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CYTOGENETIC INSTABILITY AMONG THE VEGETATIVE PROGENIES OF *A. REPENS CV. PURDUE* SOMACLONES

Tárczy H. M.[1], G. Gyulai[2], J. Janovszky[3], E. Kiss[2], L. E. Heszky[2]

(1) R. F. Research Institute, Agricultural University of Gödöllő, Kompolt, Hungary, H-3356. (2) Department of Genetics and Plant Breeding, Agricultural University of Gödöllő, Hungary, H-2103. (3) Irrigation Research Institute, Szarvas, Hungary, H-5540.

SUMMARY

The distribution of mitotic chromosome numbers and changes in the meiotic chromosome pairing in the vegetative progenies of *Agropyron repens* cv. Purdue somaclones were analyzed. Mitotic chromosome variability showed an increase at both cell (SC1-3 somaclones) and plant (SC2) level. Nevertheless, from the progenies of the somaclone SC2, a stable hipoploid clone with a chromosome number of 2n = 38 was selected. The numbers of aneuploid cells in the root tips of vegetative clones also showed an increase. In the analysis of meiotic chromosome pairing, a shift towards the appearance of multivalents (quadrivalents, hexavalents, octovalents) was observed.

Key words: somaclone, vegetative progeny, mitosis, meiosis, homogenity test

Abbreviations: Agr. - *Agropyron repens cv.* Purdue; SC1-11 - somaclones of *Agropyron repens cv.* Purdue from 1 to 11.

INTRODUCTION

The discovery of variability arising from cell and tissue cultures has led to a series of new sources of variation such as in vitro mutants (Malone and Dix 1986), somatic hybrids (Melchers et al. 1978), somaclones (Heszky et al. 1989), protoclones (Nagata and Takabe 1971), cybrids (Dudits et al. 1987), stable recombinants (Aviv and Galun 1980; Medgyesi et al. 1985), and even new types of gametoclones (Guha and Maheswari 1964). In tissue culture regenerants, a full range of karyotypic variations were observed including ploidy changes, aneuploidy, deletions, duplications, inversions, translocations. The reasons of this chromosome instability were supposed to be both genetic instability of initial plants and the *in vitro* cultivation processes of callus cultures (Larkin and Scowcroft 1981; D'Amato 1985; Ling 1987; Geier 1991). The analysis of vegetative progenies of the tissue culture regenerants showed a further change in the numbers of chromosomes (D'Amato 1985; Fehér et al. 1989; Larkin et al. 1989). In the present study, the mitotic and meiotic behaviour of the vegetative progenies of *A. repens* cv. Purdue somaclones is presented.

MATERIALS AND METHODS

Plant materials: Somaclones from new genotype *A. repens cv.* Purdue with partial sterility (a breeding material of Janovszky 1988) were initiated from callus cultures of young inflorescence origin (Gyulai et al. 1992). The vegetative progenies of eleven vigorous somaclones (SC_{1-11}) were investigated . Somaclones were propagated *in vitro* on hormone free F-medium (Gyulai et al. 1992). The entries of the tested progenies were as follows. For mitosis: *A. repens cv.* Purdue as control: 20 plants; vegetative progenies of somaclones SC_1 : 15 plants; vegetative progenies of somaclones SC_2 : 17 plants; vegetative progenies of somaclones SC_3 : 18 plants; vegetative progenies of somaclones SC_4 : 14 plants; vegetative progenies of somaclones SC_5 : 23 plants; vegetative progenies of somaclones SC_6 : 11 plants. For meosis: vegetative progenies of *A. repens cv.* Purdue as control, and the vegetative progenies of SC_{7-11} somaclones, one plant per somaclone, and at least three florets per plant.

Analysis of mitosis: Root tips were pretreated at 2 °C for 24 h, fixed in Carnoy fixative for 24 h, and stained in aceto-carmine solution (4 %) for 48 h (Hangyelné et al. 1986). The number of investigated cells was 100 in each case of somaclone. An estimation of the stability of the chromosome number was performed at both cell and plant level. At plant level, plants with at least five metaphases were considered for statistical analysis. A homogenity test (comparison of more than two empirical frequency distributions with two classes) was performed using the method of Svab (1981). The two classes were created as normal (2n = 42), and abnormal (2n = 35,38,40,41,49). Chi^2 values were counted according to Svab (1981).

Analysis of meiosis: Florets were fixed in Carnoy fixative and stained in 4 % acetocarmine. Meiotic chromosomes of at least three florets were investigated under magnification of 1000 x.

RESULTS

Variation in mitotic chromosome numbers at cell and plant level: The homogenity test of mitotic chromosome numbers at cell level *(Table 1)* showed significant differences between the investigated groups. Two groups were separated: (1) Agr., SC4, SC5 and SC6; (2) SC1, SC2 and SC3.

