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THE INFLUENCE OF THE OPEN POLLINATION ON THE INDUCING RATE ON TOP AND BOTTOM OF THE EAR ON DH TECHNOLOGY

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Abstract

Accelerating the development of homozygous lines and consequently hybrids is an important aspect of the maize breeding programs. Doubled haploid technology has successfully replaced the traditional method of obtaining homozygous lines in maize breeding programs in Europe, North America, China and Central America, due to the clear advantages in terms of timing and important reduction of the workload and costs by eliminating controlled pollination and relatively simple methodology.

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 (time and type of pollination). The study was carried out in special climatic conditions of the year 2016 (the absolute maximum temperature was 35.8^{0}C for June and 35.7^{0}C for July. The results showed that the source genotype used in the induction nursery influenced the anthocyanin coloration in both aleurone and embryo. Clear variability regarding the anthocyanin coloration was observed among the ears from the same genotype and even between aleurone and embryo from the same ear. Comparing the PHK (putative haploid kernels) number from the top and bottom of the ear, the top average is distinct significantly positively over the bottom average PHK number. Similarly to controlled pollination, the highest percentage of PHK remains on top of the ear in the case of this experiment with open pollination induction nursery.

Key words: *DH technology, inducers, open pollination, haploid kernels.*

INTRODUCTION

The *in vivo* maternal haploid induction scheme is based on a dominant anthocyanin color marker, known as *R1-Navajo* (*R1-nj*), that expresses in the aleurone as well as in the embryo of the haploid inducer, unlike the source populations, where the coloration is usually missing in both aleurone and embryo (Prassana et al., 2012). However, it must be noted that the size and intensity of the anthocyanin coloration of the *R1-nj* color marker might vary significantly depending on the genetic background of the source genotype (from which we want to obtain haploid forms) and haploid inducer, as well as environmental factors (Chase, 1952; Röber et al., 2005; Kebede et al., 2011; Prigge et al., 2011).

It is known in the literature that in case of controlled pollination, the highest frequency of haploid forms is at the top of the ear. Studies realized by Sarmaniuc and published in 2015 in her doctoral thesis titled "Improving the technology of creating homogeneous lines of maize (*Zea mays L.*)" showed that the rate of haploid kernels is much higher at the top of the ear after 2, 3 and 4 days from the controlled pollination, but in evolution of female inflorescence development and maturation, the index decreases in both versions - "top" and "bottom". Poor pollination occurs because of delayed silk emergence, after pollen shedding was complete; drying pollen or silk - all these situations occurring in periods of drought and heat. At temperatures of 35^{0}C, maize pollen loses its viability in 1-2 hours and silks begin to dry at temperatures exceeding 32-33^{0}C. In an isolation of induction with open pollination, donor sources should be grouped depending on the silking period and the inductor must be planted at different planting times for optimal pollination (Prassana et al., 2012). The year 2016, was extreme dry with high temperatures during pollination that affected both pollen and silk viability. The average for daily maximum temperatures was 29,4^{0}C in June and 31.4^{0}C in

July. The absolute maximum temperature was 35.8^{0}C for June and 35.7^{0}C for July. The aim of this study was to check if in case of open pollination (when we don't know exact the time of pollination) the highest percentage of haploids remains on top of the ear.

MATERIALS AND METHODS

The study was carried out at the National Institute of Research and Development Fundulea in 2016. A number of 15 F1 maize breeding populations from different heterotic groups were used as female sources. Each female source was crossed with the inducer MHI (Moldavian Haploid Inductor), in the field in an induction isolation nursery. Ten ears from each population were divided in two, top and bottom and for each of these two parts putative haploid kernels (PHK) were counted.
Grains resulted from crosses were divided in 3 categories in both parts (top and bottom) 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 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 and 0=lack of pigmentation (Sarmaniuc et al., 2013).

RESULTS AND DISCUSSION

Many researchers have highlighted that the source genotype influence both the antochyanin coloration as well as the rate induction (Coe, 1994; Eder and Chalyk, 2002; Kebede et al., 2011; Bitica et al., 2016). Variation in the size and intensity of the anthocyanin coloration is presented in table 1; all 15 populations showed high variability of the expression of anthocyanin coloration for both embryo and aleurone, appreciated on average with scores between 1 (identification of haploid is possible but errors could occur due to very weak staining in the embryo) and 4 (level that allows easy identification of PHK). Clear variability was observed also among the ears from the same genotype and even between aleurone and embryo from the same ear. A good example is the genotype L537 appreciated on average with scores between 3,3 - 4 for aleurone and 1- 2,3 for embryo.

