,,,,SUNFLOWER BREEDING FOR RESISTANCE BROOMRAPE (*Orobanche cernua* Loefl. /*Orobanche cumana* Wallr.)

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Abstract

The paper reports the results we have thus far achieved in introducing genes for resistance to broomrape into sunflower line HA-19. Our use of convergent crosses based on transgressive recombination has proven very suitable as a method for incorporating resistance genes into standard sunflower lines. The χ^2 test has shown the inheritance of resistance to be controlled by a single dominant gene. The results have also confirmed that the presence of broomrape in plant materials can be diagnosed very early in the season using a modification of the Pancenko method. In the present study, an assessment made 40 days after sowing showed that broomrape plants were for the most part well developed by that time.

Key words: Sunflower, broomrape, resistance, dominance.

Introduction

Broomrape, a floriferous parasite of the family *Orobanhaceae*, is one of the most dangerous parasites of sunflower. It is a typical obligate parasite that deprives the plant of water and mineral substances. Its center of origin is most likely the Black Sea basin, from where this parasite spread into the interior of the continent by *Artemisia maritima* (Horvath, 1996). According to Terjohin (1988), *O. cernua* can form as much as 235,000 seeds per plant. Broomrape is known to be spread by insects, watering water, floods, and wind, while opinions vary as to whether or not it can also be spread by seeds. Burlov and Kostjuk (1976) and Pogorelitskij and Geshele (1976) established that resistance to broomrape is controlled by the dominant gene Or. Vranceanu et al. (1981) identified a series of differential lines for distinguishing between races C, D, and E. Some researchers, however, determined that the inheritance of broomrape resistance is not monogenic. Krokhin (1980), for instance, reports finding two complementary genes for this trait, while Dominguez (1996) made crosses with inbred line R-41 and determined that the resistance is controlled by two independent recessive genes. In a study involving crosses with the variety Ždanov 8281, Saavedra del Rio (1994) showed the presence of an inhibitor gene for Or_2, and in some of the crosses he even found epistasis. Several precent papers, therefore, have shown that the inheritance of resistance to downy mildew is considerably more complex than previously thought (Alonso, 1998). Investigating two lines that carry resistance to broomrape races A-E (LC-1093 and P-1380-2), Pacureanu-Joita Maria et al. (1998) determined that P-1380-2 did not have complete resistance to broomrape in the Constanta-Tulcea-Braila area and concluded that a new race, race F, must have appeared in the region. They also found that that line LC-1093 contained a gene for resistance to race F as well as the Or_6 gene. In Yugoslavia, there had not been much interest in broomrape until the severe infestations of the late 80s and early 90s. The parasite became a major threat to the country's sunflower production in 1995, when it appeared in the

province of Vojvodina on lighter soils. Detailed studies revealed that broomrape race in question was race E, which up until that time had never been recorded in Yugoslavia (Mihaljcevic, 1996). The varieties Progres, Jubilejni 60, and Oktjabr, developed by crossing *H. annuus* with *H. tuberosus*, are resistant to race E. The introduction of new, race-E-resistant sunflower hybrids in Yugoslavia has significantly reduced the danger of this parasite's further spread in the country (Skoric et al., 1996). By testing populations of *H. annuus* and *H. petiolaris* ssp. *petiolaris* for a broomrape population containing races A, B, and E, Dozet and Marinkovic} (1998) have determined that the species *H. petiolaris* is an excellent donor of Or genes.

New races of broomrape can significantly endanger sunflower production. The development of hybrids resistant to the new races of these pathogens is therefore essential, as is the incorporation of resistance genes into standard sunflower lines that have proven themselves in large-scale production and that have good production properties and high GCA and SCA values. This is the surest and most economical way to control The objectives of this paper were to present our results so far regarding the introduction of the Or_5 gene into line HA-19 and to study the mode of inheritance of resistance to broomrape

Materials and methods

In the study, we used the convergent cross method based on transgressive recombination proposed by Mac Key in 1962, which enables one to almost fully restore the recurrent parent in a span of five generations. The recurrent parent in our program is the commercial line Ha-19. This line does not contain Or_5 gene. Donors of genes for resistance to broomrape were line CMS1 90.

Resistant plants from the broomrape test are transferred to a greenhouse, where they are first transplanted into Jiffy-7 pots, and then, after they have reached the stage of 3-4 permanent leaves, either into buckets containing the infected substrate or into infected field. The infected field is a highly infested broomrape plot where sunflowers have been grown for three years in monoculture. Such conditions ensure that the plants will be infested while enabling the resistant plants to grow normally and produce enough pollen and seed for further selection. Another advantage of this method is that it enables a large number of progenies to be both tested and utilized at the same time, which is of particular importance in the early stages of selection. During the winter, plants are tested in 12 dm^3 buckets filled with a mixture of sand and humus in the proportion of 1:1. Before transplantation, each hole to be sown with sunflower seeds is infected with broomrape seed. The seed has been collected from mature broomrape plants picked from the infested materials in the field and, once the unwanted stem parts have been removed, left to lie about for around 30 days to terminate dormancy. Four plants are transplanted into each bucket. Later, the plants are assessed for broomrape, susceptible ones are removed, while the resistant ones are used in further selection. Although this method cannot be used to test as many plants as in field studies, it is very suitable for greenhouse tests during the winter as well as for testing the later generations. In the buckets, the symptoms of broomrape infestation are extremely noticeable, as a large number of broomrape plants forms on the susceptible sunflowers. All of the breeding materials can also be double-checked for broomrape presence using another, faster method (Pancenko, 1975), since the materials can be assessed for broomrape in as little as 28 to 35 days (Mihaljcevic, 1996). Previous experiences with the tests have shown that the materials can be assessed even earlier (as shown in the results), but we choose to assess them 40 days after sowing just to be on the safe side. This method can be used to assess 200 accessions per 20 m^2 (most often 10 plants per accession are assessed), and if the assessment is done early,

