International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 8, No. 5, p. 212-218, 2016

INNSPUB

RESEARCH PAPER OPEN ACCESS

Tetraploid monogerm lines as maternal components of sugar beet hybrids

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Key words: Sugar beet, Tetraploids, Male sterility, Monogerm, Productivity.

http://dx.doi.org/10.12692/ijb/8.5.213-218 Article published on May 30, 2016

Abstract

Tetraploid monogerm lines of sugar beet with pollen sterility and their hybrid combinations with diploid multigerm pollinators are the object of the present study. Data received for the sterility of the lines and the percentage of the hybrids in their progeny confirm the thesis, that almost a full hybridization is obtained in this type of crosses, even in cases of partial pollen sterility of the female components. This is due to the higher fertilization ability of the pollinators' haploid pollen. The lower germination of the seeds formed on the tetraploid male-sterile components, is consequence of the growth of the pericarp, containing substances – inhibitors of the germination. The high germination levels of the tested crosses prove that by means of some breeding methods this basic disadvantage of the monogerm tetraploids in hybridization could be overcome. In the field tests the hybrid combinations of the tested tetraploid monogerm male steriles demonstrate significantly higher sugar content and output than the Standard of certified varieties. The high relative values of the white sugar yield from the hybrid combinations are indicative for the good combining ability of the studied tetraploid lines. This proves the expedience of tetraploid monogerm male steriles use in the sugar beet hybrid breeding.
apply half of conventional consumption of chemical fertilizers along with Azotobacter and Pseudomonas.

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Introduction

Sugar beet hybrid varieties

The modern sugar beet varieties are hybrids between monogerm male-sterile lines and multigerm diploid and tetraploid pollinators (Konoshita, 1994). The use of lines with pollen sterility as maternal components of the hybrids is forced by the hermaphrodite structure of the beet flowers and the impossibility of mass castration for the needs of seed production, and thus the hybridization process could be regulated (Antonov, 1997). All the different schemes of breeding monogerm male-sterile lines include assessment of sterility, monogermity, habit of bushes and quantity of seeds formed, seeds germination, own productivity and technological qualities of the beet row material, combining ability. The main objective is by individual combinations and systematic selection to create homozygous lines - practically fully sterile and monogerm, with good productivity and technological qualities (Balkov, 1990).

Diploid versus triploid hybrids

The triploid sugar beet hybrids have dominated the hybrid seed market for nearly 25 years. The main reason for that has been the limited genetic variability in the monogerm gene pool, caused by the need to first select for monogerm and male sterility-maintainer genotypes, and subsequently, within this restricted gene pool to develop vigorous lines, with satisfactory quality characteristics and good combining ability. For considerable number of years triploid hybrid varieties almost invariably produced a greater yield than comparable diploid hybrids. Recently most breeders have significantly increased the genetic variability in their monogerm gene pools, using various systems of selection to develop new improved type-O inbreds and their male sterile equivalents. This, together with the intensified breeding of diploid multigerm pollinator lines and the rapid expansion in molecular technology based selection approaches, has resulted in highly competitive diploid hybrid varieties and has brought to dramatic decrease in the proportion of triploid sugar beet varieties in most European countries (Bosemark, 2006).

Sugar beet hybrid varieties breeding in Bulgaria

The triploid sugar beet hybrids have proved their advantages in the conditions of our country, even if growing of high quality triploid hybrid seeds is more costly and requires more skills and experience. The monogerm triploid hybrids based on diploid monogerm male steriles and tetraploid pollinators are absolute leaders in all our tests for productivity and technological qualities of the raw material. No matter the climatic conditions (normal rainfalls quantities or droughts during the vegetation of the crop) the triploid hybrids realize the highest white sugar yield, their environmental stability is much higher than that of the tested diploid hybrids.

Breeding reciprocal triploid hybrids of sugar beet

Triploid sugar beet hybrids could be received also by crossing tetraploid monogerm male sterile females with diploid multigerm pollinator lines or populations (Fitzgerald, 1977; Pfeiffer, 1978). Now we have a large number of tetraploid monogerm lines and populations, which could be very good basis for such a direction of our breeding program.

