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NEW BREEDING METHODS IN SUNFLOWER HYBRID RESEARCH AND DEVELOPMENT

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Abstract

Sunflower cultivation is particularly important for vegetable oil production worldwide. Therefore, breeding programs have intensified research and development to increase yields by producing F1 hybrid seeds from crosses of inbred lines through the introduction of cytoplasmic androsterility. For the experiment, we used sterile sunflower inbred lines and restorer inbred lines to isolate the restorer gene in a segregation ratio of 1:2:1 in self-fertilization and 1:1 in backcrossing with PET1-type cytoplasm. The results of our research offer breeders the possibility of achieving greater dynamics in the development of inbred lines, the introgression of genetic resistances to sunflower diseases and the stimulation of a greater number of genotypes to increase the combined capacity of sunflower inbred lines. This was achieved by reducing the breeding program from three inbred lines to two inbred lines using sterility genes and heterozygous fertility genes. The use of segregating restoration genes on sterile cytoplasm enables the breeder to form valuable genotypes, adapt quickly to new herbicide technologies and efficiently introduce resistance genes to new disease nomenclatures.

Key words*: sunflower, breeding, hybrid, inbred lines, cytoplasmic androsterility, restoring genes.*

INTRODUCTION

Since it s introduction in the 17th century (Park & Burke 2020), the sunflower oil industry has produced up to 20 million tonnes of seeds per year worldwide (Havrysh et al., 2023), with breeders playing a key role in developing several strategies and technologies that increase production efficiency and seed oil quality (Lukomets, 2021; Rani, 2017; Ahmad et al., 2005). The first breakthrough in sunflower breeding came when the first high oleic genotypes were developed by V.S. Pustovoyt using mutagenesis techniques. The newly developed germplasm led to a significant increase in oil extraction from sunflower seed production and the development of several varieties (Gavrilova and Anisimova, 2017). The mutagenesis technique has also been used to increase oil quality (Radanović et al., 2018).

Varietal use then changed with the introduction of the first sunflower hybrids Romsun 52 and 53, which were released in 1971 and had a significant increase in seed and oil yield. The first hybrids in the world were produced in Romania at the Fundulea Research Institute (Bran, 2018). The first hybrids developed based on nuclear male sterility (Makarenko et al., 2019) were later replaced with cytoplasmic sterility to increase the efficiency of F1 seed production (Ahmad et al., 2005; Hladni et al., 2007). Breeding programs started to focus more on hybrid production since new sources of cytoplasmic sterility (CMS) were obtained from Helianthus petiolaris (PET1) by Leclercq P. 1969 (Goryunov et al., 2019). CMS genotypes are the result of mutagenesis developed in mitochondria (Makarenko et al., 2019), fertility that can be restored by Rf genes (Horn et al., 2003; Horn et al., 2019; Kusterer et al., 2005). Accordingly, inbred lines of the CMS-Rf system have started to be implemented in research enterprises due to the heterosis effect (Li et al., 2022; Saif et al., 2023) achieved by isolating the A+B (CMS-mentor) line development from the Rf line development program to increase the expression of the heterosis effect to its maximum biological potential of inbred line crosses (Farrokhi et al., 2011; Habib et al., 2011). For oil breeding programs, dominant Rf1 genes are used to restore CMS (PET1), which is present on linkage group 13 (Gholinezhad et al., 2014), and for

confectionary sunflower programs, Rf3 genes, both of which are capable of restoring PET1 cytoplasm fertility.

The presented selection method aims to transform the N-rf1rf1 B-line into S-Rf1rf1 with sterile cytoplasm due to Rf1 genes in heterozygous state, which is required for maintenance (self-pollination or sibling pollination). Sterile plants derived from the S-Rf1rf1 B-line (self-pollination or pollination by siblings) are used in hybrid development, in the development of new populations to increase combining ability or to integrate new technologies and new sources of disease resistance through recurrent selection. In this way, the breeding programme gains genetic flexibility, which makes it possible to maintain and preserve the inbred line efficiently, so that the breeder can choose, depending on the stage of the breeding programme, how to approach the development of high-performance hybrids in a cost-effective way.

MATERIALS AND METHODS

The experiment was carried out using the AG1001B (N-rf1rf1) line B, the AG1001A (S-rf1rf1 or CMS) line A+B and the AG121R (S-Rf1Rf1) line Rf, developed in the Timisoara research, as sources for the experiment (Figure 1). The resulting sterile genotypes (CMS) were used as non-recurrent parents and the Rf line as a recurrent parent to develop new B lines (S-Rf1rf1).