23 days after sowing, the resistant plants can even be used for further selection. The assessment is done based on the presence of nodules on the root and nodule number and size, and when it is conducted 40 days after sowing the susceptible materials already bear very developed broomrape plants. The advantage of this method is that once the broomrape seeds come into contact with the root, infection is guaranteed. The method allows a very precise analysis of the root system depending on the study's objective. In our study, Canadian line AD-66, which is used worldwide as an indicator of the presence of any broomrape race, was used as the universally susceptible line. Watering is done according to need, based on previous experiences. Prior to assessing the materials, waterin g is reduced so that the root can be released from the substrate with greater ease and without the use of water. The test-tube substrate is a mixture of humus, sand, and perlite in the proportion of 3:1:1. In our conditions, the materials tested in the test tubes are assessed based on the presence of nodules and plants formed on the root. Every plant on which nodules or already developed broomrape plants are observed is rated susceptible.

Results and discussion

Analysis of resistance showed the expected segregation ratio to be 3:1 (resistant/susceptible), assuming that the inheritance of the resistance is controlled by a single dominant gene. The χ^2 simple test confirmed the 3 : 1 ratio and that there was no deviation from the experimental and theoretical values — P=0.50 for broomrape (Tab. 1).

Table 1. x^2 values for the 3 : 1 segregation ratio of traits for resistance to broomrape.

	Resistant plants	Susceptible plants
e	77	36
t	85	28
d	-8	8
d^2	64	64
d^2/t	0.75	2.28
χ^2	3.03	

For the breeder, the confirmation of the hypothesis about the dominant mode of inheritance is above all a confirmation that the tests they conducted were the correct ones to choose, but it is also an incentive to a greater effort towards the ultimate goal, which is the incorporation of resistance genes into standard sunflower lines. Looking at the frequency of the so-called pure progenies, i.e. those with no diseased plants, we can see that the number of broomrape-free progenies increased significantly within the space of a single generation of selection. Thus, there were no pure progenies in the S_0, but already in the S_1 17 out of the 48 progenies we tested turned out to be pure (Tab. 2). This group included heterozygous as well as homozygous progenies, and the analysis of the S_2 generation will show the frequency of resistant homozygous progenies.

Table 2. Frequency of pure progenies in the S_0 and S_1 generations in the broomrape test in F_2 generation

Generation	Number of progenies	Resistant progenies	%	Susceptible progenies	%
S0	**15**	**0**	**0**	**15**	**100**
S1	**48**	**17**	**35.4**	**31**	**64.6**

Another issue is the question of the earliest possible diagnosis of broomrape. Early diagnosis enables the transplantation of plants after assessment and their further use in selection. According to Pancenko (1975), the infestation level is determined after 25 days and the materials can be assessed immediately afterwards. Using what is essentially the above Pancenko method, Mihaljcevic (1996) reports that accurate diagnosis can be made 28 to 35 days after emergence. Investigating the infestation severity in line AD-66, which was used as the susceptible control, we have determined that diagnosis can be made even earlier than 25 days after emergence. The line was studied 23-40 days after sowing, or 20-37 days after emergence, and anywhere from 1 to 43 (typically 10-20) nodules per plant were determined to have formed on the plants as early as 23 days after sowing. After only 10 days, broomrape plants had already developed, and 40 days after sowing these plants overflowed the substrate. Between three and five broomrape plants form in the test tubes on average, while in the field the number is significantly higher — not infrequently as high as 60 broomrape plants per one sunflower plant. The small volume of the test tube does not allow the formation of a larger number of plants, but in the test with buckets as much as 30 broomrape plants form in susceptible materials. At assessment after 40 days, the broomrape plants are already formed or even very well developed, while the number of plants with small and large nodules is at this stage still relatively low. In the upcoming cycle of research, we plan to re-confirm the effectiveness of genotype assessment 20 days after emergence as well as that of the further use of these plants in the selection process.

Conclusion

The use of convergent crossing according to the transgressive segregation principle has proven to be a successful method that enables us to obtain 94% of germplasm of the recurrent parent after five generations.

Using the x^2 test, we have confirmed that the mode of inheritance of resistance to broomrape is monogenic, and the experimental results were in agreement with the expected, theoretical ratio of resistant to susceptible plants.

The bucket test with four plants per bucket has been shown to be a very successful way of testing as well as utilizing the resistant plants.

The test tube test enables very early diagnosis-making, as early as 23 days after sowing.

The test tube testing and assessment after 40 days that we conducted has shown that broomrape plants are in most cases well developed by that time.

Once the plants have been taken to the homozygous stage, it takes 6-9 more generations for the lines to be translated into the cytoplasmic male sterile form. This means that from the beginning of the breeding process at least 13-16 generations have to pass before the lines reach the desired stage, which just goes to show how complex and difficult this task is.

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