The genetic variability of the tetraploid monogerm gene pool is much higher than the variability of the diploid monogerms, and should be used, no matter the scepticism of some of our colleagues. By individual combinations and systematic selection we have created some new tetraploid monogerm lines with satisfactory seed germination, which need to be tested for the levels of their pollen sterility. This would show us to what an extent the male steriles have become equivalents of the relevant type-O sterility maintainers. The next objective of our study is to establish the correlation between the level of pollen sterility of the tetraploid monogerms and the percentage of triploid hybrids of their crosses with diploid multigerm pollinator populations and lines, because the portion of the triploid hybrids in the generation directly affects the manifestation of heterosis effect in hybridization (Antonov, 1981). This could prove the findings of other authors (Magassy, 1980) for the lower viability of the diploid pollen. Of course, the final objective of our study will be the test of the combining ability of the monogerm tetraploid maternal components in crosses with multigerm pollinators. The experience with such hybrids is limited and there are almost no reports that they yield better than comparable hybrids of diploid male steriles, confirmed in more extensive tests. We have already two certified varieties of sugar beet, which are created using this scheme, and for long period of time have been basic varieties for the sugar industrial beet production in our country. There are quite a lot of preconditions for the successful realization of triploid hybrids based on male sterile monogerm lines. And the basic aim of our study is on the basis of the most important biological and economic characteristics of the new tetraploid monogerm lines and the manifested combining ability in their crosses with multigerm pollinators to prove their high breeding value.

Material and methods

Breeding Materials in the Study

The study is performed on the experimental fields of the Agricultural Institute – Shumen during the period 2011-2013. It covers the basic for breeding biological indices of thirteen tetraploid monogerm male-sterile lines and the productivity and technological qualities of their triploid hybrids with five multigerm pollinators.

Methods of Pollen Sterility determination, Monogermity and Germination Assessment

The grouping of the plants for male sterility is made according the following method: sterile – fully male-sterile plants with white, colorless or pink stamens without pollen; half-sterile – plants with pale, non-bursting open anthers, not releasing pollen. The half-sterile plants which generate pollen, no matter its quality and quantity, fall into the group of fertile plants. This method is easily applied in the large scales of the practical breeding – for a three years period 600 plants of each line were given an account of. The degree of monogermity and the laboratory seeds germination are determined by standard for our Institute methods, in four repetitions for each year of study.

Hybridization and triploid hybrids determination

The tetraploid monogerm male-sterile (MS) lines were crossed with the multigerm (MM) pollinator E, in 4:2 ratio between the plants of the MS line and of the pollinator, and with four doubled haploid pollinators, in sunflower isolation. After the harvest of the seeds the percentage of triploid hybrids was determined. The ploidy level is determined by microscope observations of apices of primary roots of 3-days germs, and by flowcytometric analysis, according to Kikindonov (1999).

Field Tests Design

The field tests for productivity and technological qualities of the triploid crosses, carried out during 2012-2013, are arranged in a randomized block design, each tested variant in 4 repetitions, 3 rows spaced 45 cm apart, experimental plot with 10.8 m^2 area, with a Group Standard of certified sugar beet varieties.

Statistical Treatment of the Field Tests Results

Dispersion analysis (Lidanski, 1988) was used for determination of the statistical significance of the differences between the test variants.

Results and discussion

We have assessed the male-sterility level of thirteen individual combinations of 5 tetraploid monogerm origins for three years (Table 1). The part of the fully male sterile plants (with white, colorless or pink stamens without pollen) in the tested monogerm tetraploids varies – from 27.3% (MS78-13) to 92.4% (MS142-17) with mean of 64.2% for all the tested lines. We would outline the high male-sterility of the monogerm tetraploid combinations MS142-17 and MS78-6 – we would say that they are on the way to become sterile analogues of the relevant type-O maintainer monogerm line. The fertile plants in these two male sterile were registered during the first year of tests, on the second one such plants were missing, because of the selection we make in the combinations. Very good male sterility shows MS146-22 – with 79.4% fully male sterile plants, and no any fertile plants. We suppose that the monogerm combinations

MS78-13 and MS015-32 should not be treated as male-sterile, because of their very low percentage of fully male-sterile plants, the high percentage of half-sterile plants could become fertile in certain climatic conditions.