Conversion of the B line N-rf1rf1 to S-Rf1rf1 was carried out by successive backcrosses (BCn) using the same initial CMS as in the non-recurrent case and resulted in progeny until complete conversion, as shown in Figure 1. Given that the initial donor is an Rf line, we recommend that the BC process be carried out from F1BC1 (75%) to F1BC10 (99.9%), as incomplete conversion of the Rf line could degenerate the B-line background of the new population. On the other hand, if the donor is a B line S-Rf1rf1 or Rf1Rf1 for another conversion, the genetic dynamics of the conversion will leave valuable progeny along the BC process that can be used in the breeding program. The new B line on sterile cytoplasm with heterozygous Rf1 genes will change the structure of the sunflower breeding program in terms of inbred line maintenance, population development and combining ability testing. However, in the seed production process, several options can be used in which the reconversion of line B on sterile cytoplasm with heterozygous Rf1 to normal cytoplasm without Rf1 can be carried out using the method proposed by Carvalho and Toledo. (Carvalho and Toledo, 2008).

The percentage of sterile and fertile plants was recorded to track the segregation ratio of the Rf1 gene during backcrossing, self-pollination, and sibling pollination.

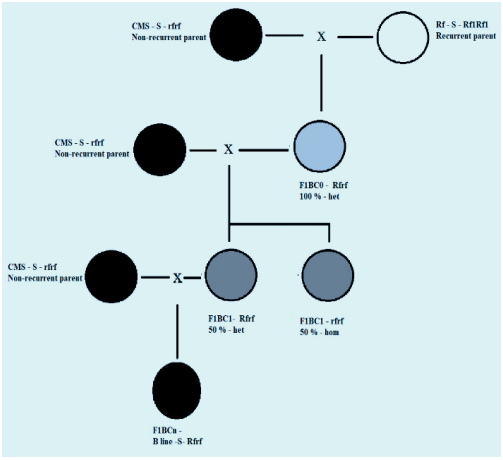

Figure 1. Conversion model of normal B-line with normal cytoplasm into B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1)

Segregation ratio analysis was performed using the $\chi 2$ test, in which the segregation ratio of fertile and sterile plants in the generations analyzed was compared to theoretical Rf1 gene segregation values of 3F:1S for self-pollinated progeny and 1F:1S for backcrossed (BC) genotypes (Sujatha & Shankar, 2011).

RESULTS AND DISCUSSIONS

According to our results in the F1 generation, depending on homozygous or heterozygous Rf1 sources, we can obtain by crossing CMS (PET1) a fully fertile offspring in the homozygous case and 50% fertile and 50%. sterile in the case where we use heterozygous Rf1 donor sources as the recurrent parent.

The F_2 ($\chi 2$=0.27) results indicate a single dominant gene for Rf1 with segregation ratio

(1: 2: 1, Rf1Rf1-fertile plants, Rf1rf1-fertile plants, rf1rf1-sterile plants). Similar results for the Rf1 segregation ratio on CMS sources (PET1) have been observed in other research papers (Horn et al., 2003).

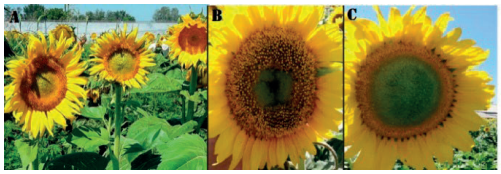

Figure 2. B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1). (A)- Sterile and fertile plants on the new B-line developed F_1BC_n, (B)-Fertile plants, (C)-sterile plants

Our results indicate a 1: 1 ratio in terms of fertile and sterile plants in the BC. In the F_2BC_1 generation a ratio of fertile to sterile plants of 1: 1 was obtained (χ^2=2.50). The same ratio can be observed for the F_2BC_2 generation (χ^2=0.40).

The results are in agreement with those of other authors (Zhang and Stewart, 2001; Yue et al., 2010) who report the same segregation ratios for the Rf_1 gene in both BC and self-pollinated. Therefore, the concept of B-S -Rf1rf1 inbred line development formation is valid (Table 1).

Table 1. Segregation ratio of fertile and sterile plants in the B-S line Rf1rf1

Generation	Number o plants	B-line S-Rf1rf1		Theoretical value		χ^2
		Fertil	Steril	Fertil	Steril	
F2	80	62	18	60	20	0.27
F2BC1 (SIB1)	40	25	15	20	20	2.50
F2BC2 (SIB2)	40	18	22	20	20	0.40
F3BC2(SELF1)	40	26	14	30	10	2.13
Test.cross(F1)	80	80	0	80	0	0

Also, in the F_3BC_2 we observed a segregation ratio of 3: 1 (χ^2=2.13) meaning that the Rf1 genes keeps the segregation ratio in normal standards when using inbred lines of B-line S-Rf1rf type (Table 1). When converting the normal B line N-rf1rf1 to S-Rf1rf1, all progenies from BC_1 to BC_2 generation crosses gave 50% fertile and 50% sterile plants (Figure 2).

