Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1320610

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 6 - Issue 5: 528-532 / September 2023

THE EFFECT OF DONORS USED IN *IN-VIVO* MATERNAL HAPLOID TECHNIQUE ON HAPLOID INDUCTION RATE

Rahime CENGİZ[1]*

[1]*Sakarya University of Applied Sciences, Faculty of Agricultural, Department of Field Crops, 54580, Sakarya, Türkiye*

Abstract: Maize is an important plant grown to obtain grain and silage, and is used in human and animal nutrition. In conventional maize breeding studies, inbred line development studies are carried out for at least 7 years if a single generation is obtained in a year, while it is possible to develop 100% homozygous lines in a short period of 2 years with the *in vivo* maternal haploid technique. The *in vivo* maternal haploid technique is widely used in advanced maize breeding programs. The choice of donor or source material to be used for haploid induction depends on the purpose of the breeding program. Generally, breeders use F_1 or F_2 populations as source material for haploid induction. In this study; 30 F_1 genotypes and their F_2s were crossed with the inducer line. The putative haploid seed was identified by considering the *R1-nj* color marker, and the haploid induction rate was determined. The effect of the generations of the donor genotypes on the haploid induction rate was compared by performing an independent sample test, and the haploid induction rate obtained from the F_1 donors was found to be higher than the haploid induction rate of the F_2 donors. It was determined that there was a change in the haploid induction rate as the genotypes changed within the F_1 and F_2 donor groups.

Keywords: Maize, Breeding, Haploid technique, Inducer line, Source material

***Corresponding author:** Sakarya University of Applied Sciences, Faculty of Agricultural, Department of Field Crops, 54580, Sakarya, Türkiye
E mail: rahimecengiz@subu.edu.tr (R. CENGİZ)
Rahime CENGİZ https://orcid.org/0000-0001-6355-7496

Received: June 27, 2023
Accepted: August 10, 2023
Published: September 01, 2023
Cite as: Cengiz R. 2023. The effect of donors used in *in-vivo* maternal haploid technique on haploid induction rate. BSJ Agri, 6(5): 528-532.

1. Introduction

Maize is an important plant that is grown for grain and silage, and is used in human and animal nutrition. Due to the support of maize production in Türkiye in recent years, there has been a remarkable increase in maize cultivation area and production. In the last 10-15 years, there has been a great increase in maize cultivation areas, especially in our Central Anatolia and Southeastern Anatolia regions. The increase in the profitability of maize production, the development of high-yielding varieties in the cultivation technique, the effective use of water and fertilizer, and ease of mechanization and marketing are the most important reasons for the increase in cultivation area and production. Today, maize is cultivated in approximately 65-70 provinces (Soylu, 2022).

The development of high-yielding and quality hybrids in maize requires the continuous development of new inbred lines. A very long time is needed to obtain the lines that are settled with conventional methods. The haploid technique provides significant advantages in shortening this period. The potential of doubled haploids in maize breeding has long been demonstrated (Chase, 1969).

Today, doubled haploids are widely used in many areas of maize research and in conventional hybrid maize breeding around the world. In research, doubled haploid genotypes have been a valuable tool in structural and functional genomics, proteomics, metabolomics, marker-assisted studies, molecular cytogenetics, genetic engineering, and other fields. In breeding, doubled haploid lines allow the efficiency of selection to increase, shorten the breeding period, and save time and effort (Geiger, 2009).

Haploids are developed from an unfertilized female egg (gynogenesis) or a male cell (androgenesis). The *in vitro* androgenesis method is performed by anther culture. There are two known methods of obtaining haploids from the embryo of the maize. These are maternal haploids and paternal haploids. In this method, some native genotypes are used, called 'inducer lines', with which maternal haploids can be obtained. The method in which inducer lines are used as pollinators is defined as *in vivo* gynogenesis. The rate of obtaining haploid from modern inducer lines is between 5-8% (Geiger, 2009).

When maize plants are crossed with specific genotypes called inducers, maize kernels with haploid and normal diploid embryos show a clear distinction. This is called *in vivo* haploid induction. Generally, kernels with haploid embryos have normal triploid endosperm. Therefore, these grains show the same germination rate and germination strength as diploid embryonic grains (Coe and Sarkar, 1964).

The inducer genotype is used as a pollinator for the production of maternal haploids. Both the cytoplasm and the chromosomes carried by the resulting haploids come

from the donor plant. Different inducer genotypes are used in paternal and maternal haploid induction methods. Both methods of *in vivo* haploid induction are much less dependent on the donor genotype structure than the *in vitro* technique (Röber et al., 2005; Spitko et al., 2006).