The laboratory germination of the seeds, formed on the tetraploid monogerm lines participating in our study is quite satisfactory, having in mind that the maternal components are still in the initial stage of their stabilization. The comparatively high values of laboratory germination of the tested hybrid combinations (mean of 79.5%) show that by breeding treatment (selection and individual combinations) the creation of forms with increased seeds germination is not so difficult. At this stage we would continue our work with the hybrid combinations whose germination exceeds 80%.

Table 1. Male sterility, monogermity and seeds laboratory germination of male-sterile lines of sugar beet (2010-2012).

Line	Male Sterility- %			Germination %	3x hybrids %
	Sterile	Half-sterile	Fertile		
MS 59-7	52.9	45.4	1.7	67.0	99.0
MS 59-9	63.1	34.2	2.7	83.0	88.6
MS 59-11	61.9	38.1	-	81.5	89.2
MS 78-6	89.2	9.1	1.7	80.0	98.2
MS 78-12	61.4	38.6	-	80.5	89.2
MS 78-13	27.3	71.6	1.1	75.5	99.0
MS 142-15	70.4	28.4	1.2	80.0	90.0
MS 142-16	73.1	25.3	1.6	85.0	88.5
MS 142-17	92.4	7.0	0.6	77.0	94.4
MS 146-22	79.4	20.6	-	79.5	84.9
MS 146-26	60.5	37.2	2.3	87.5	92.1
MS 015-32	33.3	66.7	-	72.5	100.0
MS 015-35	69.4	30.6	-	84.5	94.2

Table 2. Productivity and technological qualities test of triploid sugar beet hybrids of tetraploid monogerm male sterile lines with multigerm diploid pollinator E, 2012-2013 - in % of Group Standard of certified varieties.

MS line	Root Yield	Sugar content	Output	White sugar yield
MS 59-7 x E	92.2	115.7***	120.2***	110.6
MS 59-9 x E	94.2	113.5***	117.4***	110.5
MS 59-11 x E	102.2	108.0*	109.8*	112.2
MS 78-6 x E	90.5	114.4***	117.6***	106.2
MS 78-12 x E	98.2	106.7	108.6	106.6
MS 78-13 x E	95.6	111.4**	114.3**	109.1
MS 142-15 x E	91.7	112.4**	116.2**	106.4
MS 142-16 x E	99.5	106.0	106.0	105.3
MS 142-17 x E	105.7	110.2*	111.2*	117.4*
MS 146-22 x E	91.1	105.4	107.4	97.7
MS 146-26 x E	95.1	105.8	107.4	102.0
MS 015-32 x E	79.9	112.2**	116.3**	92.9
MS 015-35 x E	100.6	104.5	105.3**	105.8

In the generation from the crosses of the tested lines with the multigerm diploid pollinator E are registered mean of 92.9% triploid hybrids. Some researchers in the past have reported (Zakhariev and Mihaylov, 1977) the possibility of obtaining high percentage of hybrid seeds using tetraploid monogerm MS lines with incomplete pollen sterility. Our test results show that the lower male-sterility of the maternal components is not an obstacle for their hybridization with diploid MM pollinators, probably because of the much higher vitality of the diploid pollinator's pollen in comparison with that of the tetraploid females. In our observations during the flowering period we noted that the fertile monogerm plants among the females release their pollen later in the morning than do the multigerm pollinator plants. Lets note that the higher percentage of hybrid seeds is formed on the tetraploid females, which we refused to call male steriles – from MS015-32 (33.3% male sterile plants) the percentage of the obtained triploid hybrids is 100, and 99% of the seeds formed on MS78-13 (27.3% male-sterile plants) are triploid. The lack of correlation between the percentage of the male-sterile plants and the portion of the hybrids in the generation shows that there is a specific for the lines receptivity of the maternal plants towards the more vital haploid pollen of the pollinator. And with such a degree of maternal component's male sterility the high percentage of obtained triploid hybrids is guaranteed.