Successive backcrossing with the CMS version of line B was carried out using the fertile plants from the resulting progeny. It is very important to know that if we want to achieve a complete conversion of the B line, we need to ensure that there are enough CMS seeds that can stand in for the non-recurrent parent until the new S-Rf1rf1 B line is fully developed. A complete transformation of the line will be carried out in parallel with a fertility testing procedure that can be controlled by crossing with CMS tests or using molecular markers.

Maintaining of the B-line S-Rf1rf1 is mandatory on the heterozygous allelic state of the Rf1 genes, otherwise the homozygous state of the Rf1 genes will not allow their maintenance and it will be necessary to reintroduce the CMS form again of the normal B line to resume the heterozygous allelic state. The method of maintaining new inbred lines (Figure 3) can alternate either through self-pollination and sib-pollination, it can be only sib-pollination, or it can be only through self-pollination. Thus, the breeder can use a method depending on the goal pursued.

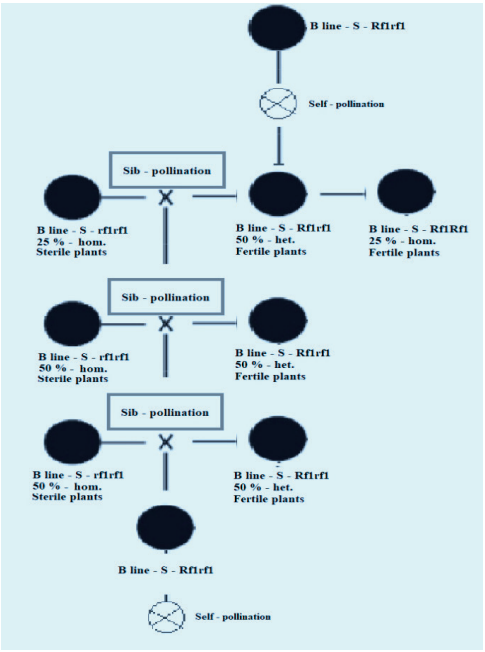

Figure 3. Maintenance procedures of the new B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1rf1)

For testing the combining ability, sterile forms resulted plants during maintenance will always

be used as non-recurrent forms together with an Rf-tester inbred line. The sib-pollination maintenance form will only continue if the test hybrid genotype is good enough to be maintain until the final evaluation of the combining ability of the new inbred line B-S-Rf1rf1. Inbred lines intended for registration can be propose for registration under B - S-Rf1rf1 form, or they will be re-converted into traditional inbred lines of type A+B. (CMS-maintainer B-N- rf1rf1) (Figure 4).

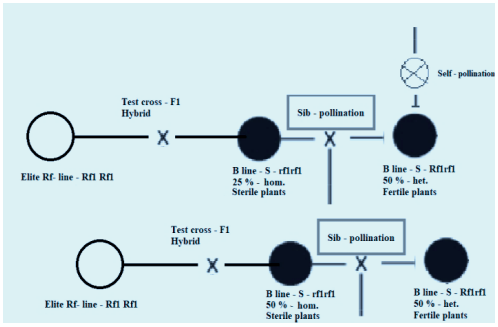

Figure 4. Testing the combining ability of the new B-line with sterile cytoplasm and heterozygous fertility restoring gene (Rf1rf1)

CONCLUSIONS

The Rf1 gene confirmed as single dominant gene being able to restore sterile cytoplasm PET1 according to our results in a segregation ratio for self-pollination of 1: Rf1Rf1 - fertile plants, 2: Rf1rf1 fertile plants and 1: rf1rf1- sterile plants in all our breeding procedures of conversion of the B line N-rf1rf1 into S-Rf1rf1. Also, sib-pollination register progenesis in rapport of 1: Rf1rf1 fertile plants (50%) to 1: rf1rf1- sterile plants (50%) when crossing sunflower genotypes with cytoplasm PET1 concluding the essential need of a single dominant gene for the conversion of B line N-rf1rf1 into S-Rf1rf1. Developing B-lines with S- Rf1rf1. can increase the dynamics of the sunflower breeding program reducing the workforce by reducing the breeding program from 3 inbred lines to 2 inbred lines, eliminating the emasculation process for developing new populations and conversions processes and testing in preliminary stages the combining ability of the new B-lines without CMS conversion. Other advantages consist is in avoiding unwanted effects that appear on CMS forms by protecting and maintaining a single Rf1 restorer gene to its exact segregation ratio and avoiding the formation of recessive restorer genes.

The method of maintaining new B-inbred lines S-Rf1rf1 can alternate either through self-pollination and sib-pollination, it can be only sib-pollination, or it can be only through self-pollination. Thus, the sunflower breeder can use these genetic dynamics of the method of maintenance depending on the goals of the research program.

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