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Genetics and Breeding

Economical qualities of crosses between doubled haploid sugar beet lines

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Abstract. *The gynogenesis of isolated in vitro ovules is a basic method for the receipt of haploid plants and doubled haploid forms of sugar beet. The application of the induced haploidy is an excellent possibility for enrichment of the sugar beet gene fund and allows accelerated creation of highly homozygous and genetically stable lines. Ten years field tests were conducted on the experimental fields of Agricultural Institute – Shumen to study the practical value of dihaploid monogerm and multigerm lines for the hybrid breeding of sugar beet. All the tested dihaploid lines formed higher root yields than their initial lines, maintained by classical methods. The monogerm dihaploid 150-4 and the multigerm tetrahaploid 48 combined that with proven higher sugar content. The diploid hybrids of the monogerm dihaploid 150-4 and the multigerm dihaploid 58 lines realized the highest white sugar yields, significantly higher than the yield of the Standard varieties. The triploid hybrids of the monogerm dihaploid lines 54-4 and 150-4 and of the multigerm tetrahaploid 55 pollinator gave the highest white sugar yields, and could be presented to the State Agency for tests and certification as new Bulgarian sugar beet varieties.*

Keywords: sugar beet, monogerm, multigerm, dihaploid, hybrid

Introduction

Self-pollinated (inbred) lines are used in the heterosis breeding of sugar beet. The obtainment of homozygous lines by enforced self-pollination is connected with some difficulties (Bosemark, 2006). The self-sterile biotypes, being a basic part of the populations, almost don't set seed. At the other hand, bringing the genes, part of which are recessive, in homozygous condition, is accompanied by the characteristic inbreeding depression of the lines. And not the last, the inbreeding brings to significant narrowing down the hereditary basis of the initial population.

The application of the dihaploidy (Bossoutrot and Hosemans, 1985) in the breeding program allows considerable shortening of the time for creation of highly homozygous lines (Atanassov, 1988). The obtainment of plants from gametes, not from zygote, by induced haploidy (Steen, 1987), is an excellent possibility to find out and stabilize into lines valuable gene recombinations, determining the expression of useful technological indices and increased disease resistance (Zakhariev and Kikindonov, 1997; Kikindonov, 2003, Eujayl et al., 2016).

The aim of our long-term research is to study some productivity parameters of monogerm and multigerm sugar beet dihaploid (doubled haploid) lines. The inclusion of these lines in the cross breeding schemes gives ideas for their genetic and breeding values.

Material and methods

Our field tests during the period 2004 – 2014 cover monogerm and multigerm dihaploid lines and the hybrid combinations between them. All the tested lines descend from isolated *in vitro* unpollinated ovules of individually selected plants. From individually selected plant of the diploid multigerm population 316 were isolated non-fertilized ovules, which were cultivated *in vitro* according to the method for induced *gynogenesis* of Slavova (1993). The diploidization of the haploid explants was made in the 8-leaves stage of the explants. During the process of diploidization of the haploid regenerants 4 tetrahaploids arose – two of them participate in our study. The same *in vitro* techniques were used for the creation of the monogerm dihaploid lines. The stabilized lines 19-27 and 19-66 descend from a selected plant of the monogerm diploid line 19, while the initial line 760, maintained by means of microclonal propagation followed by seed reproduction, descends from individually selected plant of a monogerm hybrid combination (31113 x A_3). And the same is the origin of the other two participants in our study – the monogerm dihaploids 54-4 and 150-4. The high self-fertility and monogermity of the obtained genotypes determine their use as "O" types of monogerm male-sterile lines (Kikindonov, 2003).

The seed reproduction groups for joint flowering were planted in isolation crop of sunflower, as well as the crosses between the monogerm and the multigerm dihaploids (proportion 4:2 between the maternal monogerm component and the multigerm pollinator).

The tests for technological qualities were carried out during the period 2004-2014. The soil type of the experimental fields of the Agricultural Institute – Shumen is carbonate black-earth with a good mechanical structure and weakly alkaline reaction of the soil solution.

Experimental design

The randomization of the field tests is according the two-seated lattice method, in four replications, having a plot size of 10.8 m^2 with 3 rows each at a distance of 45 cm, with a Group Standard of certified varieties of sugar beet. The tested parameters are the root yield (t/ha), sugar content (%) and the white sugar yield (t/ha).