In haploid inducer lines commonly used today, the *R1-nj* allele has been combined with other genes required for anthocyanin biosynthesis. Most maize germplasms used in breeding programs do not have the anthocyanin biosynthesizing genes or the *R1-nj* allele, which imparts the red-violet color in grain or plant tissue. When the source material that does not contain the anthocyanin color gene and the inducer lines used as the male are crossed, the *R1-nj* gene is dominant to the colorless *r1* allele, so the emergence of the Navajo phenotype in the embryo and endosperm is expected in all the seeds obtained. However, differential expression of the *R1-nj* allele allows the differentiation of maternal haploids from diploids. When inducer lines with a high haploid induction rate are used for induction cross, maternal haploids usually occur between 6-10% (Chaikam and Boddupalli, 2012).

The choice of donor or source material to be used for haploid induction depends on the purpose of the breeding program. Generally, breeders use F_1 or F_2 populations as source material for haploid induction. Prigge et al. (2011) used single hybrids, village populations, and open-fertilized varieties as source material for *in vivo* maternal induction. In addition, haploid induction rate (HIR) and false haploid selection rates were compared when these materials were used as source material according to kernel type (dent and flint maize). Significant variations were detected among the source materials used in terms of response to haploid induction. Higher HIR was determined in single hybrids compared to other source materials.

Significant differences in induction rate were determined between donor genotypes (eg, dent, flint maize, local variety) (Roux, 1995; Eder and Chalyk, 2002; Röber et al., 2005). However, the range of variation determined for these differences was small compared to the response to anther and microspore culture. Environmental conditions also affect the success of the *in vivo* haploid technique.

Hu (2014) used six inducer lines and 10 different F_1 as donors in his study to determine the induction rates of different inducer lines under the same conditions. The haploid induction rate ranged from 2.17 to 5.33%, and the induction rate of the haploid inducers was ranked from low to high as KMS-3<WY-1<PR-2<YP-13<KMS-2<KMS-1. Considering the haploid seeds obtained from different donors, the average haploid induction rate differed significantly between donors and ranged from 1.26% to 10.27%.

In vivo haploid technique has been widely used in maize breeding studies in recent years as a method that shortens the breeding period and increases its efficiency. The success of obtaining haploid seeds with the *in vivo* technique using haploid inducers varies according to the characteristics of the inducer line and the genotypes used as the donor.

In our country, the use of *in vivo* maternal haploid method in hybrid maize breeding has become increasingly widespread. This work was planned to determine the effect of donor genotypes used in *in vivo* maternal haploid technique on the haploid induction rate (HIR). This study determined the effect of 30 F_1 donors and their F_2s on HIR. The study's results aimed to facilitate breeders using this technique in choosing donor generations.

2. Materials and Methods

This study was carried out in the Sakarya location in 2020-21. MHI (Moldovian Haploid Inducer) available from Maize Genetics and Genomics Database (Maize GDB) was used as the inducer in the *in vivo* maternal haploid technique. The MHI inducer line has *R1-nj*, *B1*, and *Pl1* alleles. It creates anthocyanin in the seed and the rootlets during the germination period. According to Chalyk (1994), it has an average haploid induction rate of 4.5%. As source material for *in vivo* maternal haploid technique, 30 numbers of F_1 (single hybrid) and 30 numbers of F_2 (30 of F_2 derived from F_1 were used) were counted and packaged, and sprayed against subsoil pests and fungal diseases. Hybrid maize varieties used as donors are planted which were for grain and silage purposes, in the dent kernel type, early, mid-late, and late maturity groups. F_2s of donor F_1s were obtained by the bulk selfing method in 2020. Each of the F_1 and F_2 donors was planted in 2 rows, 70 cm row spacing, and 18 cm in row spacing, in 5 m long plots with a hand sowing machine on two times in 2021.

After the experimental area was prepared with suitable tillage tools before planting, a composite fertilizer containing 10 kg of pure nitrogen (N) and 10 kg of pure phosphorus (P_2O) was applied per decare. The second fertilizer of nitrogen (12 kg N da-1) was given when the plants were 40-50 cm. Cultural struggle (hand hoe) was made with weeds. Plant tags are attached before flowering.

