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Aberrant doubled haploid lines in maize

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Doubled haploid (DH) maize lines generally are created from *in vivo* induced haploid plants by artificial chromosome doubling using mitosis inhibiting compounds such as colchicine (Wan *et al.,* 1989; Gayen *et al.,* 1994). DH lines therefore should be homozygous; however, we have observed a significant phenotypic variation in the progeny of some DH lines.

Possible reasons for this phenomenon include:

1. Mutagenesis

a) Colchicine does not only act as an inhibitor of mitosis but may also induce mutations. If this happens in the diploid sporophytic tissue of a treated haploid plant, selfed progenies will be heterozygous leading to segregation in subsequent generations.

b) Since the diploidy is the natural ploidy level of maize, the mutation rate of haploids, due to the influence of environmental conditions, might be higher than that of diploids.

2. Paternal gene transfer

Haploid inducers acting as pollinator generally do not transmit any genes to the resulting haploids, i.e. the induced haploids carry genes from the maternal genotype only. Yet, occasionally limited male gene transfer (DNA introgression) was reported in the literature (Fisher, 2004; Liang Li *et al.,* 2009). This leads to a transformation of genetic material, no heterozygousity will occur at the DH level. However, if a male chromosome segment is added to the female genome leading to aneuploidy, segregation may occur in subsequent selfing generations.

Our studies showed a significant influence of inducers on the manifestation of quantitative traits in haploid plants, and that, most likely, was associated with the DNA introgression (Rotarenco *et al*, 2009).

Our objective was to reveal the most possible reason leading to the instability of DH lines.

A well-known inbred line A619 and a DH line, 134, were used in the study. The 134 line is one of the DH lines derived from our breeding synthetic population, SP, and after three generations, we noticed a significant phenotypic variation within this line. In contrast, the inbred line A619 is characterized as a rather stable genotype. Both lines were crossed with a haploid-inducer line MHI (Chalyk, 1999).

Haploids produced from each line were divided into two groups. The first group was planted in the field; the second group was subjected to a chromosome - doubling treatment (Deimling and Geiger, 1997). There were about 150 haploid kernels in each group. Haploids planted in the field were randomly pollinated with a bulk of pollen from their diploid lines, doubled haploids were self-pollinated.

By the pollination of haploids with their diploid analogues, eight new lines, called reconstituted lines, have been obtained from each initial line. Six DH lines have been produced from both A619 and 134 lines by chromosome doubling.

Reconstituted and DH lines have been compared with the initial genotypes in S_2 and S_3. Plant height, ear length and coefficients of variation of these traits were estimated. The experiments were carried out in three replications on two - row plots.

No significant differences were revealed between the line A619 and its derivatives, whereas among the lines produced from the line 134, a significant variability for the estimated traits was detected. We did not reveal any significant differences between the S_2 and S_3 generations. Results of comparing the initial lines with their DHs in S_3 are presented in the table below.

Means of plant height and ear length, coefficients of variation of these traits in the lines A619 and 134 and their DHs (S_3)

Initial lines and DH progenies (S_3)	Plant height		Ear length	
	Mean	Coef. var.	Mean	Coef. var.
A619	202.2±2.2	6.6	15.7±0.7	20.7
A619DH1	198.2±2.8	6.4	14.2±0.5	13.5
A619DH2	200.5±2.6	5.8	16.1±0.7	17.0
A619DH3	200.3±1.9	4.7	16.7±0.7	15.9
A619DH4	201.8±1.4	3.8	15.5±0.3	8.2
A619DH5	207.1±2.6	5.4	16.0±0.7	17.3
A619DH6	197.4±2.4	6.1	17.7±0.9	13.5
134	269.5±5.6	10.6	15.1±0.19	10.7
134DH1	275.2±2.9	4.9	16.9±0.21***	7.6
134DH2	267.8±2.0	3.5	14.5±0.16	7.6
134DH3	270.2±3.9	6.6	16.8±0.26***	9.1
134DH4	227.3±2.2***	5.4	15.1±0.13	5.8
134DH5	223.4±2.5***	6.4	14.7±0.16	7.1
134DH6	268.0±2.1	3.7	15.1±0.17	7.1

*** Significantly ($P < 0.001$) different from initial line

Lines 134DH4 and 134DH5 were significantly inferior to the initial line, 134, for plant height; 134DH1 and 134DH3 significantly exceeded the initial one for ear length. Four reconstituted lines, either for plant height or for ear length, differ significantly from the initial genotype 134 (data is not presented). Additionally, among those 14 lines derived from the line 134, we have noticed a variation for the beginning of flowering – up to 10 days.

In all DH lines, the coefficients of variation have reduced in comparison with the initial genotypes (Table). Thus, most likely, the influence of colchicine is not the main reason leading to the instability of DH lines; the same can be said about the influence of environmental conditions since we did not obtain any significant differences among the reconstituted lines derived from the line A619 (data is not presented).

Differences among the lines produced from the line 134 may be connected with the fact that the initial genotype represents a heterogeneous material. At the moment, aneuploidy is the most preferred version of the segregation in the progeny of some DH lines.

Every year, we notice a high frequency of unstable DH lines, named *aberrant doubled haploid* lines, among so-called spontaneous doubled haploids - there might be something in common between these phenomena and we are assuming that that is aneuploidy.

We are expecting that further work will bring us more answers on this topic.

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