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THE INFLUENCE OF THE FEMALE PARENT ON THE INDUCING RATE WITH FIVE DIFFERENT INDUCER LINES IN MAIZE DH TECHNOLOGY

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Abstract

In the last decade, DH technology has been integrated by many maize breeding programs in Europe, North America and China due to the releasing of lines with inducing efficiency up to 15 %, developed for temperate and tropical areas as well as due to improved selection of the putative haploid kernels (PHK) on the basis of the expression of anthocyanin coloration conferred by R1-nj as a genetic marker. Even though the R1-nj marker system offers an efficient way to identify haploids, the expression given by this genetic marker could be influenced by several factors such as the genetic background of the female parent. According to the literature, haploids obtained from dent genotypes are more easily recognized and both anthocyanin coloration and intensity of the coloration are better expressed than in flint genotypes; anthocyanin coloration could be also affected by the moisture of kernels at time of harvest and may vary from a small patch to covering the entire aleuronezone, exception kernel basis. Intensityof anthocyanin can range from very poor to strong anthocyanin both in the embryo and aleurone.

In 2014 at NARDI Fundulea, 27 populations were used as female parent in crosses with five different inducers and inducing efficiency was determined for each cross to see the influence of the female parent on the inducing efficiency.

Key words: *doubled haploid technology, female parent, inducer line, anthocyanin coloration.*

INTRODUCTION

Releasing maize hybrids in the classic system is a process that takes 5-7 years only to obtain homozygous lines, to which at least 4-6 years are needed for testing and registration of the hybrids (Rotarenco et al., 2010).

Hence the need to approach new methods that make possible to obtain a greater number and more diverse hybrids in a short time corresponding to the current needs. In vivo maternal haploid induction offers undoubtedly great advantages to modernize maize programs by simplifying the protocol for obtaining homozygous lines in a period of 2 years but also by reducing the costs involved in this process. The procedure for the production of maternal haploids allows obtaining haploidsfrom different genotypes on a large scale (Deimling et al. 1997; Chalyk and Rotarenco1999; Eder and. Chalyk, 2002).

DH technology requires an inducer with a good induction rate (HIR) up to 15% and a marker system that can provide an easier recognition of the haploid forms at different stages of vegetation.

The most common marker system used in obtaining in vivo maternal haploid is based on *R1-nj* gene that is involved in the synthesis of anthocyanin(Nanda and Chase, 1966; Chase, 1969; Neuffer et al., 1997; Eder and Chalyk, 2002; Röber et al., 2005).

The main limitations of this system are the presence of the *C1-I* gene in the maternal germplasm, which inhibits the expression of anthocyanin coloration, and the influence of different maternal germplasm (dent and flint forms) on the size and intensity of anthocyanin

coloration. Consequently, several attempts were made to improve the marking system by incorporating other two marker genes as *B1* and *Pl-1* in the new inducer lines recently released. This improved system makes possible, the recognition of haploid forms, post doubling, in the first stages of vegetation (Prasanna et al., 2012).

The objective of the present study was assessing the influence of different female parents within each inducer on the inducing efficiency.

MATERIALS AND METHODS

The study was carried out at the National Institute of Research and Development Fundulea in 2014. A number of 27 F2 maize populations from different heterotic groups were used as female sources. Each female source was crossed with 5 inducer lines as MHI (Moldavian Haploid Inductor), Td RhA, Td RhR, Td RhAPM, Td RhRPM, in the field under controlled pollination. Grains resulted from crosses were divided in 3 categories based on the expression of the anthocyanin coloration coded by *R1-nj* gene on the kernel as follows: category 1, kernels with no coloration on both aleurone and embryo; category 2, kernels with coloration of both aleurone and embryo and category 3 considered as PHK (Putative haploid kernels) with purple coloration only on the aleurone and uncolored embryo. A scale 0-4 was used for visual assessment of the intensity of anthocyanin coloration on aleurone and embryo from category 2 (kernels with coloration in both aleurone and embryo): 4 = intense pigmentation, 3=normal pigmentation, 2 = poor pigmentation, 1 = very weak pigmentation and 0 = lack of pigmentation). Cytological analysis was performed to check visually selected PHK. Root tips were cut from a random sample of selected PHK from each of 12 populations. The chromosome complement of plantlets was established by means of chromosome counts on root-tips squashed, stained by Feulgen method.

