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New inducers of maternal haploids in maize

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On the basis of the first inducer of maternal haploids in maize (*Zea mays* L.), Stock 6 (Coe, 1959), a number of new inducer lines have been created (Tyrnov *et al*, 1984; Lasharmes *et al,* 1988; Sarkar *et al,* 1994; Shatskaya *et al,* 1994; Chalyk, 1999; Rober *et al,* 2005). The inducers possess dominant anthocyanin marker genes allowing haploids to be identified at different stages (dry seeds, seedlings and mature plants), and their haploid-inducing rate was significantly increased in comparison with the initial inducer (Stock 6). Nevertheless, some essential disadvantages of the existing inducers have been noticed.

Due to a small plant size, most of the inducer lines cannot be used for the production of haploids by open pollination at isolated fields. To overcome that, hybrids between inducers are often applied; however, the following problems may appear in hybrid inducers: (1) reducing the frequency of haploid induction, and (2) changing the expression of marker genes (unpublished).

The *R1-nj* marker gene (purple scutellum and a "purple crown" of the aleurone) is widely used for the screening of haploids in dry seeds. However, the expression of this gene has a strong female influence: sometimes the screening of haploids might be very confusing or even impossible, especially in those cases when there are inhibitor genes (*C1-I)* in females (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 impossible as well.

The aim of our work was to create new inducers which would have (1) improved plant traits, (2) good expression of marker genes and (3) high rates of haploid induction.

Two inducer lines have been selected as an initial material – MHI (Chalyk, 1999), as a source of favorable plant traits and the high frequency of haploid induction, and Stock 6 (Maize Genetics Cooperation Stock Center), as a source of the *B1* and *Pl1* marker genes (sunlight-independent purple pigmentation in plant tissues) allowing haploids to be identified by the lack of anthocyanin coloration in seedlings.

During the F_2 - F_5 generations, selection for desirable characteristics has been carried out, as well as for resistance to logging and diseases. Only those plants which had the haploid-inducing rates more than 10% have been selected in each generation.

From 92 lines (F_5), 9 new inducers have been selected. By phenotype and other characteristics, they have been divided into four groups and named PHI – Procera Haploid Inducer (Picture 1).

For the estimation of the haploid-inducing frequency, the following females have been used: two inbred lines - A464 and A619, their hybrid (A464/A619), and a synthetic population - SP. Previously; it has been revealed that the screening of haploids in these females, by the expression of the *R1-nj* gene, had a very high accuracy.

From five to ten plants of each female have been crossed with each inducer. There were about 1000 kernels in both hybrid and population and about 400 kernels in each female line obtained as a result of crossing with each inducer. The main characteristics of the initial and PHI inducers are presented in the table.

Picture 1. Initial and PHI inducers, (from left to right) Stock 6, MHI, PHI-1, PHI-2, PHI-3 and PHI-4

Main characteristics of the initial and PHI inducers

Inducer, Male	Planting-flowering, days	Plant height, cm.	Haploid-inducing frequency, %				
			Female				Mean
			A464 line	A619 line	A464/A619	SP popul.	
Stock 6	60	158	0.7	1,3	2.1	0.9	1.2
MHI	65	192	9.1	5.5	8.2	6.0	7.2
PHI-1	55	151	11.1	12.5	12.1	12.7	12.1
PHI-2	60	198	15.6	12.3	12.0	12.0	13.0
PHI-3	70	180	14.3	14.5	14.2	15.1	14.5
PHI-4	65	200	11.8	10.7	12.0	16.8	12.8

By flowering time, rather contrast inducers have been obtained - there was a two-week interval between the earliest inducer (PHI-1) and the latest one (PHI-3). By plant height, a transgressive effect has been revealed in the PHI-2 and PHI-4 inducers. In some cases, haploid-inducing rates were almost twice higher in the PHI inducers in comparison with the initial line - MHI. However, the percentage of haploids may significantly vary due to different reasons (Rotarenco, 2002; Rotarenco and Eder, 2003; Rober *et al*, 2005; Rotarenco and Mihailov, 2007). The lowest rate of haploid induction in all the PHI inducers was 10%.

Selection for good expression of the *R1-nj* gene has been carried out among the PHI inducers. For this kind of test, the B73 and Mo17 lines have been used as females (Picture 2).

Picture 2. B73 pollinated with (from left to right) Stock 6, MHI, PHI-2 and PHI-4

The PHI inducers have been crossed with a carrier of the *C1-I* gene (inhibitor of the *R1-nj* gene). The expression of the *R1-nj* gene was completely suppressed; however, haploids were easily screened by the lack of purple pigmentation in 4-day seedlings (Picture 3).

Picture 3. Screening of haploids by the lack of purple pigmentation in roots

The PHI inducers had large tassels with good pollen production. After their self-pollination, a rather good seed set (for inducers) has been obtained (Picture 4).

Picture 4. Ears of the PHI-4 inducer

Thus, both quantitative and qualitative traits of inducers can be improved, as well as their haploid induction frequency. The authors consider that these achievements are able to increase the efficient of the technology of *in vivo* haploid induction.