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Ratios of Phenotypes at the Adh1 Locus in the Apozygotic Offspring in Sugarbeet (C_1 Generation)

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Phenotypic class frequencies for alcohol dehydrogenase (ADH) isozyme patterns yielded by experimental and control sugarbeet seeds produced through agamospermy were analysed. Experimental apozygotic seeds were obtained from C_0 plants treated with 0.1% colchicine (C) during seed germination. The controls were obtained from untreated plants. The numbers of phenotypic classes and their ratios in the offspring of control and colchicine treated sugarbeet plants provided evidence indicating that the main mode of agamospermous reproduction is sporophytic in the studied sugarbeet hybrid. Two of the 7 control offspring showed a 3:8:3 phenotypic ratio characteristic of gametophytic agamospermy and the experimental group did not show it. Based on the obtained results it is suggested that colchicine treatment increases the proportion of cells with high level of chromosome endoreduplication providing of embriogenesis through sporophytic agamospermy.

KEY WORDS : Epigenetics, isozymes, endoreduplication, agamospermy, colchicine, sugarbeet

INTRODUCTION

Studies performed on sugarbeet demonstrated that isozymes are good genetic markers providing a better understanding of the modes of agamospermic reproduction. Thus, isozymes are efficient tools in identification of sporophytic agamospermy in higher plants (Levites et al., 1998), and characters that allowed us to assume that sugarbeet may also reproduce by gametophytic agamospermy (Maletskii and Maletskaya, 1996; Maletskii et al., 1997; 1998). According to the hypothesis of Maletskii and Maletskaya (1996), plant tissue mixoploidy underlies gametophytic agamospermy, i.e., the presence of tetraploid cell admixtures among the bulk of diploid cells. The existence of mixoploidy was demonstrated in many plant species (D'Amato, 1985). Reduction division of admixed tetraploid cells results in the formation of diploid embryo sac cells capable of parthenogenetic development.

The 3:8:3 ratio of phenotypic classes observed in agamospermous offspring not different from the one in tetraploid gametes resulting from meiosis and chromatid segregation mode support the idea that tissue mixoploidy is the basis of gametophytic agamospermy (Maletskii, 1997; Maletskii et al., 1998). Another support came from the more than 2.5-fold larger average DNA mass in nuclei of sugarbeet line SOAN-41 (tending to agamospermy) than the one of line SOAN-210 (tending to gamospermy) (Maletskaya and Maletskaya, 1999).

Acceptance of Maletskii's hypothesis raises the question of how colchicine treatment, giving rise to the formation of polyploid cells, may affect autosegregation pattern in agamospermous offspring.

Taking the above in consideration, the aim of this study was to analyse ratios of phenotypes for isozyme patterns in the agamospermous C_1 offspring of sugarbeet.

MATERIALS AND METHODS

Seeds obtained through agamospermy from sterile F_1 Klein Wanzleben (msKW) hybrid sugarbeet plants were used. The experimental seeds were obtained from C_0 plants which were grown from seeds treated with colchicine (C) at the germination stage (Maletskii, 1999). The experimental seed batch was soaked in 0.1% C solution according to Savitsky's

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method (1966), the control seeds were soaked in running water. After C exposure, the seeds were germinated in a thermostat (at 29°C) on moist filter paper, then sown in the field. The plants were grown all summer. In autumn, the plants were harvested and stored in cellars to vernalization. In spring, the roots were planted in an isolated plot; the fertile plants were discarded during early flowering, and only pollenless plants of the ms0 and ms1 phenotypes according to Owen's classification (1942) were left on the plot. Offspring seeds were taken individually from each plant.

Alcohol dehydrogenase (ADH; E.C.1.1.1.1.) was used as marker enzyme. ADH isozyme patterns were analysed by applying extracts from individual seeds into the starch gel. Electrophoretic separation and histochemical staining were performed according to the standard methods (Meizel and Markert, 1967; Levites, 1985). The conformance of the observed phenotype frequencies to those expected was determined by the χ^2 test.

RESULTS AND DISCUSSION

Three types of ADH patterns were distinguished in apozygotic sugarbeet seed offspring, similar to those observed in the zygotic ones resulting from fusion of male and female gametes (Figure 1). Single-banded ADH patterns designated as FF and SS phyenotypes corresponded to the fast (FF) or slow (SS) variants of electrophoretic mobility. Such electrophoretic patterns are characteristic of Adh1-F/Adh1-F (FF) and Adh1-S/Adh1-S (SS) homozygotes, as well as of seeds of Adh1-F/Adh1-0 (F0) and Adh1-S/Adh1-0 (S0) genotypes whose Adh1-0 allele is either the inactivated Adh1-F or Adh1-S allele. The three-banded ADH pattern, designated as FS phenotype, is characteristic of Adh1-F/Adh1-S heterozygotes.