Table 1. The antochyanin coloration for aleurone and embryo, 15 genotype (10 ears for each genotype), NARDI Fundulea 2016

Genotype	Ear	1	2	3	4	5	6	7	8	9	10
L502	A	3	3	2.7	3	2.5	3	3	3	3	3
	E	3	3	3	3	3	3	3	3	3	2.7
L507	A	2	2.5	2	2	2	2.5	2.5	2	2	3
	E	3	3	3	3	3	3	3.5	2.5	2.5	2.7
L508	A	2.7	2.5	3	2	3	2.5	2.7	3	2.7	2.5
	E	3	2	2.5	3	2.7	2.5	3	3	3	2.7
L519	A	2	3	2.3	2	1.7	2	1.3	3	3	1
	E	3	3.3	2.7	2.3	2	2.3	2	3.7	3.3	2.0
L529	A	3	3	3	3	3	3	2.7	3	3.3	3
	E	3	3	3	3	3	3	3.3	3	3	3
L535	A	4	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
	E	2	2.7	2.7	3	2.7	2.7	3	3	3	2.7
L537	A	4	4	4	4	3.7	4	4	3.3	4	3.7
	E	2	1.7	2	2	2.3	1.7	1	1	1	1.3
L552	A	2	3	3.3	3	3	3	3	2.7	3.3	3.3
	E	3	3	3	3	3.3	3.3	3.7	2.7	3.7	4
L560	A	3	2.3	3	3.7	3	3.3	3.3	3	3	3
	E	3	2.7	2.7	3.7	3.7	3.7	3	3	3.7	3.7
L567	A	3	3	3.7	2.7	2.7	3	3.3	3.3	3.7	3.3
	E	4	3.3	2.7	3	2.7	3.7	3	3	3	3
L573	A	3	3.3	3	2.7	3	3	3	3.3	3.7	3
	E	3	3	3	3	3	2.7	3.3	3	3	3
L576	A	3	4	4	3.7	4	4	3.3	4	3.7	4
	E	3	2.7	3	3	4	3.7	4	3.3	3.3	3
L584	A	4	4	4	4	4	3.3	4	3.7	4	3.7
	E	4	3	3	3	4	2.7	4	3	4	3
L586	A	3	2.7	2.3	3	2.7	3	2.7	3	3	3
	E	3	2.3	2.3	2.3	2	2.7	2.7	3	2.3	2.7
L587	A	3	2.3	3.3	3.3	3.7	4	4	3	3.3	3.3
	E	3	3	3.3	2.7	4	4	4	4	3.3	2

*A= aleurone, E= embryo

Furthermore, the analyses of variance for anthocyanin coloration intensity of the aleurone and embryo (table 2) confirmed that all the variance sources - genotype, kernel components (aleurone and embryo) as well as the interaction between genotype and kernel

components have significant effect on the anthocyanin coloration intensity.

Table 2. ANOVA for the anthocyanin coloration intensity of the aleurone (A) and embryo (E), 15 maize populations submitted to induction, NARDI Fundulea, 2016

Source of variation	Degrees of freedom	Sum of squares	Mean square	F Value	Probability
Replications	9	1,510	0.168	0.844	
Genotype(G)	14	34,220	2,444	12,295	0.0000 (***)
Error(G)	126	25,049	0.199		
Aleurone/ Embryo(A/E)	1	2,576	2,576	29,601	0.0000 (***)
GxA/E	14	34,845	2,489	28,599	0.0000 (***)
Error	135	11,749	0.087		
Total	299	109,949			

As it can be seen in table 3, showing the anthocyanin coloration for aleurone and embryo for the genotypes submitted to induction, the aleurone expressed much better anthocyanin coloration than the embryo. However, there were genotypes distinct significantly positively compared to the average experience as populations L537, L576, L584 regarding the aleurone anthocyanin coloration and significant, populations L584 and L586 for the embryo.

There were also genotypes witch coloration for the aleurone was very poor compared to the average experience as L507 and L519. The most stabile genotypes regarding the anthocyanin coloration are L576 and L 584. In case of population L 537 the anthocyanin it is much better expressed in the aleurone then in the embryo.

Table 3. Anthocyanin coloration for aleurone and embryo, 15 genotypes, NARDI Fundulea, 2016