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Statistical analysis of the test results

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

Results and discussion

The induced haploidy differentiates the initial sugar beet population or hybrid form into highly homozygous lines. Of course, the homozygousity of the doubled haploids is a basic, but not the only one advantage of these lines. The presence of only sister chromosomes in the doubled haploids copies and stabilizes the initial form, and allows separation of unique recombinants, arisen in result of the crossing-over during the meiosis. Thus the gene fund could be enriched with genotypes, showing combinations of traits, valuable for the sugar beet breeding (Kikindonov, 2003, Forster et al., 2007). This is proved by our long-term field test results of the studied dihaploid (DH) lines (Table 1). All the tested DH lines have higher root yield than the relevant initial lines. This confirms the proposition that during the dihaploidy accumulation of additive factors for productivity is realized. We would underline the proved higher productivity (root yield) of the monogerm dihaploid 19-66 than the yield of the initial line 19. The proved positive differences between the yield of the tetrahaploid (TH) lines and that of the initial line are not a surprise, as well as the higher root yield of the multigerm TH lines compared to the DH multigerm lines' yield of roots. There are quite indicative differences between the sugar content values of the tested lines. The monogerm DH line 150-4 has proven higher sugar content than the initial monogerm line 760, significantly higher than the sugar content of the other monogerm DH line 54-4. Impressive is the proved higher sugar content of TH 48 – it is significantly higher than the sugar content of TH 55, DH 58 and the initial multigerm line 316. Both results confirm our thesis that for our study have been chosen some valuable recombinants in regards to that quality trait. The white sugar yield from a unit of area is a resultant index (Carter, 1987), which is determining for the economical and the breeding value of the tested sugar beet lines. The DH line 19-66 exceeds significantly the initial monogerm line 19, and DH 19-27, with its high white sugar yield, and this is due to its significantly higher productivity (root yield). In the other group of monogerm lines DH 150-4 realizes significantly higher white sugar yield than the initial line 760, but in that case this is due to the proved higher sugar content of the DH line. All the multigerm DH and TH lines have given higher white sugar yield than the initial multigerm line 316. The excess is proved for the white sugar yield of TH 48 (due to the proved higher sugar content), and of TH 55 (due to its significantly higher root yield). In summary, we could make the tentative conclusion, that the best performing monogerm DH lines are the DH 19-66 (with proved higher productivity) and DH 150-4 (with proved higher sugar content), and the highest white sugar yielding multigerm lines are TH 48 (with proved higher sugar content) and TH 55 (with proved higher productivity). Of course, we are making this with the proviso that the DH lines have been compared with the relevant initial monogerm and multigerm lines. All the studied DH lines show relative stability of the inheritance of the basic economical traits in the process of their reproduction without any selection. This facilitates significantly their use in the breeding and seed production.

Compared with a Group Standard of certified sugar beet varieties all the tested diploid hybrids of the dihaploids realize higher root yields (Table 2). And the excess is very well proved – 111.4 to 113.7% of the Standard's yield. Such a higher yield of roots was registered in almost every year of the test period (long enough to cover years with whatever extreme deviations from the normal climatic conditions during the vegetation). Regarding the sugar content of the tested diploid crosses we could say most of them are on the same level like that of the Standard's sugar content. Only the crosses of the lines 19-27 and 19-66 with DH 52 fall back the Standard's sugar content. And that is probably the reason for the lowest white sugar yield of these two crosses (compared to the white sugar yield from the remaining diploid crosses in the study). It is obvious, that the highest sugar content, registered for the monogerm DH line 150-4, reflects not only in the higher white sugar yield of this line, but in the white sugar yield of this line's diploid hybrids too. The

Table 1. Root yield, sugar content and white sugar yield of dihaploid sugar beet lines (2004 – 2014)

Dihaploid line	Root Yield % to the initial line	Sugar content % to the initial line	White sugar yield % to the initial line
Monogerm dihaploid lines			
DH 19-27	103.1	101.5	105.2
DH 19-66	112.5 +	101.5	113.1* ++
19 /initial-abs.values/	37.32 t/ha	14.80 %	4.39 t/ha
*P ≤ 0.05	8.9	6.1	7.0
DH 54-4	105.7	97.9	101.9
DH 150-4	104.2	104.8 +	110.7 ++
760/initial-abs.values/	42.83 t/ha	13.69 %	4.80 t/ha
*P ≤ 0.05	6.7	3.8	9.4
Multigerm dihaploid lines			
DH 52	102.7	100.9	106.6
TH 48	107.9	105.4 +	113.6
DH 58	109.0	98.2	107.9
TH 55	117.9 ++	99.4	116.3 ++
316 /initial-abs.values/	44.81 t/ha	15.76 %	5.79 t/ha
*P ≤ 0.05	10.1	5.3	12.9

