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THE EFFICIENCY USE OF DOUBLED-HAPLOID TECHNOLOGY IN MAIZE BREEDING – OBTAINING DH PARENT LINES AND HYBRIDS

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Abstract

The current challenge faced by the breeders and maize growers worldwide is the pressure determined by the climate changes causing the expansion of desertification phenomenon and aggravating the draught in soil and the atmospheric heat. Considering these aspects, for market introduction of the new maize hybrids improved in terms of their main characters, breeders have perfected and developed doubled-haploid technology, so now all the multinational companies use this technology widely in obtaining doubled-haploid lines.

The aim of this paper is to present the doubled-haploid parent lines and hybrids obtained through using doubled-haploid technology in maize breeding program. In this respect, there were used Procera Haploid Inducers (PHI) which have been obtained into Procera Genetics maize breeding program. Researches were conducted within the maize breeding fields belonging to the Procera Genetics Ltd Company.

Key words*: maize, doubled-haploid, haploid inducer, heterosis, hybrids.*

INTRODUCTION

The use of haploid plants in the breeding was possible after detection of the line Stock 6 (Coe, 1959), which has the ability to induce maternal haploids with a frequency of 2%, while the appearance through the natural way of the maize haploids is 0.1%.

The doubled-haploid method greatly reduces the time required to develop new parent inbreds. Observation, testing and selection work is conducted on these newly developed lines. The goals of the traditional method and the doubled-haploid technology are the same. However, the doubled-haploid technology reaches those goals much faster.

Advantages of using DH lines in hybrid breeding include: maximum genetic variance in line per se and testcross trials, high reproducibility of early-selection results, high efficiency in stacking targeted gene arrangements and simplified logistics (Röber et al., 2005).

The first objective is to develop an inbred parent line that has the same genes on both the left and right chromosome of the pair. This is called a genetically stable or homozygous inbred.

The second stage is to select the best of the newly developed inbreds through an intense breeding and testing program.

An inbred parent line must be homozygous or genetically stable so that it will produce the hybrids year by year and it can be certain the hybrids are the same genetically, and they have the same agronomic performance potential, each time they are produced.

The double haploid technology is much faster and can produce a new, genetically stable inbred line in one year. The plants in the genetic population (germplasm pool) are pollinated with a haploid inducer.

When the harvested kernels are planted, they produce haploid plants. A haploid plant has only one chromosome from each pair. One can say that a haploid plant has ten single chromosomes, while a normal plant has ten pairs of chromosomes.

The haploid plants are subjected to a special treatment compound that causes the single chromosome to double – think of it as making a "photocopy" of the chromosome. This "photocopy" is genetically identical to the original.

Doubled-haploid (DH) technology versus Traditional breeding technology

The DH technology shortens the breeding cycle significantly by rapid development of completely homozygous lines (in 2-3 generations), instead of the conventional inbred line development process, which takes at least 6-8 generations to derive lines with ~99% homozygosity (Forster and Thomas, 2005; Geiger and Gordillo, 2009; Chang and Coe, 2009).

Doubled-haploid technology:

1. First generation: obtaining haploids: donors x haploid inducers; haploids selection.
2. Second generation: doubling haploids; obtaining DH lines.
3. Third generation: increase DH lines and their crossing with testers for combining ability, marker assisted selection (MAS).
4. Fourth generation: hybrids testing for level of high heterosis and selection of the advanced DH lines.

Traditional breeding technology:

1. First generation: selfing F1 populations.
2. Second generation: selfing F2 populations, phenotypic selection.
3. Third generation: selfing F3 lines, phenotypic selection, marker assisted selection (MAS) and early testing for general combining ability (GCA).
4. Fourth generation: Hybrids testing for level of high heterosis, selfing F4 lines, phenotypic selection.
5. Fifth generation: selfing F5 lines, phenotypic selection, testing for specific combining ability (SCA).
6. Sixth generation: hybrids testing for level of high heterosis and the main morphological, physiological and agronomic traits, selfing and selection F6 lines.
7. Seventh generation: maintaining and increase parental lines, seed production for advanced hybrids, testing advanced hybrids in a large network.