When the flowering period of the F_1 and F_2 genotypes came, all tassels were cut and removed. Ears are closed with shoot bags without flowering. The hybridization process was carried out according to Russell and Eberhart (1975). Plants of the inducer genotype were covered with tassel bags when the anthers started pollinating 50% in the main axis after the tassel emergence. When the silk was 3-5 cm in the ears covered with shoot bags in the source material, the isolation bags at the tassel of the inducer line were collected and put together and poured onto the silks of the donors. The process continued until the hybridization of all source material was complete. At least 5 ears were crossed in each donor. Crossed ears were kept in isolation papers until harvest. At harvest, the ears of each donor were

taken into separate mesh bags and labels were attached. When the grain moisture decreased to 14%, grains of each genotype were separated from the cob, and the total number of seeds was determined. Haploid seeds were selected considering the *R1-nj* color marker carried by the inducer line (Chaikam and Boddupalli, 2012).

Four different types of kernel samples are obtained from induction cross; i) normal diploid or hybrid seeds (F_1) have purple-colored endosperm and embryo, ii) seeds considered haploid (H) have purple endosperm and colorless embryo, iii) seeds with diploid endosperm (DE) have no color in the endosperm but have color in the embryo, iv) considered non-hybrid (MD) are seeds without coloration in the embryo and endosperm (Figure 1).

The haploid induction rate for each genotype was determined according to the formula below.

HIR = (Haploid seed number/Total number of seeds) x 100

The t-Test analysis of the data obtained from the study was made using the MSTAT-C package program.

3. Results

30 different F_1s used as trial material was planted in 2020, and their F_2s were obtained. In 2021, 30 F_1 and 30 F_2 source materials were planted according to the method. Induction cross was performed using the *in vivo* maternal haploid technique. The images of the cobs obtained at the harvest are given in Figure 2. In the seeds taken from each source material, haploid seed selection was made according to the method (Figure 3).

Figure 1. Four different categories of seeds from induction crossing.

Figure 2. Ear samples from induction crossing.

Figure 3. Identification of haploid seeds according to the *R1-nj* color marker.

Although the *R1-nj* marker system is an effective way of distinguishing haploids, the expression of the *R1-nj* allele is highly influenced by the genetic background of the source material. Navajo crown coloration ranged from a small dot (at the point where the silk attaches to the grain) to coloration that spanned the entire endosperm. In addition, the darkness of color in the endosperm and embryo also differed from very light to dark and deeper (Figure 4).

HIRs of each genotype were calculated according to the method. HIR of F_1 genotypes varied between 3.2-7.1%. HIR of F_2 genotypes was found to be the lowest at 2.8% and the highest at 6.5%. Two groups were compared using a t-test in terms of HIRs of 30 F_1s and their F_2s used as donors. According to the results of the analysis, F_1 donors were superior to F_2 donors and took place in group a (Table 1).

HIR obtained from induction crossing of F_1 genotypes used as donor source material was found to be statistically significant at the 5% level compared to HIR values of F_2s derived from the same F_1s (Table 2). These results obtained from the study by Prigge et al. (2011) are in line with the findings. At the same time, it was determined that there was a change in the induction rate as the genotypes changed within the F_1 and F_2 donor groups. The haploid induction rate was not affected by whether the cultivars were early or late, grain or silage. Since all of the genotypes used in the study are in dent kernel type, they do not carry an inhibitor gene that will prevent the emergence of the *R1-nj* allele. The expression of the *R1-nj* allele in F_1 and F_2 donor genotypes supports this situation.

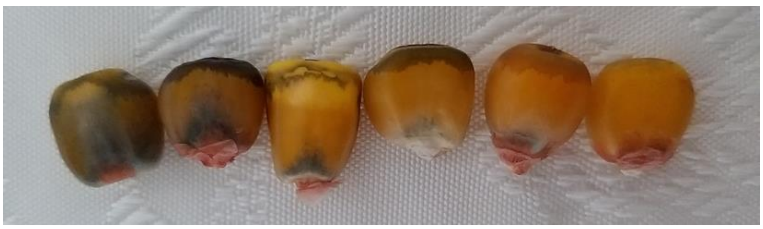

Figure 4. The expression of the *R1-nj* allele in the kernel from different donors.

Table 1. t-Test group analysis of F_1 and F_2 donor source materials

	Groups	Number of Donors	Mean	Std. Deviation	Std. Error Mean
F_1 and F_2 Donors	1.00 F_1	30	5.3500 [a]	1.09379	0.19970
	2.00 F_2	30	4.7267 [b]	1.00032	0.18263

Table 2. Independent sample test for HIR between F_1 and F_2 donor source materials (t-Test).