Table 2. Root yield, sugar content and white sugar yield of diploid hybrids of dihaploid sugar beet lines (2004 – 2014)

Hybrid	Root yield % to the standard	Sugar content % to the standard	White sugar yield % to the standard
DH 19-27 x DH 52	111.7 ++	95.6 +	104.6
DH 19-66 x DH 52	111.5 ++	95.5 +	103.7
DH 54-4 x DH 52	113.7 ++	96.8	109.2
DH 150-4 x DH 52	111.8 ++	100.4	112.0 +
DH 19-27 x DH 58	112.7 ++	97.4	109.1
DH 19-66 x DH 58	113.2 ++	100.1	113.7 ++
DH 54-4 x DH 58	111.4 ++	99.1	110.9 +
DH 150-4 x DH 58	109.5 +	102.1	111.2 +
Group standard /absolute values/	49.21 t/ha	16.08 %	6.55 t/ha
*P ≤ 0.05	8.5	4.1	9.4

Table 3. Root yield, sugar content and white sugar yield of triploid hybrids of dihaploid sugar beet lines (2004 – 2014)

Hybrid	Root yield % to the standard	Sugar content % to the standard	White sugar yield % to the standard
DH 19-27 x TH 48	102.3	103.0	107.3
DH 19-66 x TH 48	105.1	104.3	109.3
DH 54-4 x TH 48	109.5	102.3	111.8 +
DH 150-4 x TH 48	109.8 +	101.6	111.5 +
DH 19-27 x TH 55	115.4 ++	97.8	112.3 +
DH 19-66 x TH 55	104.9	104.5 +	110.6 +
DH 54-4 x TH 55	111.7 +	100.4	112.2 +
DH 150-4 x TH 55	110.2 +	102.7	113.3 +
Group standard /absolute values/	50.22 t/ha	15.28 %	6.23 t/ha
*P ≤ 0.05	9.6	4.5	10.6

hybrids of the monogerm DH line 19-66 also realize comparatively high white sugar yield, but that is due to not only the good productivity of the line, but probably to the effect of hybridization with high white sugar yielding multigerm pollinators as well – DH 58 is the multigerm dihaploid with the highest white sugar yield. We could summarize that the best white sugar yield is realized by the diploid hybrids of the monogerm DH line 150-4 and of the multigerm DH pollinator 58.

Half of the tested triploid hybrids of DH lines have higher productivity than the Standard (Table 3). The excess is proved for the hybrids of the monogerm DH lines 54-4 and 150-4 with both TH pollinators. The highest root yield is realized by the hybrid 19-27 x TH 55, which is due to the high productivity of the TH pollinator. The highest sugar content is registered in the triploid hybrids of the monogerm DH line 19-66. The high sugar content of the TH 48 dominates in the triploid hybrids of this pollinator. That is not valid for the monogerm DH line with the highest sugar content – DH 150-4. Obviously the participation of the multigerm pollinator with two genomes in the triploid hybrids (Rostel, 1981) determines the dominance of paternal heritage factors of this important qualitative trait in the triploid hybrids. And the high values of the sugar content in the hybrids of the monogerm DH line 19-66 is sooner manifestation of its good combining ability with the two TH pollinators.

The triploid hybrids realize higher white sugar yields than the Group Standard of certified varieties in this long term tests. With the exception of the hybrids of the DH lines 19-27 and 19-66 with the pollinator TH 48, all the tested triploid hybrids exceed the Standard varieties with significant differences. We have observed such higher values of the white sugar yield in our previous, 3-5 years tests of crosses of the same DH lines with conventional male sterile lines and pollinators. Some of these crosses also realized higher productivity and sugar content than the Standard varieties, but the excess regarding the white sugar yield was not so categorical.

Conclusion

The experimental dihaploidy is an excellent method for sugar beet gene fund enrichment. The studied homozygous lines feature unique combinations of genes, expressing very good productive and quality traits of sugar beet, with great value for the hybrid breeding of the crop. The best performing lines in our study are the monogerm lines DH 19-66 and DH 150-4, and the multigerm pollinators DH 58 and TH 55. The highest white sugar yield is realized by the crosses of the monogerm DH line 150-4, and the crosses of the multigerm pollinators DH 58 and TH 55. These diploid and triploid hybrids are potential new varieties of sugar beet.

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