During maize breeding program, the new inbred parent lines are crossed to make experimental hybrids that are tested for a number of traits, including:

- Yield;
- Moisture;
- Dry Down;
- Stay Green;
- Thousand Grain Weight (TGW);
- Grain Percentage;
- Test Weight;
- Emergence;
- Vigor;
- Phenotypic Uniformity;
- Anthesis-Silking Interval (ASI);
- Stalk Strength;
- Root Strength;
- Disease Resistance;
- Harvest Appearance;
- Plant Height;
- Ear Height;
- Various Grain Quality Traits (protein, starch, oil content).

Haploidy is frequently used in recurrent selection to haploid level.

Single doubled haploid descent recurrent selection will be one of the most efficient methods for low heritabilities and with a rapid development of doubled haploid lines (Gallais, 1990).

The aim of this paper is to present the DH parent lines and hybrids obtained through using doubled-haploid technology in maize breeding program.

MATERIALS AND METHODS

For producing maternal haploids, the haploid inducers are used as the male parents in induction crosses, with the source germplasm or donors as the female parents. Maternal haploids carry both cytoplasm and chromosomes from the donor.

In this study there were used Procera Haploid Inducers (PHI; Figure 1) (Rotarenco et al., 2010), with high inducer rate (HIR), enough and good pollen, good phenotype and very adapted to temperate climate conditions (Table 1).

PHI have been obtained into Procera Genetics maize breeding program, with own funds.

Three synthetic populations belonging to the most important heterotic groups were used as donors, in crossing with PHI for obtaining haploids, respectively doubled-haploid parent lines (Table 2).

Table 1. Main characteristics of the initial and PHI inducers

Inducer	Planting-flowering, days	Plant height, cm.	Haploid-inducing frequency
Stock 6	60	158	1.2%
MHI	65	192	7.2%
PHI-1	55	151	12.1%
PHI-2	60	198	13.0%
PHI-3	70	180	14.5%
PHI-4	65	200	12.8%

Figure 1. Procera Haploid Inducers (PHI): a) PHI-1; b) PHI-2; c) PHI-3; d) PHI-4

Table 2. Obtaining DH parent lines and experimental maize hybrids

Synthetic population	Heterotic group	No. of haploids selected	No. of DH parent lines obtained	No. of DH parent lines selected	No. of DH parent lines selected	No. of hybrids obtained for GCA	No. of hybrids obtained for SCA
SP-01-012SSS	SSS	970	92	60	20	40	100
SP-02-012Lanc.	Lancaster	1025	108	80	20	40	100
SP-03-012Iod.	Iodent	1010	98	70	20	40	100

Stages of obtaining DH parent lines and experimental hybrids were the following:

1. 2012: Fundulea season-SP as donors were crossed with PHI;
2. 2012: winter-haploids seeds have been selected;
3. 2013: Fundulea season-haploids were doubled and have been obtained DH parent lines;
4. 2013-2014: winter season (Chile)-DH parent lines were increased and crossed with two testers each for testing general combining ability;
5. 2014: Fundulea season-DH parent lines were increased, phenotypic selected and hybrids have been tested for the main agronomic traits and high level of heterosis; twenty DH parent lines from each heterotic groups were selected and used in crossing with two testers each;
6. 2015: Fundulea season-DH parent lines selected have been per se tested and crossed with five testers each for testing specific combining ability.

Each SP population was made up of four elite parental lines, very used in registered and commercial Procera maize hybrids.

Researches were conducted within the maize breeding fields belonging to the Procera Genetics Ltd Company, which are located in Fundulea city, Calarasi County, Romania.

RESULTS AND DISCUSSIONS

This paper presents the results obtained with DH parental lines versus parental lines that formed synthetic populations as donors.

