Influence of parentage on in vivo induction rate of haploid-tropical maize (*Zea mays L.*)

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Introduction

Double haploid (DH) lines have been used on past years for many genetic and molecular studies moreover to become the backbone in major commercial maize breeding programs. Advances in the in vivo production of haploids through the use of specific lines known as inducers made the land mark for its application. In-vivo induction of haploid plants was first recognized as a potential method for rapid production of DH lines in maize breeding when the inbred line stock 6 was identified as a haploid inducer line by Coe in the 1960s (Coe,1959). Progress in increment of the induction rate of stock 6 line led to the creation of more efficient inducer lines. Inheritance studies (Lashermes and Beckert, 1988) and QTL analysis (Röber, 1999) revealed that in vivo haploid induction is a genetically controlled character that is under the control of a few to many genes. Despite the mechanism of inheritance of haploid induction rate of the paternal genotype being extensively investigated, studies focusing on the maternal parent (genotype) are scarce. The objective of this study was therefore to elucidate the genetics of induction rate of maternal genotypes inferred by estimating the general combining ability (GCA) and specific combining ability (SCA).

Materials and methods

Plant material and experimental conditions. Ten white seeded tropical inbred lines (Table 1) from the International Maize and Wheat Improvement Center (CIMMYT) were crossed in a half diallel design. The 45 F1s obtained were planted in 5x9 alpha lattice design with two replications in the 2008 summer cropping season at CIMMYT's Agua Fria experimental station, Mexico (CIMMYT, 2003). F1 hybrids were crossed with a temperate inducer hybrid (RWS X UH400) obtained from the University of Hohenheim, Stuttgart, Germany.

After harvesting, seeds were screened to identify putative haploid seed using the purple coloration of the embryo phenotypic marker system (Figure 1) that is controlled by the R1-nj gene (Nanda and Chase, 1966).

Figure 1. The three different categories of seed harvested and classified based on purple coloration of the endosperm and embryo

The putative haploid seeds (category 3) were planted on the field. Further, plants having purple stem color, which is the character of the inducer genotype (purple plant), were eliminated.

Induction rate (IR) was calculated as:

$$IR(\%) = \frac{catagory\ 3 - purple\ \_\ plant}{catagory\ 1 + catagory\ 2 + catagory\ 3} x100$$

Statistical analysis. Statistical analysis was done using DIALLEL-SAS program (Zhang and Kang, 1997) employing the Griffing's diallel analysis model 2 method 4 (Griffing, 1956)

Results & Discussion

The analysis of variance for the induction rate of the single crosses was highly significant (Table 1). Mean values of induction rate ranged from 1.4 – 14.5% with overall average induction rate of 6.1% (Figure 2). The highest induction rate was obtained from the cross P2xP9 followed by P5xP7 and the lowest induction rate were from crosses P3xP4 and P2xP5 (Figure 2). This finding is in agreement with studies in temperate maize by Lashermes and Beckert (1988) which used stock 6 as an inducer line and found that induction rate varied according to the maternal genotype that ranged from 2.7% - 8%. In addition, our findings in tropical maize concur with those reported by Eder and Chalyk (1999) in temperate maize who reported that the maternal genotype used had a significant effect on the number of haploid seeds obtained ranging from 2% - 5%. The difference in the range of induction rate of the temperate materials obtained in the studies mentioned above as compared to the tropical materials in our trial suggest that the tropical materials have much genetic diversity in terms of their effect on haploid seed production through in-vivo haploid induction method.

Table 1. Analysis of variance for induction rate

Source of variation	df	MS
Crosses	44	12.154**
GCA	9	20.916**
SCA	35	9.901**

* Significant at the 0.05 level
** Significant at the 0.01 level

The seeds selected based on the embryo marker system controlled by the R1nj gene were planted and the seeds that were presumed to be haploid seeds but were misclassified were identified by the purple stem color maker. Percentages of falsely classified haploids (false positive) are given in Figure 3. Misclassification based on the embryo marker system ranged from 1.6 – 44.2% with an average of 12.5%. In this experiment the use of the R1nj gene for haploid seed identification has resulted in misclassification of a considerable amount of seeds. Literature indicates that the R1nj gene has very high misclassification rate in tropical material (Kato, 2002). More report on misclassification of haploid seeds was obtained in tropical maize genotypes by Belucuas and coworkers (Belucuas *et al*., 2007). Further results also support misclassification of haploid seeds in tropical maize genotypes (Belucuas *et al*., 2007). Furthermore, the purple color can not be easily identified in small seeds and seeds with thicker seed coat around the embryo. This may contribute to the high misclassification rate. Therefore a more efficient phenotypic marker system should be developed to decrease the misclassification rate. The use of the Pl gene with purple color of the root has been suggested (Röber *et al*., 2005)