Table 3. Productivity and technological qualities test of triploid sugar beet hybrids of 4x monogerm male steriles with multigerm dihaploid pollinators, 2012-2013 - in % of Group Standard of certified varieties.

4x line/pollinator	Dh 52	Dh 57	Dh 58	Dh 63	Average
Root Yield	P≤0.05 – 11.1				
MS 59	90.4	88.2	93.2	93.9	91.4
MS 78	98.5	86.5	102.3	96.3	95.9
MS 142	95.6	99.5	94.3	98.6	97.0
Average	94.8	91.4	96.6	96.3	94.8
		Sugar Content	P≤0.05 – 7.8 P≤0.01 – 10.3		
MS 59	108.8*	113.1**	113.8***	115.8***	112.9**
MS 78	112.5**	106.0	108.1*	113.1**	109.9*
MS 142	105.0	110.7**	102.4	104.6	105.7
Average	108.8*	109.9*	108.1*	111.2**	109.5*
		Output	P≤0.05 – 9.8 P≤0.01 =- 13.0		
MS 59	111.8*	115.7**	117.3***	118.4***	115.8**
MS 78	115.8**	107.0	108.4	115.8**	111.8*
MS 142	107.0	113.4**	100.4	104.9	106.4
Average	111.5*	112.0*	108.7	113.0**	111.3*
		White Sugar Yield	P≤0.05 – 15.1		
MS 59	100.9	102.0	109.2	110.9	105.8
MS 78	113.9	92.4	110.7	111.4	107.1
MS 142	102.1	112.7	94.6	103.2	103.2
Average	105.6	102.4	104.8	108.5	105.4

The results of the field tests for productivity and technological qualities of the same thirteen hybrids are given on Table 2. The root yield from these crosses is on the level of the Standard's yield (exception is the combination MS015-32 x E). And a proved higher yield, compared to the remaining crosses, form the hybrid combination MS142-17 x E (105.7% of the Standard's root yield). At the same time the majority of the tested hybrids are with proved higher values of the technological qualities sugar content and output than those of the Standard. In result of that the mean white sugar yield from the tested hybrids is 6.4% higher than the yield of the Standard (the hybrid combination MS142-17 x E – with proved difference of the higher white sugar yield).

The test of hybrids of some tetraploid monogerm lines (we have chosen the combinations with the highest seeds germination) with multigerm doubled haploid pollinators (Table 3) allows better assessment of the same lines as parental components in the hybridization, regarding their combining ability. The root yield here is also on the level of the Standard's yield. If the word is about the technological indices impressive are the proved higher mean values of sugar content and output of the hybrids of MS59-9 and MS78-12. And also, the mean values of these two indices for all the tested hybrids of the dihaploid multigerm pollinators exceed the Standard's values with proved differences. Normally, the white sugar yield from the tested crosses is 105.4% of the value of this resultant index of the Standard varieties. Magassy (1980) reports, that the hybrids of crosses between monogerm tetraploid MS lines and diploid pollinators exceed the relevant Standard variety in their white sugar yield.

Triploid hybrids of tetraploid monogerm population with incomplete pollen sterility, tested by Zakhariev and Mihaylov (1987) have also exceeded the Standard's white sugar yield with proved differences because of the very well proved heterosis effect in their productivity. In our study, the proved higher values of the technological parameters sugar content and output determine the realization of higher than Standard's yield of white sugar from the triploid hybrids of the tested monogerm tetraploid MS lines.

Conclusion

High percentage of triploid hybrids is received when crossing monogerm tetraploid lines with incomplete pollen sterility with diploid multigerm pollinators, no matter the extent of maternal component's sterility. Monogerm tetraploid male-sterile lines with increased seeds germination could be differentiated by purposeful selection and individual combinations. In certain conditions the hybrids of monogerm tetraploid lines with multigerm diploid pollinators realize higher white sugar yield than the Standard's on the basis of their proved higher technological indices.

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