The results were obtained in 2014 when DH lines were tested in the observation plots in Fundulea location.

Each plot was made up of two rows, row length was 4.8 meters, the distance between rows 75 cm, and the plant density was 68,000 plants/ha.

Each trial was made up of 24 lines, 20 DH parent lines and the 4 initial parental lines that were the controls of the trial.

In total, there were three trials, one for each set of DH parental lines belonging to the three synthetic populations.

Important per se traits that have been taken in the initial study were the following:

- phenotypic uniformity;
- anthesis-silking interval (ASI);
- stay green;
- uniformity of insertion of ear;
- prolificity (two cobs per plant).

For the following traits: phenotypic uniformity, stay green, and uniformity of insertion of ear, the scoring system was with notes: 1-very weak, 9-very good. Anthesis-silking interval (ASI) was noted in days between anthesis and silking, and prolificacy (two cobs per plant) was noted in percent.

For the first set of DH lines belonging to synthetic population SP 01-012SSS the results are presented in Table 3.

It can be observed that for all traits studied DH parent lines are superior comparative with parental lines components of synthetic population.

The best results were obtained for ASI and prolificity, two traits involved in atmospheric heat tolerance. When these two traits appear in normal conditions, it means that in conditions of heat stress parental line will have a high tolerance. For ASI trait, number of days noted with minus (-3, -2, -1) that means the lines silking before anthesis.

For the second set of DH parent lines which belong SP-01-012 synthetic population, the results are presented in Table 4.

The best lines for ASI were: SP 02-DH-108 (-2), SP 02-DH-110(-2), SP 02-DH-111 (-3), SP 02-DH-115 (-2), SP 02-DH-116 (-1), and for prolificity: SP 02-DH-104 (78%), SP 02-DH-110 (80%), SP 02-DH-111 (82%), SP 02-DH-113 (76%).

Table 3. Results obtained for first set of DH parent lines comparative with based lines belonging SP-01-012SSS

Line number	**Line code**	**Plants uniformity, note**	**Anthesis-silking interval (ASI), number of days**	**Stay-green, note**	**Uniformity of insertion of ear, note**	**Prolificity (two cobs per plant), %**
1	SP 01-DH-01	9	0	8	9	55
2	SP 01-DH-02	8	0	8	9	68
3	SP 01-DH-03	9	1	8	9	65
4	SP 01-DH-04	9	0	8	8	60
5	SP 01-DH-05	8	0	9	9	78
6	SP 01-DH-06	9	0	7	9	80
7	SP 01-DH-07	8	1	9	9	82
8	SP 01-DH-08	8	-1	9	8	75
9	SP 01-DH-09	8	-2	7	8	68
10	SP 01-DH-10	9	-1	8	9	55
11	SP 01-DH-11	8	-2	8	9	59
12	SP 01-DH-12	9	-2	8	9	60
13	SP 01-DH-13	8	-2	9	9	75
14	SP 01-DH-14	8	-3	7	8	66
15	SP 01-DH-15	8	0	9	9	78
16	SP 01-DH-16	9	0	8	8	82
17	SP 01-DH-17	9	0	8	9	80
18	SP 01-DH-18	9	-1	8	8	67
19	SP 01-DH-19	9	-2	8	9	55
20	SP 01-DH-20	9	-3	9	9	75
21	PL-01SSS	7	0	8	8	33
22	PL-02SSS	6	2	7	7	59
23	PL-03SSS	7	1	7	8	67
24	PL-04SSS	7	3	8	8	56

Table 4. Results obtained for second set of DH parent lines comparative with based lines belonging SP-02-012Lanc

