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Improvements of *in vivo* haploid induction in maize

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Currently, doubled haploid technology is the main way homozygous inbred lines are developed in maize breeding. It became possible after the creation of haploid inducers - specific genotypes which are used as pollinators to produce maternal haploids *in vivo*.

Modern inducers have relatively high rates of haploid induction and a system of marker genes allowing haploids to be identified at different stages - dry kernels, seedlings and mature plants (Röber *et al.,* 2005; Rotarenco *et al.*, 2010). However, there are some limiting factors in the haploid induction technique namely: the frequency of haploids has a significant variation in different donors (Prigge *et al.,* 2009); the identification of haploids among dry kernels might be very complicated or ever impossible - in cases when the donors are homozygous for certain genes (*R1*, *C1-I)* (Belicuas *et al.*, 2007; Geiger *et al*., 2009); the marker genes *B1* and *Pl1* are frequently affected by paramutations both in crosses with donors and in inducers themselves (Chandler, 2000). Additionally, in some inducers, we have noticed partially male sterility negatively influencing their pollen production. Poor seed set in inducers complicates their maintenance and reduces the yield of haploids in crosses with donors.

Chalyk *et al.* (2003, MNL-77) found a high frequency of aneuploid cells in haploid inducers. The authors concluded that aneuploidy could be the reason of the haploid-inducing ability. Based on this hypothesis, it is possible to assume that there should be a positive correlation between the haploid-inducing rate and the frequency of aneuploid cells in inducers. On the other hand, aneuploidy can lead

to irregularities during meiosis and be the reason of both partially male sterility and poor seed set.

In 2010, as a result of the estimation of new haploid inducers PHI (Rotarenco *et al,* 2010), there were genotypes with the high rates of haploid induction (more than 10%) and good both pollen production and seed set.

In 2011, the main objectives were (1) to estimate the connection between the haploid-inducing ability and partially male sterility in the PHI inducers, (2) to determine the correlation between the frequency of haploids and the yield of haploids per ear in different donors.

Besides inducer lines, PHI-1, PHI-2, PHI-3 and PHI-4, two hybrid inducers, PHI–3 × PHI–2 and PHI–3 × PHI- 4, have been tested. Five donors - two hybrids, A619 × A464 and B73 × Mo17, a synthetic population, SA, and two inbred lines, B73 and A619, have been used in the experiment.

To synchronize the flowering time, both the inducers and donors were planted with different delays. The donors were pollinated on the third day after the emergence of silks, which is the best time for haploid induction (Rotarenco *el al*., 2007). In each donor, from 5 to 10 ears were pollinated with the pollen of each inducer.

Partially male sterility (segmental shedding) was revealed in the inducers PHI-3 and PHI-4, whereas the inducers PHI-1 and PHI-2 had a good shedding. As checks, we used the initial inducers - MHI (Chalyk, 1999) and Stock6 (Coe, 1959) (Fig. 1 and 2).

Figure 1. MHI: segmental shedding

Figure 2. Stock 6: good shedding

In three donors, A619 × A464, B73 × Mo17 and the SA population, the highest rate of haploid induction had an inducer with a good shedding - PHI-1. The same inducer line had the highest yield of haploid kernel per ear in those donors (Table 1, Fig. 3).

Table 1.

Haploid induction rates, average numbers of haploid kernels per ear and the coefficients of correlation between these traits in three donors obtained by crosses with six inducers (2011)

Inducer	Donor					
	A619 × A464		B73 × Mo17		Population SA	
	Haploids %	Haploids per ear	Haploids %	Haploids per ear	Haploids %	Haploids per ear
PHI–1	15.3	17.0	14.7	18.1	17.1	28.0
PHI–2	10.7	7.2	10.2	11.3	11.7	9.6
PHI–3	12.2	8.1	10.1	8.0	15.2	6.7
PHI–4	12.0	8.8	8.4	10.0	14.3	14.0
PHI–3 × PHI–2	9.1	9.2	-	-	-	-
PHI–3 × PHI–4	14.1	19.0	-	-	-	-
Coefficient of correlation	0.77*		0.89*		0.72	

* Significant at $P < 0.05$

Figure 3. Female: synthetic population SA

Remarkably, the hybrid inducer PHI-3 × PHI-4 did not have any signs of male sterility in contrast with the parent lines. Moreover, its haploid induction rate, 14.1%, was higher than in the parents and the number of haploids per ear was the highest, 19.0, in the donor A619 × A464.

The inducer PHI-3 had the highest haploid induction rate, 24.6%, in B73; however, the average yield of haploids was the lowest - 6.6. The highest yield of haploids, 36.8, in B73 was obtained by the PHI-1 inducer with the haploid induction rate of 21.6%. The same inducer showed the best result in the second donor line - A619 (Table 2, Fig. 4).

Table 2.

Haploid induction rates, average numbers of haploid kernels per ear and coefficients of correlation between these traits in two donors obtained by crosses with four inducers (2011)

Inducer	Donor			
	B73		A619	
	Haploids %	Haploids per ear	Haploids %	Haploids per ear
PHI–1	21.6	36.8	15.2	12.2
PHI–2	14.0	10.0	7.4	1.6
PHI–3	24.6	6.6	14.3	3.4
PHI–4	18.5	18.3	10.3	4.6
Coefficient of correlation	0.13		0.71	

All coefficients of correlation had positive values. In two donors, A619 × A464 and B73 × Mo17, they were statistically significant ($P < 0.05$).

Figure 4. Female: B73

In conclusion, our results revealed that the haploid-inducing ability, partially male sterility and seed set are not such strong connected inducers' characteristics. Thus, we are able to create inducers with the high rates of haploid induction good both shedding and seed set.

Using hybrid inducers can be the way to improve these inducers' properties as well. However, the hybridization of different inducer lines, based on our experience, might have a negative effect on the haploid induction rate and the marker genes. Therefore, first, the most successful hybrid combinations have to be identified.