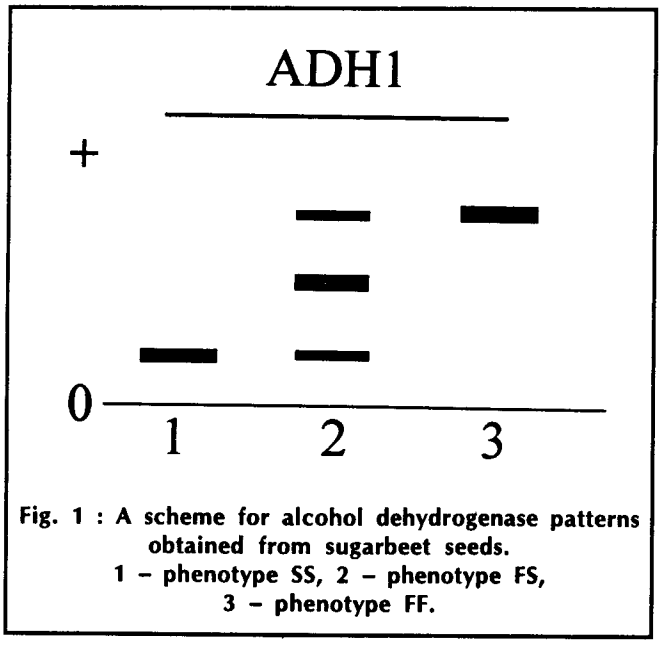

Fig. 1 : A scheme for alcohol dehydrogenase patterns obtained from sugarbeet seeds.
1 – phenotype SS, 2 – phenotype FS,
3 – phenotype FF.

Among the agamospermous offspring of control and experimental plants, along with seeds showing the active enzymes, there were also seeds with null ADH activity. Their phenotypes were designated as "00".

To simplify the analysis, all the agamospermous offspring was subdivided into groups A,B,C, D and E depending of the number of detected phenotypic classes showing the active ADH (see Tables 1 and 2). Group A is composed of offspring lacking phenotypes FS and SS. Group B consists of offspring lacking phenotype SS. Offspring whose seeds lacked phenotype FF was referred to group C. Group D included offspring with phenotypes FF, FS and SS. Offspring in which a single phenotype class FS was identified was referred to group E.

The ratios of type A are almost the same in control and C_1 offspring; the presence of a single FF phenotypic class suggested that the starting maternal plants were homozygous for the Adh1-F allele. The B type ratios in offspring of control and C-treated plants do not differ significantly, when estimated on the basis of all, including null, phenotypes (χ^2 = 0.1774; $P>0.05$) and also excluding them (χ^2 = 0.0043; $P>0.05$).

The C type ratios are very close in offspring of the control and C-treated plants when the null phenotypes are included in comparisons (X = 4.3041; $P>0.05$). However, this ratio type becomes significantly different in offspring of the control and C-treated plants, when their estimates are based only on FS and SS phenotypes (χ^2 = 4.3039; P 0.05).

Both B and C type ratios are quite similar to the ratios previously demonstrated for offspring resulting from sporophytic agamospermy (Levites et al., 1998). In such a case, polymorphism is due to epigenetic variability; which is the result of the inactivation of one of the alleles at the heterozygous enzyme locus. Polymorphism of this kind we designated as

Table - 1 : Ratios of phenotypes at the Adh1 locus in apozygotic offspring in control sugarbeet hybrid plants ms Klein Wanzleben

Plant number	Phenotypic classes				$\chi^2_{(3:8:3)}$	Offspring ratio type
	FF(F0)	FS	SS(S0)	00	(FF:FS:SS)	
msKW – 21	9	-	-	1	32.97	A
msKW – 35	105	107	-	13	123.99	B
msKW – 11	-	21	2	7	11.37	C
msKW – 13	-	79	8	18	41.97	C
msKW – 30	26	44	13	2	5.33	D
msKW – 34	22	44	13	1	2.46	D
msKW – 33	15	20	3	2	9.16	D

