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GENERAL ASPECTS REGARDING THE RECOGNITION OF MAIZE HAPLOID KERNELS ACCORDING TO THE SIZE AND INTENSITY OF ANTHOCYANIN COLORATION

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Abstract

In the last decade, the use of double haploid lines in maize breeding programmes has become a standard procedure. This has become possible due to the substantial progress achieved by using in vivo maternal haploid technology induction. Currently, there are in use haploid inducers with inducing efficiency up to 15 %, making possible utilization of double haploid (DH) technology on a large scale. The most important advantages of this system is to shorten the period for obtaining homozygous lines (only 2 years) as compared to the conventional system (5-7 years) and high recognition precision of the putative haploid kernels. The anthocyanin coloration of the aleurone can vary from a small patch to covering the entire aleurone zone, exception kernel basis. Intensity of anthocyanin can range from very poor to strong anthocyanin both in the embryo and aleurone .
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. Since 2013, at NARDI Fundulea, we made some observation regarding the size and intensity of the anthocyanin coloration for the dent and flint genotypes studied. Annotations done on both aleurone and embryo were assessed with scores from 0-4: 0 for the lack of pigmentation and 4 for intense pigmentation. The objective of the present study was to see what score corresponds to a good choice of the haploid kernels.

Key words: *anthocyanin coloration, doubled haploid lines, haploid inducers.*

INTRODUCTION

Every year the DH technology advances more and more, the needs of haploid plants are becoming larger, but inducers are showing disadvantages in achieving this goal. The reason might be the fact that the rate of induced putative haploid kernels (PHK) is not high enough or females express instability in the manifestation of the anthocyanin coloration based on *R1-nj* gene that is involved in the synthesis of anthocyanin and used as genetic marker (Sarmaniuc M. et al., 2013). For the above system to work, an efficient screening system for separating the PHK from non-haploid seeds was needed. The anthocyanin marker gene, *R1-nj* (Nanda and Chase, 1966; Chase, 1969; Neuffer et al., 1997; Eder and Chalyk, 2002; Röber F.K. et al., 2005) was used for this screening process. However, the expression of this gene has a strong female influence sometimes the screening of PHK might be confusing or even impossible, especially in cases when there are inhibitor genes (*C1-I*) in female genotypes (common for flint maize). Even if there were no inhibitors of the *R1-nj* gene, but the moisture of kernels during the harvesting was high, the screening of haploids might be more difficult as well (Rotarenco V. et al., 2010). Since the material to be induced is very diversified it is necessary to find a marker system that allows more precise recognition of PHK from the mass of diploid dried kernels harvested from inducing fields and thereby saving costs involved in artificial chromosomal doubling and saving greenhouse and field space and labor allocated to this process (Prasana B.M.. et al., 2012). The procedure for the production of maternal haploids allows obtaining haploids

from different genotypes on a large scale (Deimling et al. 1997; Chalyk and Rotarenco 1999; J. Eder., S. Chalyk, 2002).
The aim of this study was to determine the behavior of different female sources used (dent and flint forms) with regards to the size and intensity of anthocyanin coloration and influence of these two parameters in the precise selection of the haploid/diploid forms.

MATERIALS AND METHODS

The study was carried out at the National Institute of Research and Development Fundulea in 2013.
As female sources, 22 synthetic and F2 populations were used. Each female sources was crossed with the inducer line in the field. Grains resulted from crosses were divided in 3 categories based on the expression of the anthocyanin coloration given by *R1-nj* gene on the kernel as follows: category 1, kernels with no coloration on the aleurone and embryo; category 2, kernels with coloration in both, aleurone and embryo and category 3, PHK with purple coloration only on the aleurone. A 0-4 scale was used for visual assessment of the intensity of anthocyanin coloration on aleurone and embryo within 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) at the level of total induced kernel bulk for each population.
The visual selection of the PHK was verified using cytological analysis. Root tips were cut from a sample of 20 PHK of each of 7 populations selected randomly.
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. The need to obtain haploid plants from a large spectrum of genetic backgrounds determined detailed analysis of phenotypic manifestation of anthocyanin markers. As it can be seen in table 1 and 2, the percentage of haploid varied between 0.64 and 6.68 and the scores received for the size and anthocyanin intensity varied between 1 and 4, for both embryo and aleurone.
Best percent of PHK were obtained from dent populations that received score 3 on the embryo, level that allows easy identification of PHK. Populations 3, 11 and 15 that have been scored 1-2 for anthocyanin coloration intensity on embryo, produced an induction efficiency of less than 2%. In this case the identification of haploid is possible but errors could occur due to very weak staining in the embryo. Population 3 that had an induction efficiency less than 1% (0.65%), has flint kernel and received the score 1 for anthocyanin coloration of the embryo; the anthocyanin coloration was almost inhibited in this population (table 1).

Table 1. Categories of kernels by type of coloration and efficiency of the inducer expressed as % PHKs from total kernels analysed.

