Gamma-irradiation of seeds with haploid and diploid embryos

--Rotarenco, VA; Maslobrod, SN; Romanova IM; Mihailov, ME

Haploid plants have recently gained wider utilization in maize breeding programs. This was feasible after highly effective inducers of maternal haploids were discovered (Coe, 1959).

All genes, both dominant and recessive, are expressed at the level of haploid plants due to the absence of the second gene allele. Thus, the use of haploids for induced mutagenesis may allow more efficient identification of mutations. In addition, a higher mutation number may appear at the level of haploid plants in comparison with diploids--the reduction of gene repair efficiency in haploids might be one possible reason.

In order to induce mutations in haploids, their reaction to a mutagenic factor should be evaluated. The aim of this work was to establish the impact of different γ -irradiation doses on seeds with haploid embryos. Both haploid and diploid seeds of the heterogeneous SA population were irradiated. Dry seeds were irradiated at doses of 20, 40, 60, 80 and 100Gy. Soaked seeds (24 hours) were irradiated at doses of 2, 4, 6, 8 and 10Gy. 50 kernels were used in each treatment. Following irradiation, seeds were divided into two replications and grown in a growth chamber for 4 days (28°C). Root (main root) and coleoptile length were measured in seedlings, and the number of roots was counted. The results of the experiment are shown in the Table.

The irradiation of haploid seeds (soaked) resulted in a significant decrease of root length at doses of 4, 6, 8 and 10Gy as compared with control. A similar regularity was found for coleoptile length; however, significant differences were observed only at doses of 6 and 10Gy. This tendency was maintained for the parameter of root number, but the differences were not significant. The dose of 2Gy showed a tendency to stimulation for the three indices mentioned.

The irradiation of haploid dry seeds resulted in a decrease in root length, accompanied by intensification of the inhibiting effect beginning from a dose of 40Gy to 100Gy. As for coleoptile length, a significant difference at a dose of 100Gy was found. A significant reduction was discovered for root number beginning from a dose of 40Gy. A tendency toward stimulation was found at a dose of 20Gy.

The irradiation of diploid seeds (soaked) resulted in a decrease

Table. The parameters of 4-day seedlings after γ -irradiation of seeds

Haploids					Diploids		
#	Dose,	Coleoptile	Root	Number of	Coleoptile	Root	Number of
	Gy	length, mm	length, mm	roots, no.	length, mm	length, mm	roots, no.
Soaked seeds							
1	Control	53.0±4.97	104.4±6.81	3.4±0.23	70.4±4.62	124.6±7.61	3.7±0.27
2	2	60.8±3.97	107.0±3.43	3.6±0.18	73.1±4.36	133.4±6.54	3.9±0.18
3	4	55.0±4.89	75.9**±5.79	3.0±0.22	71.9±3.66	132.1±4.70	4.3±0.18
4	6	42.5*±4.26	66.3***±5.49	3.0±0.22	73.1±5.11	114.6±7.42	3.3±0.20
5	8	53.0±3.26	76.0**±5.94	3.3±0.17	78.0±3.47	118.5±5.43	4.1±0.16
6	10	44.6*±2.92	50.7***±4.42	3.0±0.16	54.2*±5.25	75.3***±7.50	2.8*±0.26
Dry seeds							
1	Control	23.7±2.56	71.3±3.79	3.1±0.15	28.2±2.85	74.3±6.06	3.0±0.19
2	20	29.0±2.83	69.2±3.59	3.4±0.14	38.3±2.70	90.2*±4.58	3.3±0.15
3	40	18.9±2.05	49.1***±3.80	2.7±0.17	31.7±2.43	86.5±5.12	2.9±0.17
4	60	21.9±2.26	40.4***±3.30	2.3***±0.18	34.3±2.99	76.3±5.88	3.0±0.24
5	80	17.6±2.19	41.0***±3.06	2.3***±0.16	29.9*±2.74	79.9±5.23	2.9±0.19
6	100	11 0**+2 00	27 7***+2 62	1 8***+0 16	19.5*+2.23	53 1**+5 06	1 8**+0 16

*, **, *** significant at 5%, 1% and 0.1% level, respectively

in root length beginning at 6Gy; however, a significant difference was observed only at 10Gy. A similar trend towards stimulation at a dose of up to 8Gy, and a significant decrease at 10Gy was found for coleoptile length. A similar regularity was discovered for root number. The tendency towards the stimulating effect was observed up to 4Gy for root length, and up to 8Gy for coleoptile length and root number.

The irradiation of diploid dry seeds yielded an increase in root length, the reliable increase being found at 20Gy and 40Gy. A dose of 100Gy yielded a significant decrease. The tendency towards stimulation was observed at a dose of up to 80Gy for coleoptile length, but significant stimulation was established at a dose of 20Gy. A significant decrease was found at a dose of 100Gy. A significant decrease was observed at 100Gy for root number. Significant differences were not observed in germination rate between the treatments and regardless of the ploidy of the kernels; average germination rate was 95%.

Based on the results obtained, some conclusions can be made: a significant difference in sensitivity to irradiation was recorded in haploids in comparison with diploids; stimulation of seedling growth was found at minimal doses of irradiation in both diploid and haploid seeds, however, the stimulation was insignificant in most cases. This experiment allowed us to identify doses of γ -irradiation that can be employed to induce genetic variation using haploids.