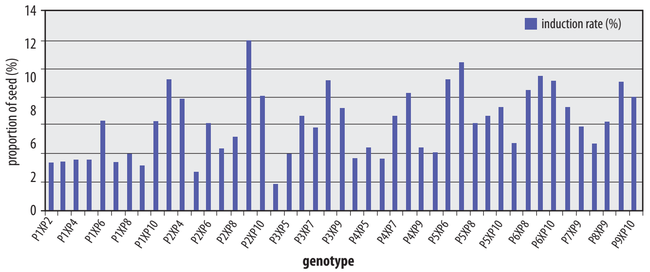

Figure 2. Mean induction rate of the 45 single crosses

Substantial amount of seeds with no purple coloration on the endosperm as well as the embryo were observed in the experiment. This indicates the presence of inhibitor gene in the maternal genotypes. Proportion of colorless seed ranged from 0.69 - 53.9% with an average of 14.9% (Figure 3). The occurrence of large amount of colorless seed in this experiment led us to believe that an inhibitor gene exists in some of the maternal parents. Especially the highest proportion of the colorless seed was obtained from crosses involving P1. There are reports on color inhibitor gene C1-I that were observed in previous studies (Coe, 1962) which inhibit the expression of the purple color on seed of maize. This leads us to suppose that the inhibitor gene C1-I could exist in Pl genotype and its presence may have also affected the embryo color marker system that is used to calculate the induction rate of this genotype since it was the worst combiner for induction rate in our study.

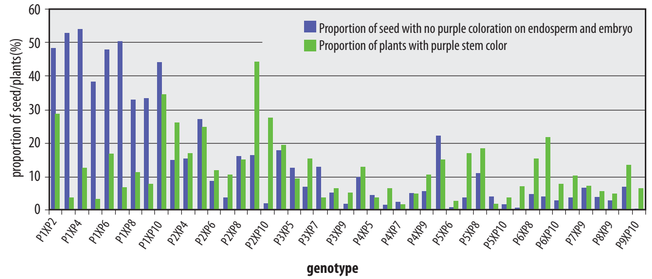

Figure 3. Percentage of while seeded end purple stemed plants

The variance due to general combining ability (GCA) and specific combining ability (SCA) were highly significant (Table 1), indicating that both additive and non additive genes control induction rate in maize. The mean square for GCA was higher than that of SCA indicating that additive genes were more important than non additive genes in controlling induction rate in the present study. GCA values of the parents and SCA values of the F1 crosses are given in Table 2.

Table 2. GCA and SCA values of the 45 single crosses

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
P1	**-2.339****	-0.916	0.239	1.068	-0.095	1.485	-0.075	-0.901	-1.583	1.789
P2		**0.375**	3.235*	2.664	-3.659**	-1.469	-1.899	-2.385	4.534**	0.905
P3			**-0.620**	-2.401	-1.474	-0.016	0.566	2.560	0.729	-2.460
P4				-1.389**	-0.175	-2.175	2.115	2.479	-1.353	-1.211
P5					**-0.196**	2.203	4.633**	-0.854	-0.285	0.716
P6						**0.994***	-2.138	0.276	1.405	1.406
P7							**-0.316**	0.386	-0.865	-1.714
P8								**0.990***	-1.851	1.300
P9									**0.931***	0.279
P10										**0.560**

* Significant at the 0.05 level
**Significant at the 0.01 level

The highest GCA value was obtained from P6, P8 and P9 showing the contribution of these three inbred lines for high induction rate. On the other hand, P1 was the worst general combiner followed by P4 consequently leading to their low contribution to induction rate. P2xP3 and P2xP4 were the best crosses for SCA for induction rate. Inheritance studies by Lashermes and Beckert (1988) and QTL analysis studies by Röber (Röber, 1999) indicate that in vivo haploid induction is a genetically controlled character controlled by a few to many genes. The results from our study and the works by the aforementioned researchers (mostly with temperate maize) suggest that induction of haploidy is a complex character controlled by many genes in both parents.

Conclusion

Our results showed that the single crosses as well as the parents have highly significant differences in induction ability. More important the present study revealed that induction rate varied according to the maternal genotype used for induction (1.4% – 14.5%). The fact that the maternal genotype is as important in determining the induction rate as the inducer line gives an opportunity to maximize the potential of the double haploid breeding technique through manipulation of methods that increase the ability of the maternal genotype for high induction rate. This could be achieved through identification of the proper time or environmental conditions for pollination so as to obtain maximum induction rate. Our study revealed that both additive and non additive genes condition the expression of induction rate in maternal genotypes. Further studies on the genetics of this trait are required to provide more information that could be used to effectively exploit this trait in maize breeding. Particularly the effect of growing conditions on the rate of induction should be assessed. This study provides insights on what kind of gene interaction is involved in the control of induction rate from the maternal side and how a higher induction rate may be achieved.

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