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
F_1 and F_2 Donors	Equal variances assumed	0.189	0.666	2.30	58	0.025*	0.62333	0.27062	0.0816	1.16503
	Equal variances not assumed			2.30	57.5	0.025	0.62333	0.27062	0.0815	1.16513

* Significant at 5%.

4. Discussion and Conclusion

It has been stated in other studies that the effect of donor genotypes on the success of obtaining haploids in the *in vivo* maternal haploid technique is less compared to the *in vitro* haploid technique. Although the success rates of obtaining haploid plants are not very different between the two techniques, they depend on the genotype in both methods (Beckert, 1994). In our study, it was determined that there was a change in the haploid induction rate as the donor genotypes changed.

The study was conducted under greenhouse conditions using 12 different local maize landraces and ADAIL-I inducer line to develop doubled haploid lines with different oil and zein content. Haploid induction rates of donor materials ranged from 6.08% to 11.71%, and the mean HIR value of the ADAIL-I inducer line was determined as 8.20% (Kahriman et al, 2022).

The nature of the source material to be used as a donor in the development of doubled haploid lines can change the breeding scheme. Hybrids created for special purposes and populations obtained from them by inbreeding can be used in induction hybridization as donors by choosing appropriate for the purpose. However, a strong selection is required in terms of the characteristics of the source material. The development of homozygous maize lines has an important place in hybrid maize breeding. Breeders use different source materials to develop

genetically different maize lines. It is quite common to use different F_1 (single hybrid) source materials in breeding studies for this purpose. Considering the haploid induction rate in studies of obtaining doubled haploid maize lines using the *in vivo* maternal haploid technique, the use of F_1s comes to the fore.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	R.C.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

Acknowledgments

The MHI (Moldovian Haploid Inducer) inducer line used in this study was obtained from Maize Genetics and Genomics Database and I would like to thank Maize GDB.

References

Beckert M. 1994. Advantages and disadvantages of the use of in vitro/in situ produced DH maize plants. In: Y. P. S. Bajaj (ed.). Biotechnology in Agriculture and Forestry. Vol. 25. Maize. Springer Verlag, Berlin, pp: 201-213.

Chaikam V, Boddupalli MP. 2012. Doubled Haploid Technology in Maize Breeding: Theory and Practice. CIMMYT, Mexico, pp: 20-23.

Chalyk ST. 1994. Properties of maternal haploid maize plants and potential application to maize breeding. Euphytica, 79: 13-18.

Chase SS. 1969. Monoploids and monoploid derivates of maize (Zea mays L.). Bot Rev, 35: 117-167.

Coe EH, Sarkar KR. 1964. The detection of haploids in maize. J Heredity, 55: 231-233.

Eder J, Chalyk ST. 2002. In vivo haploid induction in maize. Theor. Appl Genet, 104: 703-708.

Geiger HH. 2009. Doubled Haploids. In: Bennetzen JL, Hake S (eds.). Maize Handbook-Volume II: Genetics and Genomics. Springer Verlag, New York, US, pp: 641-657.

Hu G. 2014. Study on haploid induction rates in different maize inducers. Agri Sci Tech, 15(4): 554-556.

Kahrıman F, Kahrıman A, Güz AM, Yüksel NN. 2022. Development of homozygous maize lines differing in oil and zein content using in-vivo maternal haploid technique. Biotech Stud, 31 (2), 79-86.

Prigge V, Sanchez C, Dhillon BS, Schipprack W, Araus JL, Banziger M, Melchinger AE. 2011. Doubled haploids in tropical maize: I. Effects of inducers and source germplasm on in vivo haploid induction rates. Crop Sci, 51: 1498-1506.

Röber FK, Gordillo GA, Geiger HH. 2005. In vivo haploid induction in maize-performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica, 50: 275-283.

Roux SR. 1995. Züchterische Untersuchungen zur in-vivo-Haploideninduktion bei Mais. PhD. Thesis, University of Hohenheim, Stuttgart, Germany.

Russel WA, Eberhart SA. 1975. Hybrid performance of selected maize lines from reciprocal recurrent and testcross selection programs. Crop Sci, 15: 1-4.

Soylu S. 2022. Mısır Yetiştiriciliği. Mısır; Islah Teknikleri ve Yetiştiriciliği. İKSAD Yayınevi, Ankara, Türkiye, pp: 547-574.

Spitkó T, Sági LJ, Pintér LC, Barnabás MB. 2006. Haploid regeneration aptitude of maize (Zea mays L.) lines of various origin and of their hybrids. Maydica, 51: 537-542.