pseudosegregation (Levites et al., 1998). The finding of B and C type ratios in the control and C-treated offspring, as well as their similarity, is an evidence to indicate that C-treatment to does not affect the mechanism of epigenetic variability in plants reproducing by the agamospermous mode. If epigenetic variability were absent in sporophytic agamospermy, the offspring of FS heterozygotes would consist of only FS heterzygotes. SS(S0) phenotypes can arise only as a result of the F-allele inactivation, while FF(F0) phenotypes are the result of the inactivation of the S-allele. For this reason, when designating phenotypes, it is implied that phenotypic FF and SS classes, in some offspring may be represented by F0 and S0 genotypes, respectively. The presence of phenotypes with null ADH activity evidences that both alleles of this enzyme marker gene may become inactivated (Table 1 and 2). In this particular case, absence of ADH activity does not appear to be due to insufficient maturity of seeds. In fact, ADH activity was analysed only in well-filled seeds and ADH is one of the enzymes with very high activity in seeds (Schwartz, 1996). It was also observed that seeds with null ADH activity show

Table - 2 : Ratios of phenotypes at the Adh1 locus in apozygotic offspring (C_1) of colchicine treated (C_0) sugarbeet hybrid plants ms Klein Wanzleben.

Plant number	Phenotypic classes				$\chi^2_{(3:8:3)}$	Offspring ratio type
	FF(F0)	FS	SS(S0)	00	(FF:FS:SS)	
msKW – 90c	10	-	-	-	36.72	A
msKW – 88c	25	26	-	4	29.44	B
msKW – 11c	-	101	23	28	39.87	C
msKW – 1c	69	115	23	16	24.06	D
msKW – 7c	12	2	1	5	30.63	D
mKW – 9c	34	49	10	17	15.20	D
msKW – 22c	19	19	3	7	16.51	D
msKW – 44c	50	47	3	5	55.74	D
msKW – 63c	27	22	4	2	28.57	D
msKW – 77c	-	10	-	-	7.5	E

normal activity of another enzyme (malate dehydrogenase) we used as an additional marker to evaluate the physiological state of seeds.

In D type ratios, where all the phenotypic classes are maximally represented (with the active and inactive forms), the manifestation of the C-effect is more diverse. The 3:8:3 ratio of the FF, FS and SS phenotypic classes in control msKW-30 and msKW-34 offspring is an argument in favour of the existence of autosegregation in agamospermous offspring caused by meiosis. This suggested that such offspring were represented by seeds developed directly from eggs without the involvement of male gametes. Phenotypic ratio in msKW-33 offspring of the control group is at variance from the 3:8:3 ratio. It cannot be excluded that this offspring arose through sprophytic agamospermy associated with an inactivation of not a particular allele, but by an inactivation of either Adh1-F or Adh1-S. The same may be suggested for the offspring msKW-1c, msKW-7c, msKW-9c, msKW-22c, msKw-44c and msKW-63c of the experimental plant group.

The E type ratio found only in the experimental group (msKW-77c) indicating the absence of polymorphism and uniformity for the heterozygous phenotype is evidence that this offspring also arose through sporophytic agamospermy.

Thus, in the control group, of the 6 polymorphic offsprings, 4 may be classified as during arisen through sporophytic agamospermy, while in the experimental group, all the polymorphic offspring arose through it. There is a reason to conclude that this is the main mode of agamospermous reproduction is sporophytic in the sugarbeet hybrid studied.

This conclusion raises the question of why there arose in the control group two offsprings in which a phenotypic ratio conforming to the one expected in the case of gametopytic agamospermy according to the Maletskii's and Maletskaya's hypothesis (1996). There are, as yet, no grounds for refuting the possibility that the initial plants from which the offspring derived were capable of reproduction through gametophytic agamospermy. However, any phenotype class ratios may be assumed in the sporophytic offspring, even the one close to 3:8:3 which is not the case with gametophytic mode. The assumption is acceptable, when one considers epigenetic variability in agamospermous offspring manifests as inactivation of any one allele at the Adh1 locus.

Thus, the current results demonstrated that a phenotypic class ratio in all the polymorphic offspring of colchicine-treated plants conforms to the one in offspring arisen through sporophytic agamospermy, i.e., the percentage of offspring showing the distinctive features of sporophytic agamospermy reached 100%. Other observations are relevant:1) the existence of cells in mixoploid tissues having chromosomes with high level of endoreduplication (4, 8 and more chromatids) in many higher plants species (D'Amato, 1985); 2) the increase in cell size in the C-treated plants (Maletskii, 1999) and 3) the higher values of the DNA mass in cell nuclei in the line tending to agamospermy (Maletskaya and Maletskaya, 1999). These observations justify the suggestion that C-treatment causes an increase in the proportion of germinative tissue cells having a high level of the endoreduplication of chromosomes and, as a consequence, capable of embryogenesis through sporophytic agamospermy.

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