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EFFECTS OF ANTIMITOTIC AGENTS ON HAPLOID PLANT PRODUCTION FROM UNPOLLINATED OVULES OF SUGAR BEET (*Beta vulgaris* L.)

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ABSTRACT
*The effects of antimicrotubule agents on haploid embryo formation from unpollinated ovules of sugar beet (*Beta vulgaris L.*) were investigated. The antimitotic agent colchicine (at 100 and 150 mg/l) and trifluralin (at 5.0 mg/l) increased the frequency of haploid embryo formation whereas pronamide (at 76.9 and 128.2 mg/l) and trifluralin (at 3.4 mg/l) decreased. Ovules that were non-treated with antimicrotuble agents (i.e., ovules of the control treatment) produced higher percentages of haploid embryos (4.25 %) when compared to the pronamide and trifluralin at 3.4 mg/l concentration. Toxic effects of these agents on embryo formation from ovules were evident. A significant genotypic variation among the lines used was observed. The line M4 produced the highest yield with a mean of 14.71% haploid embryo production while the line M2 producing no embryos at all.*

Introduction
Sugar beet (*Beta vulgaris* L.) is one of the major crops in the world and, therefore, the effort on bitechnological developments has been rather intensive. Many different approaches have been suggested for the induction of haploid and dihaploid plants. Classical techniques such as natural polyembryony and crosses between diploid and tetraploid lines or with wild species yielded very low numbers of haploids (1). *In vitro* androgenesis has been attempted numerious times (2) but with very limited success. Gynogenesis, however, has been the most successful method for the production of haploid plants in quantities attractive for breeding purposes although it is more labor intensive than the others (3-8).

Colchicine is known to inhibit mitosis in a wide variety of plant and animal cells by interfering with the structure of the mitotic spindle, but it is highly toxic and often induces chimeric plants (9) and induced embryogenesis. Other antimitotic agents such as trifluralin, oryzalin, pronamide and amiprophosmethyl (APM) have also been tested in sugar beet (8) and their effects were inconsistent in terms of both haploids and doubled haploids. The present study reports the effects of pretreating unpollinated ovules of some sugar beet breeding lines with three antimitotic agents on the production rate of

haploid embryos instead of their chromosome doubling effects.

Materials and Methods

Haploid Plant Production

Ovule explants of 7 diploid male fertile sugar beet families, listed in Table 1, were used for haploid plant production. Plants were grown in the field during the normal season in Ankara (Turkey) and unopened flower buds were excised from bolted plants in June and July, and surface sterilised in 2% NaOCl + 0.03% Tween 20 for 20 min followed by several rinses with sterile distilled water. Ovules were isolated by using dissecting microscope under sterile conditions and cultured on full-strength MS (10) basal salt medium supplemented with 10% sucrose and 0.75% agar (Oxoid No. 3).

For haploid embryo induction, firstly, flower buds were exposed to cold (4 °C) for 5 days in dark conditions and then isolated ovules were cultured on MS medium containing 2.0 mg/l BAP and 0.1 mg/l IBA (**Table 1**). After 15 days of incubation ovules were immersed in liquid medium, all containing colchicine (100 or 150 mg/l), trifluralin (3.4 or 5.0 mg/l) or pronamide (76.9 and 128.2 mg/l) for 5 hours at 27±2 °C. All media were composed of full-strength MS salts and supplemented with 1.0 mg/l BAP and 3% sucrose. Treated ovules were then transferred to an agar-solidified MS medium supplemented with 2.0 mg/l BAP without rinsing. Embryos developed from the cultured ovules were then transferred to MS medium containing 0.5 mg/l BAP and 3% sucrose for further growth. Cultures were incubated at 27±2 °C with a 16-hour photoperiod under warm white fluorescent tubes (40-50 mE m^{-2} s^{-1}). Experiments were repeated three times, and every treatment of each repeat used 15 ovules. Number of ovule explants giving rise to haploid plants was recorded and expressed as the mean % of haploid plants per treatment. For statistical analysis, ANOVA and Duncan's Multiple Range Test were carried out (**Table 2**).

