape varieties but could also concern any other crop in which different systems of hybridity are used to create hybrids (sunflower, maize, wheat …).

Three situations presently encountered in DUS tests for oilseed rape are described and discussed.

1st situation : Male sterile lines (used as female parental lines in hybrids) are frequently applied for PBR's. How should we conduct the test and take a decision on such a material ?

According to our experience in sunflower, the answer seems quite easy for lines with a genocytoplasmic sterility, whereas the situation is not so evident for lines with a nuclear sterility.

	Female lines A created using a genocytoplasmic sterility system (ex : OGU INRA)	**Female lines A created using a nuclear sterility system** (ex : PGS)
Material to be submitted by the breeder	- A line (100 % male sterile) - B line (100 % male fertile and used in seed production as the maintainer of the A line)	- A line (50% male sterile and 50 % male fertile) - B line (100 % male fertile but not used in practise to produce the A line). Should it be submitted and studied ?
Tests to be conducted	- A line : D U S (necessity to study B) - B line : D (conformity to A) U S	- A line : D (pb : 2 phenotypes) U (pb : 2 phenotypes) S - B line ? : D (conformity to A) U S
Positive Decision on line A	- A line must be D and U - B line must be U and S and «very similar» to A	- A line : ? - B line : ?

Concerning the right case (nuclear sterility), our experience in the last two years lead us to foresee two possibilities :
- either to refuse to protect the A lines (which would force the breeder to protect the B lines).
- or to accept to protect the A lines under the condition that the B line is submitted and fullfills the DUS criteria.

The arguments behind these positions are the following :

- **It seems to us impossible to protect A pure (100 % ms) :** pure seeds of this material do not exist. We can observe A pure only after applying a chemical treatment on A in mixture (in the case of the PGS system). But when doing this chemical treatment, we not only reduce by half the density of plants, but also create big irregularities in the plot (sometimes, plants mf are killed on one meter long in a plot …). Our experience leads us to conclude that we cannot make a correct description of A pure, neither a correct estimation of off-types, because of the irregular growing which obviously affects the phenotype of the surviving plants.

- **If we accept the principle of protecting A (50 % ms - 50 % mf),** it means that we accept to protect a mixture of two varieties. Does it mean that we protect at the same time the two varieties ?

  * **If no** (only the mixture is protected), we can imagine to consider that all the A lines (50 % - 50 %) form a special group when conducting D, U, S tests. We would check DUS criteria on the mixtures themselves.
    Such a procedure is possible ; its limits lay on the less accuracy of the technical work which would be done, and on the less value of the title of protection (it is always more difficult to make a good assessment of uniformity and distinctness in a mixture , than in a pure variety). Another inconvenient for breeders is the possibility to give PBR's to a mixture which could be made from an existing B line, owning to another breeder …

  * **If yes** (the 2 varieties present in the mixture are protected is, when the mixture is protected), then we think that we have to study the two components separatly :

    - The A line pure (after chemical treatment)

    - The B line pure (submitted by the breeder).

    In this case, a positive decision on A (50 % ms - 50 % mf) would require the following conditions :

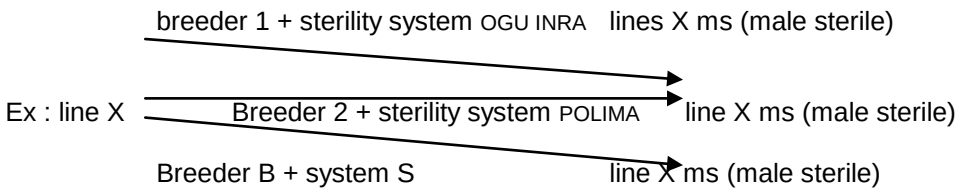
    . A pure is uniform and «similar to B».

    . A (50 % ms - 50 % mf) has effectively 50 % of male sterile plants and 50 % male fertile plants.

    . B is distinct, uniform and stable.

    If we would take such a position, why not just protect the B form ?

2nd situation : Two (or more) male sterile lines are selected from the same initial line.



Question : is it possible to declare the different X ms «distinct», knowing that X is already protected ?

The answer will be discussed after 3rd situation is exposed.

3nd situation : Two lines X ms and X R are selected from the same line X to make it respectively male sterile (X ms) and restorer (X R). In this case, X ms is used in some combinations as a female parental line, and X R is used in other combinations as a male parental line.

breeder 1 + sterility system OGU INRA → lines X ms (male sterile)

Ex : Line X

Breeder 1 + restauration system OGU INRA → line X R (restorer line)

Question : is it possible to declare X, X ms and X R distinct, knowing that X is already protected ?

In France, several cases of varieties illustrating situation 2 and 3 are presently under test or have already been under test.

We want to underline that up to now, we have been able to treat all these cases by applying the current UPOV rule at the level of the phenotype :

- in situation 2, any one of the different X ms taken independently can be declared distinct from X, because there is a clear difference on at least one characteristic of the UPOV guideline : n° 15 : presence or absence of pollen.

- in situation 2, the different X ms can be declared distinct from each other if at least one morphological characteristic allows to discriminate them. To give an exemple, we have observed that a line X ms with the OGU INRA sterility gene generally has wider and larger petals that «the same line» X ms with the POLIMA sterility gene. Up to now, we did not observe cases where there were no differences between two X ms derived from the same X.

- in situation 3, X and X ms are clearly different by characteristic «absence or presence of pollen», as well as X ms and XR.

It might happen in future that there are no morphological differences between two lines X ms derived from the same X, or between XR and X ; thus, the question would arise to accept or not to take into account a phenotypical difference which would require the intervention of another line and another generation : by crossing the two «non distinct» lines with a well chosen line (maintainer or restorer for one system), it would be easy to show that the two lines do not behave in the same way. Distinctness would then be achieved at the level of the phenotype observed or the progenies of the two lines after crossing, and not directly on the phenotype of the lines.

Although we think that it would be very difficult to refuse such a demonstration of evidence of differences between two lines, if it was necessary, we also think that there is no need to adopt right now a definitive and theoretical position in favour or against «distinctness based on different sterility or restoration genes» :

- it would be unrealistic to refuse, on principle, to protect different lines derived from the same one and modified for sterility or restoration. We see with the application tested up to now that distinctness can be easily achieved on the basis of the UPOV rule, with differences observed on several morphological characteristics (width of petals, lenght of petals, production of pollen, flowering date …).

- on the other hand, a definitive position in favour of distinctness based only on the identity of sterility or restoration genes would not be in agreement with the present UPOV rule which requires differences to be observed at the level of the phenotype.

- finally, we propose to adopt an open and pragmatic position saying that each individual case will be studied ; the current procedure in the field will be applied and does not exclude additionnal tests which could show distinctness at the level of the phenotype. Differences observed only at the level of the genotypes (molecular markers) are clearly not sufficient for a positive decision.

We would be very interesting in knowing other expert's point of view on these different questions, which are very important in terms of decisions taken on varieties between different member states, but also in term of organizing the DUS tests for all types of varieties applied. In a crop like oilseed rape where more and more distinctness problems occur, we cannot ignore the interest offered by characteristics linked to the expression of sterility or restoration genes which could help us to group the material and, then, to be more efficient within each group when assessing distinctness of varieties.

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