RESULTS AND DISCUSSIONS

According to the results obtained by Eder and Chalyk (2002); Kebede et al. (2011), the female sources influence the haploid rate, and those obtained by Coe (1994) demonstrated the influence of the female on the expression of the marker gene*R1-nj.*

All 27 populations showed a high variability of the expression of anthocyanin coloration for both embryo and aleurone, appreciated on average with scores between 2 (identification of haploid is possible but errors could occurdue to very weak staining in the embryo) and 4 (level that allows easy identification of PHK), the variation in the size and intensity of the anthocyanin coloration it can be seen in table 1.

Table 1.Type of kernel and the expression of the anthocyanin coloration of each maternal form for the aleurone and embryo depending of the haploid inducer

Genotype	Td. RhR	Td. RhA	Td RhR P.M	Td. RhA P.M	MHI
*P 1	**D/3/4	D/3/4	D/3/3	D/3/3	D/4/4
P 2	D/2/3	D/3/3	D/3/3	D/3/3	D/3/3
P 3	D/3/3	D/4/4	D/3/4	D/3/4	D/3/3
P 4	D/4/4	D/3/4	D/3/4	D/3/3	D/4/4
P 5	D/2/3	D/2/3	D/2/2	D/2/2	D/3/3
P 6	D/3/4	D/3/3	D/4/4	D/3/4	D/3/3
P 7	D4/3	D/3/4	D/3/3	D/4/4	D/4/4
P 8	D/2/3	D/3/3	D/3/3	D/3/3	D/3/4
P 9	D/3/4	D/4/4	D/3/4	D/3/4	D/4/4
P 10	D/3/2	D/3/3	D/3/2	D/3/3	D/3/3
P 11	D/2/2	D/3/3	D/2/3	D/3/3	D/3/2
P 12	D/4/4	D/3/4	D/3/4	D/3/2	D/4/4
P 13	D/3/4	D/3/3	D/3/4	D/3/3	D/4/4
P 14	D/3/4	D/4/4	D/3/4	D/2/4	D/4/3
P 15	D/2/3	D3/4	D/3/4	D/3/3	D/2/3
P 16	D/2/2	D/3/3	D/3/3	D/3/3	D/2/4
P 17	D/3/3	D/3/4	D/3/3	D/4/4	D/3/4
P 18	D/3/2	D/3/3	D/2/3	D/3/3	D/3/3
P 19	D/3/4	D/3/3	D/3/3	D/4/4	D/3/3
P 20	D/3/3	D/3/4	D/3/3	D/3/4	D/3/3
P 21	D/3/4	D/3/4	D/3/3	D/3/4	D/3/3
P 22	D/3/4	D/3/4	D/4/4	D/3/4	D/4/4
P 23	D/3/3	D/3/3	D/3/3	D/3/3	D/3/3
P 24	D/3/4	D/3/4	D/2/3	D/4/4	D/3/3
P 25	D/3/2	D/2/2	D/2/2	D/2/3	D/3/3
P 26	D/3/4	D/3/3	D/3/4	D/3/4	D/3/3
P 27	D/3/3	D/2/3	D/3/3	D/4/4	D/4/4

*P = Population; **D=Dent type

Populations that received scores over 3 on the embryo, allowed easy identification of PHK, demonstrated also by cytological analysis (table 2). In populations 1, 14 and 15 correct recognition of PHK was 100%. For the variants with scores 2 for embryo coloration, the proportion of correct recognition was a little over 50% (populations 11 and 5). This might be explained by the fact that the score 2 for the embryo means a very weak intensity of the anthocyanin coloration so errors could occur, recognition of the haploid forms from the diploid one require an increased attention from staff.