Line number	**Line code**	**Plants uniformity, note**	**Anthesis-silking interval (ASI), number of days**	**Stay-green, note**	**Uniformity of insertion of ear, note**	**Prolificity (two cobs per plant), %**
1	SP 02-DH-101	8	1	9	8	53
2	SP 02-DH-102	8	0	9	9	48
3	SP 02-DH-103	8	0	8	8	66
4	SP 02-DH-104	9	0	9	8	78
5	SP 02-DH-105	7	0	8	9	66
6	SP 02-DH-106	9	0	9	9	56
7	SP 02-DH-107	9	1	8	9	53
8	SP 02-DH-108	9	-2	7	8	60
9	SP 02-DH-109	8	0	9	8	72
10	SP 02-DH-110	8	-2	9	8	80
11	SP 02-DH-111	8	-3	8	9	82
12	SP 02-DH-112	9	0	8	7	56
13	SP 02-DH-113	8	0	7	9	76
14	SP 02-DH-114	9	0	9	8	73
15	SP 02-DH-115	8	-2	7	9	74
16	SP 02-DH-116	9	-1	9	8	67
17	SP 02-DH-117	8	0	9	9	59
18	SP 02-DH-118	9	0	9	8	68
19	SP 02-DH-119	9	1	9	8	55
20	SP 02-DH-120	8	2	8	9	72
21	PL-101Lanc.	8	2	7	7	48
22	PL-102Lanc.	7	1	8	8	61
23	PL-103Lanc.	8	0	7	8	51
24	PL-104Lanc.	8	2	7	7	43

Table 5. Results obtained for second set of DH parent lines comparative with based lines belonging SP-03-012Iod

Line number	Line code	Plants uniformity, note	Anthesis-silking interval (ASI), number of days	Stay-green, note	Uniformity of insertion of ear, note	Prolificity (two cobs per plant), %
1	SP 03-DH-201	9	-1	8	8	67
2	SP 03-DH-202	9	0	8	8	73
3	SP 03-DH-203	9	0	8	8	71
4	SP 03-DH-204	8	1	9	9	76
5	SP 03-DH-205	7	0	8	9	70
6	SP 03-DH-206	8	-2	9	9	69
7	SP 03-DH-207	8	-2	9	9	78
8	SP 03-DH-208	9	0	8	9	78
9	SP 03-DH-209	8	1	7	8	73
10	SP 03-DH-210	9	0	9	9	70
11	SP 03-DH-211	9	0	9	8	69
12	SP 03-DH-212	9	2	9	9	81
13	SP 03-DH-213	8	1	8	7	75
14	SP 03-DH-214	9	-2	9	9	77
15	SP 03-DH-215	8	0	8	9	68
16	SP 03-DH-216	9	0	9	9	72
17	SP 03-DH-217	8	1	8	8	56
18	SP 03-DH-218	9	-2	9	9	73
19	SP 03-DH-219	7	-3	9	9	68
20	SP 03-DH-220	9	-1	9	8	80
21	PL-201Iod.	7	1	8	8	50
22	PL-202Iod.	7	0	6	7	52
23	PL-203Iod.	8	1	7	7	61
24	PL-204Iod.	7	1	7	7	49

For the third set of DH parent lines which belongs SP-03-012Iod synthetic population, the results are presented in the Table 5.

The DH parent lines that performed for ASI were: SP 03-DH-201 (-1), SP 03-DH-206 (-2), SP 03-DH-207 (-2), SP 03-DH-214 (-2), SP 03-DH-218 (-2), SP 03-DH-219 (-3), SP 03-DH-220 (-1), and for prolificity performed the DH parent lines: SP 03-DH-207 (78%), SP 03-DH-208 (78%), SP 03-DH-211 (81%0, SP 03-DH-214 (77%), SP 03-DH-220 (80%).

CONCLUSIONS

From our study it can be concluded that haploid technologies are characterized by the following:

- Allow to reduce time and expenses in maize breeding and to increase the efficiency of selection procedures significantly.
- Reduced expenses.
- Complete homozygosity of doubled-haploid lines
- Phenotipic and genotypic uniformity of doubled-haploid and hybrids
- Increase anthesis-silking interval (ASI), prolificity, stay green.

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