Ploidy Determination

Young leaves of 45-50 days old shoots were soaked in 8-hydroxyquinoline (0.002 mol/l) for 4 h followed by several rinses with tap

TABLE 1

Main characteristic of sugar beet (*Beta vulgaris* L.) breeding lines used

Lines	Main Characteristics
M1	Diploid monogerm, medium root yield, medium sugar yield
M2	Diploid monogerm, medium root yield, high sugar yield
M3	Diploid monogerm, high root yield, medium sugar yield
M4	Diploid monogerm, medium root yield, medium sugar yield
M5	Diploid monogerm, medium root yield, medium sugar yield
E107	Diploid monogerm, high root yield, high sugar yield
E113	Diploid monogerm, medium root yield, high sugar yield

TABLE 2

Analysis of variance of haploid embryo production from unpollinated ovules of six sugar beet breeding lines (M1, M2, M3, M4, M5, E107 and E113) treated with three antimitotic agents (colchicine, trifluralin or pronamide)

Source of Variation	D.F.	Sum of Squares	Mean Square	F Value
Replication	2	461,775	230,887	13,7776**
Treatment (A)	6	348,070	58,012	3,4617*
Error	12	201,099	16,758	-
Genotype (B)	6	3347,315	557,886	12,3101**
A X B	36	2412,105	67,003	1,4785
Error	84	3806,820	45,319	-

water. Treated leaves were then fixed in a 2:1 fixative solution (two parts 96% ethanol : one part HCl) for 15 minutes. The leaves were washed thoroughly with tap water to remove the fixative and kept in distilled water at room temperature. A small piece of the leaf tissue was excised and placed on a glass slide. After applying a drop of 3% aceto orcein, the tissue was squashed before counting chromosomes under a light microscope.

Results and Discussion

Certain treatments applied to ovules, plants or plant parts from which ovule explants have been taken prior to culture may have a strong influence on embryo induction (11). When the overall means were taken into account, it was observed that treating ovules with colchicine at 150 mg/l produced more embryos (5.48 %) than with 100 mg/l colchicine (4.45 %) although the difference was not significant (**Table 3**). This result is in contrast to the findings of Hansen et al. (6) who reported that colchicine was toxic with prolonged periods of treatment. Treating ovules with trifluraline at 5.0 mg/l also produced more embryos (4.98 %) than with 3.4 mg/l (1.91%). On the other hand, treating ovules with pronamide (at concentrations of 76.9 or 128.2 mg/l) decreased embryo formation (1.06% and 3.1%) from ovules when compared to the control treatment (i.e., no antimitotic agents applied). Our results are partially in agreement with those of Hansen et al. (8) who observed that the oryzalin and trifluralin had more toxic effects, which reduced the embryo formation in sugar beet ovules, than pronamide.

Genotypic variation was also evident in the experiments (Table 3). The highest embryo yield was obtained from the line M4 (14.71%) followed by the lines E107 (4.21%) and E113 (3.81%). Line M5 did not produce embryos at all. On the other hand, colchicine, trifluraline and pronamide inhibited embryo production completely from ovules in the line M1 when compared to the control treatment which produced a mean of 4.8% embryo. The highest percentage of haploid embryos production (30.9%) was obtained from the line M4 with the application of colchi-

TABLE 3

Effects of different breeding lines and concentrations of different antimitotic agents on % haploid plant production from unpollinated ovules sugar beet

		Antimitotic Agents (mg/l)						
		Colchicine		Trifluralin		Pronamide		
Lines	Control	50	100	3.4	5.0	76.9	128.2	Means
M1	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.68 b
M2	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.37 b
M3	8.9	3.0	0.0	0.0	0.0	0.0	0.0	1.70 b
M4	5.2	23.6	30.9	6.7	18.2	7.4	11.1	14.71 a
M5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00 b
E107	6.9	0.0	0.0	0.0	14.1	0.0	8.3	4.21 b
E113	5.3	5.0	7.4	6.7	0.0	0.0	2.2	3.81 b
Means	4.45 ab	4.51 ab	5.48 a	1.91 bc	4.98 a	1.06 c	3.10 abc	

cine at 150 mg/l. Genotypic variation has been recognised as a serious problem not only with ovule cultures (5, 7, 12) but also with other tissue cultures of sugar beet (13, 14).

Antimicrotubule herbicides including trifluralin are regarded as an effective alternative to colchicine for chromosome doubling in many species (8, 15-18). Treatments of ovules with colchicine, trifluraline and pronamide did not induce spontaneous chromosome doubling in ovules of sugar beet for all lines used. In contrast to our findings, Hansen et al. (8) demonstrated that different antimitotic agents (amiprophosmethyl, pronamide, trifluralin and oryzalin) had an inducing effect on doubled haploid plant production from sugar beet ovules. To obatin higher rates of embryo induction and/or spontaneous double haploid plant production, further refinements of the treatments would be necessary.

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