Table 2. Cytological analysis for 12 random female parents with all 5 inducers

Genotype	Inducer	Total kernels analyzed	Number of confirmed real haploid kernels		Scores for the antocyanin coloration (aleurone /embryo)
			No.	%	
*P 1	MHI	12	12	100	**D/4/4
P 14	Td.RhR P.M.	12	12	100	D/3/4
P 15	Td.RhR	11	11	100	D/2/3
P 6	Td.RhR	9	8	88.8	D/3/4
P 4	Td.RhR	12	10	83.3	D/4/4
P 7	Td.RhA	12	10	83.3	D/3/4
P 9	MHI	12	10	83.3	D/4/4
P 13	MHI	12	10	83.3	D/4/4
P 10	Td.RhR	11	9	81.8	D/3/2
P 2	MHI	12	10	83.3	D3/3
P 11	MHI	12	7	58.3	D/3/2
P 5	Td.RhA P.M.	9	5	55.5	D/2/2
Total		136	114	Average 83.41	

*P = Population**D=Dent type

An evident interaction between inducers and populations is suggested by the data. Inducing capacity of each inducer is highlighted in table 3. On the average, the best inducing efficiency was registered for inducer MHI, recommended to be used intensively. The lowest inducing efficiency was obtained with the inducer Td. RhA. With regards to population, better inducing efficiencies (over 3%) were produced by populations 4, 17, 19 and 26 since populations 6, 7, 14, and 18 had efficiencies under 1%. The best percentage was obtained by the population 17 with the inducer MHI, 9.8% scored 4 for the embryo, level that allows easy identification of the PHK kernels.

Table 3. Inducing efficiency of the inducers over 27 female populations

Genotype	Inducer					Population Average
	MHI	Td. RhR	Td. RhA	Td.RhR P.M	Td.RhA P.M	
*P 1	5.51	0	0.8	3.81	1.22	2.26
P 2	2.36	3.11	0.19	3.27	0.65	1.91
P 3	3.15	3.03	0.5	4.44	0.89	2.40
P 4	3.04	0.4	3.43	5.39	4.43	3.33
P 5	2.1	***0.27***	3.15	0	1.15	1.33
P 6	0.67	1.14	1.16	0.84	0.56	0.87
P 7	1.54	0	0.62	1.29	0	0.69
P 8	2.46	2.03	1.09	2.18	1.26	1.80
P 9	2.33	1.04	2.36	0	2.65	1.67
P 10	2.36	2.36	2.63	2.31	2.52	2.43
P 11	3.05	1.73	3.25	1.97	2.82	2.56
P 12	1.91	0.28	1.06	2.56	0.81	1.32
P 13	0.56	1.78	1.22	5.25	2.8	2.32
P 14	1.21	0.41	1.74	0.78	0	0.82
P 15	2.18	2.27	2.02	2.5	2.86	2.36
P 16	1.49	0	0.63	0.8	2.32	1.04
P 17	9.8	3.42	5.73	0	0	3.79
P 18	3.35	0	0.65	0	0.91	0.98
P 19	4.57	5.22	1.25	3.45	2.78	3.45
P 20	1.93	1.03	1.91	0	1.55	1.28
P 21	3.92	1.39	0.69	1.17	4.1	2.25
P 22	2.26	1.31	2.09	2.33	3.07	2.21
P 23	1.66	0.33	5.14	1.37	0.74	1.84
P 24	3.63	0.98	2.7	2.01	1.5	2.16
P 25	3.5	4.44	0	2.3	0	2.04
P 26	6.76	4.29	6.09	5	0	4.42
P 27	5.72	0.37	0.19	0	0	1.25
Average	**3.07**	**1.58**	**1.94**	**2.1**	**1.6**	

*P = Population

Furthermore, the analyses of variance for inducing efficiency (table 4) confirmed that all the variance sources - inducers, female populations, as well as the interaction between inducers and female populations have significant effect on the efficiency of haploid induction.

Even when an inducer with a high average efficiency is used, the haploid induction rate

(HIR) is significantly influenced by the genetic background of the maternal forms.

Table 4. ANOVA for inducing efficiency

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Inducers (I)	4	41.68	10.42	12.41***
Populations (P)	26	112.66	4.33	5.16***
I x P	104	241.63	2.32	2.77**
Error	104	87.30	0.84	

, * - significant at P=0.01 and P=0.001, respectively

CONCLUSIONS

Haploid inducing efficiency is influenced by the genotype of the population submitted to the induction, haploid inducing capacity of the inducer and the inducing protocol. High efficiency of the inducer is essential insuchprotocol, but increasing the number of pollinated plants within crosses between maternal populations and inducer is highlyrecommended also to ensure the identification of sufficient PHK in the populations with genetic lower inducing capacity.

Introduction and assimilationof an improved marker system based on *R1-nj*, *B1* and *Pl-1*, capable of detecting and separation of haploid from diploid forms in different vegetation stages (field separation at plantlets or mature plants stages).

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