Genotype	Score for anthocyanin coloration			
	Aleurone (A)	Embryo (E)	A-E	Average
L502	2.92	2.97	-0.05	2.95
L507	2.25***	2.92	-0.67$^{\$\$\$}$	2.59$^{\&\&}$
L508	2.66*	2.74	-0.08	2.7$^{\&}$
L519	2.13***	2.66	-0.53$^{\$\$\$}$	2.395$^{\&\&\&}$
L529	3.00	3.03	-0.03	3.02
L535	3.7**	2.78	0.92$^{\$\$\$}$	3.24
L537	3.87***	1.6***	2.27$^{\$\$\$}$	2.735$^{\&}$
L552	2.99	3.27	-0.28$^{\$}$	3.13
L560	3.03	3.29	-0.26$^{\$}$	3.16
L567	3.17	3.11	0.06	3.14
L573	3.10	2.97	0.13	3.04
L576	3.8***	3.30	0.5$^{\$\$\$}$	3.55$^{\&\&\&}$
L584	3.87***	3.37*	0.5$^{\$\$\$}$	3.62$^{\&\&\&}$
L586	2.81	2.53*	0.28$^{\$}$	2.67$^{\&}$
L587	3.32	3.30	0.02	3.31$^{\&}$
Average	**3.11**$^{+++}$	**2.92**		**3.02**
LSD for factor A (genotypes) average=5%=0.28; 1%=0.37; 0.01=0.48 (&,&&,&&&-significant for 5, 1, and 0.1 level, respectively)				
LSD for factor B (kernel components, aleurone and embryo) average: 5%=0.02 1%=0.03; 0.01=0.04 (+++-significant for 0.1 level)				
LSD for factor B (kernel components, aleurone and embryo) at the same level of factor A (genotypes)-horizontal comparison: 5%=0.26; 1%=0.35; 0.01%=0.44 ($,$$,$$$-significant for 5, 1, and 0.1 level, respectively)				
LSD for factor A (genotypes) at the same level of (kernel components, aleurone and embryo)-vertical comparison: 5%=0.39 1%=0.51; 0.01=0.66 (*,**,***-significant for 5, 1, and 0.1 level, respectively)				

Recent researches related to the rate of induction have highlighted that the highest percentage of haploid forms is found at the top of the ear induced and is influenced by time of pollination. In case of open pollination, analysis of variance (table 4) for the PHK number showed that all the sources of variation (genotype, position on the ear-top/bottom) as well as the interaction between genotype and position on the ear have significant effect on the number of haploid forms.

Table 4. ANOVA for PHK (Putative haploid kernel) number at the top and bottom of the ear, 15 genotypes submitted to induction,NARDI Fundulea, 2016

Source of variation	Degrees of freedom	Sum of squares	Mean square	F Value	Probability
Rplications	9	54,883	6,098	1,540	0.1409 ns
Genotype(G)	14	138,567	9,898	2,499	0.0036 **
Error (E)	126	498,967	3,960		
Part of the ear (T/B)	1	180,963	180,963	98,887	0.0000 ***
G x T/B	14	56,487	4,035	2,205	0.0104 ***
Error	135	247,050	1,830		
Total	299	1176.92			

*T= top, B= battom

The evaluation of the PHK number for the top and bottom of the ear have shown that some genotypes like L502, L573, L576 were significantly positively for the top PHK

number as compared to the experiment average. Comparing the PHK number from the top and bottom of the ear, the top average is distinct significantly positively over the bottom average PHK number. At some genotypes the PHK number from the top was much higher than the PHK number from the bottom of the ear as genotypes L502, L507, L573 and L576.

Table 5. PHK number at two positions on the ear (top and bottom), 15 genotypes, NARDI Fundulea, 2016

Genotype	PHK number			
	Top (T)	Bottom (B)	T-B	Average
L502	4.8*	2.0	$2.8^{\$\$\$}$	3.4
L507	3.4	1.2	$2.2^{\$\$\$}$	2.3
L508	4.4	2.5	$1.9^{\$\$}$	3.5
L519	2.4	1.1	$1.3^{\$}$	1.8
L529	3.0	2.4	0.6	2.7
L535	2.4	1.2	$1.2^{\$}$	1.8
L537	2.7	1.2	$1.5^{\$}$	2.0
L552	2.8	1.9	0.9	2.4
L560	2.4	1.2	$1.2^{\$}$	1.8
L567	2.2	1.3	0.9	1.8
L573	5*	1.2	$3.8^{\$\$\$}$	3.1
L576	4.6*	2.3	$2.3^{\$\$\$}$	3.5
L584	2.3	1.6	0.7	2.0
L586	1.7	1.0	0.7	1.4
L587	3.3	2.0	$1.3^{\$}$	2.7
Average	**3.2^{+++}**	**1.6**		**2.4**
LSD for factor A (genotypes) average: 5%=1.25; 1%=1.65; 0.01=2.12				
LSD for factor B (position on the ear(top, bottom) average: 5%=0.31 1%=0.41; 0.01=0.53 (+++-significant for 0.1 level)				
LSD for factor B (position on the ear (top, bottom) at the same level of factor A (genotypes)-horizontal comparison: 5%=1.20; 1%=1.58; 0.01%=2.03 ($,$$,$$$-significant for 5, 1, and 0.1 level, respectively)				
LSD for factor A (genotypes) at the same level of (position on the ear (top, bottom)-vertical comparison: 5%=1.22; 1%=1.99; 0.01=2.56 (*-significant for 5 level)				

CONCLUSIONS

Anthocyanin coloration is influenced by the genotype of the population submitted to the induction; a good coloration on the aleurone and embryo allows easy identification of the PHK forms.

Moreover, similarly to controlled pollination, the highest percentage of PHK remains on top of the ear in the case of this experiment with open pollination induction nursery. For this reason it is recommended to know the silking period of the sources, and flowering of the inducer to ensure optimal induction by handling planting time of the parents.

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