Genotype	Kernel type	Total kernels analyzed	Categories of kernels by type of coloration			% PHK
			CAT. 1	CAT. 2	CAT. 3	
Population 1	D*	3932	730	3103	99	2.51
Population 2	D	4862	674	4047	141	2.90
Population 3	F**	1395	719	667	9	0.64
Population 4	D	3208	637	2416	155	4.83
Population 5	D	2826	736	1943	147	5.20
Population 6	F	789	100	667	22	2.78
Population 7	D	1852	425	1394	33	1.78
Population 8	D	2052	590	1398	64	3.11
Population 9	D	583	70	484	29	4.97
Population10	D	884	78	786	20	2.26
Population 11	D	2483	230	2207	46	1.85
Population 12	D	3728	522	3127	79	2.11
Population 13	D	1457	415	987	55	3.77
Population 14	D	938	279	616	43	4.58
Population 15	F	1497	408	1071	18	1.20
Population 16	D	4055	667	3208	180	4.43
Population 17	F	1286	221	979	86	6.68
Population 18	F	2459	447	1914	98	3.98
Population 19	D	2435	234	2147	54	2.21
Population 20	D	1718	533	1140	45	2.61
Population 21	D	2164	494	1628	42	1.94
Population 22	F	2569	274	2216	79	3.07
TOTAL		49172	9121	38145	1544	Average: 3.15

*D= Dent type
**F= Flint type

As shown in table 2, the majority of populations with dent kernel were scored 3 and 4 on embryo with one exception, the population 11, which registered a score of 2 on the embryo. In the other hand, flint kernel populations showed a lower intensity, embryo coloration being generally scored 2, but population 3 with a score of 1 and reversely population 17, that was scored 3, at the level of dent populations.

Table 2. The anthocyanin pigmentation influence of the female on embryo and endosperm

Genotype	Type of kernel	Score of intensity of anthocyanin pigmentation	
		Embryo	Aleurone
Population 1	D*	3	2
Population 2	D	4	3
Population 3	F**	1	3
Population 4	D	3	2
Population 5	D	3	3
Population 6	F	2	3
Population 7	D	3	2
Population 8	D	3	3
Population 9	D	3	3
Population 10	D	3	3
Population11	D	2	3
Population 12	D	3	3
Population 13	D	3	3
Population 14	D	4	2
Population 15	F	2	2
Population16	D	4	4
Population17	F	3	2
Population18	F	2	3
Population 19	D	3	3
Population 20	D	4	3
Population 21	D	3	3
Population 22	F	2	2

* D= Dent type
**F=Flint type

Cytological analysis (table 3; figure 1 and 2) showed that scored 3 for anthocyanin pigmentation of the embryo, conferred the best efficiency in selecting real haploid kernels. Score 2 for anthocyanin pigmentation of the embryo, the visual selecting of PHK has a lower level of confidence, a relative large number of errors can occur, as in the case of population 18 and 22.
According to the literature, haploid obtained from dent genotypes are more easily recognized and both anthocyanin coloration and intensity of the coloration on bouth aleurone and embryo are better expressed than in flint genotypes.

Table 3. Cytological analysis for 7 random sources females

Population	Kernel type	Intensity of anthocyanin pigmentation – score embryo/aleurone	Number of kernels analyzed	Number of confirmed real haploid kernels	% confirmed haploid kernels
Population 1	D*	3/2	20	15	75
Population 18	F**	2/3	20	11	55
Population 7	D	3/2	20	18	90
Population 22	F	2/2	20	7	35
Population 13	D	3/3	20	19	95
Population 16	D	4/4	20	14	70
Population 17	F	3/2	20	16	80
Total			140	100	Average=71

*D= Dent type
**F= Flint type

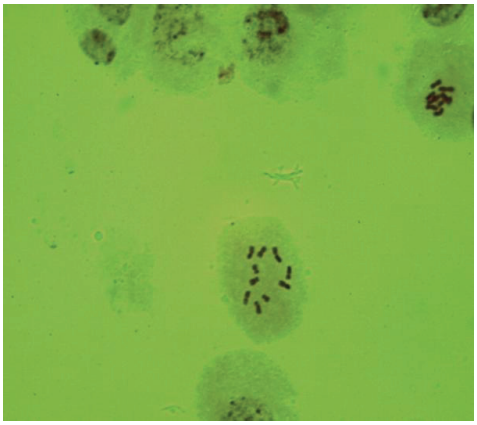

Figure 1. Mitotic metaphase; haploid plant with 10 chromosomes (photo taken during cytological analysis)

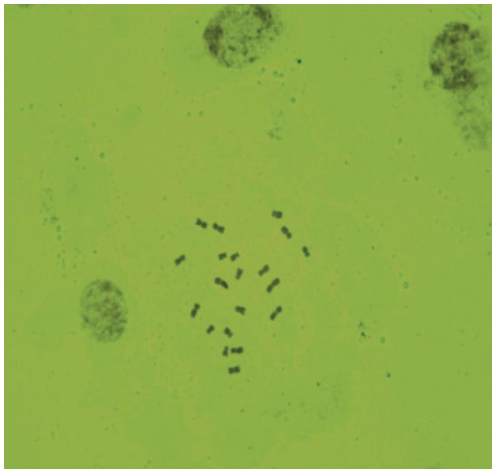

Figure 2**.** Mitotic metaphase; diploid plant with 20 chromosomes (photo taken during cytological analysis)

CONCLUSIONS

The anthocyanin coloration in both aleurone and embryo is significantly better expressed in dent populations than in flint populations; identification of haploid kernels is more confident in dent type, the real kernel percent (cytological confirmed), being over 70%.

Scores 1 and 2 for anthocyanin pigmentation of the embryo, registered generally only in flint populations resulted in a lower precise selection of PHK.

There is a stringent necessity to improve a DH procedure at NARDI-Fundulea in the very next years, by utilization of appropriate protocol to ensure a better anthocyanin pigmentation as well as to increase the inducing efficiency by testing more inductor sources.

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