Table 1. Distribution of mitotic chromosome numbers of the new genotype of *Agropyron repens* cv. Purdue (Agr.) and in the vegetative progenies of its somaclones (SC1-6) at cell level (100 cells per plant group)

Chr. No.	Agr.	SC_1	SC_2	SC_3	SC_4	SC_5	SC_6
	%						
35	3.0	15.0	14.0	4.0	4.0	8.0	4.0
38	6.0	12.0	20.0	23.0	18.0	4.0	-
40	11.0	15.0	20.0	20.0	11.0	21.0	18.0
41	10.0	-	-	-	-	-	-
42	70.0	54.0	46.0	53.0	67.0	67.0	78.0
49	-	4.0	-	-	-	-	-

Chi^2= 32.8 $P_{0.1\%}$ = 22.5

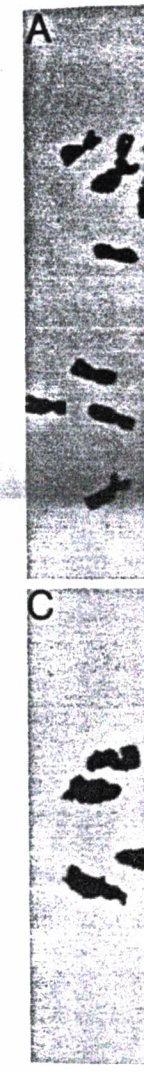

Fig.1. **(A)**: Mitot
(2n=42). **(B)**: Di
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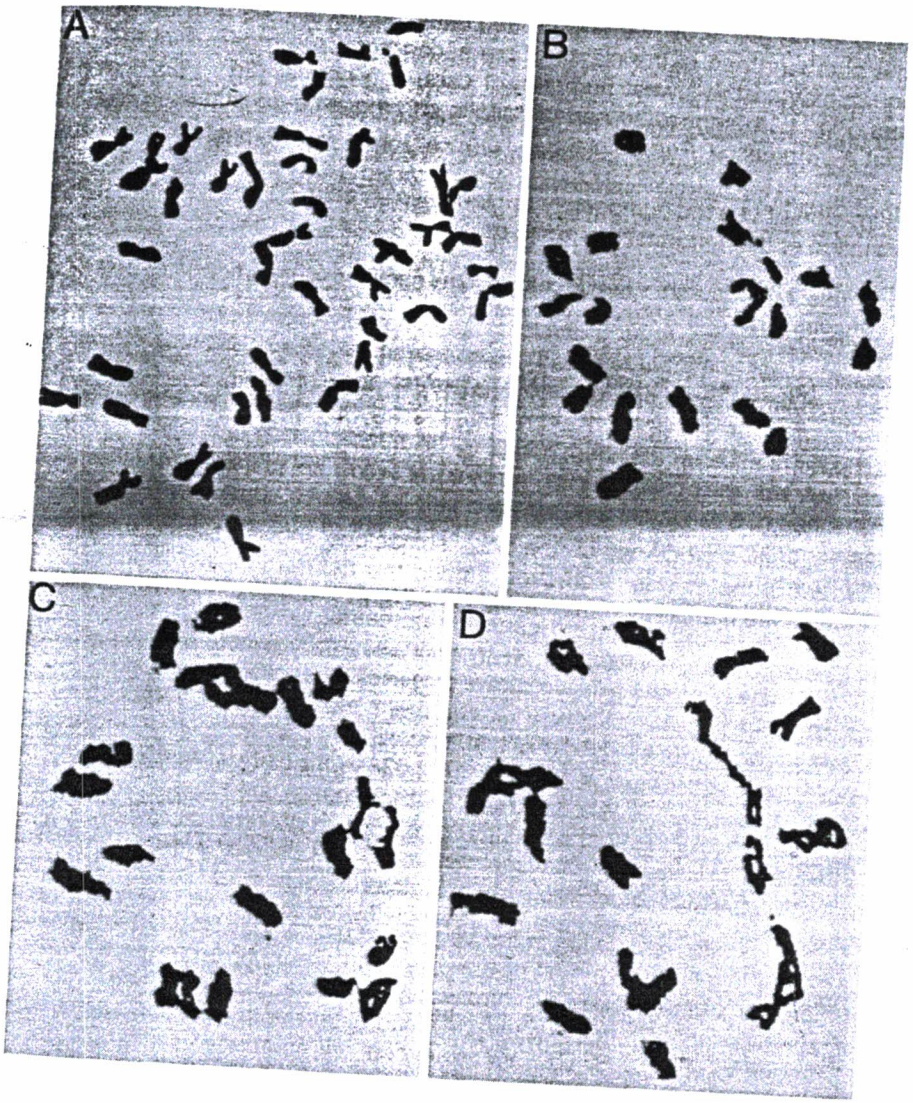

Fig.1. **(A)**: Mitotic chromosome set of the initial plant of *Agropyron repens cv. Purdue* (2n=42). **(B)**: Diakinesis in the vegetative progeny of the somaclone SC8 of *Agropyron repens cv. Purdue* (21 bivalents). **(C)**: Diakinesis in the vegetaive progeny of the somaclone SC10 of *Agropyron repens cv. Purdue*(15 bivalents and 3 quadrivalents). **(D)**: Diakinesis in the vegetative progeny of the somaclone SC11 of *Agropyron repens cv. Purdue* (13 bivalents, 2 quadrivalents, and 1 octovalent)

Meristems in the plants of the first group contained fewer cells with abnormal chromosome numbers than in the plants of the second group. Progenies of the SC2 somaclone contained the lowest numbers of cells with normal (2n = 42) chromosome numbers. Fig. 1. A. shows the normal mitotic chromosome constitution (2n = 42) of the initial *A. repens cv.* Purdue plant.

The estimation of genetic homogenity at plant level (Table 2.) was performed after the determination of the chromosome numbers of each plant.

Table 2. Distribution of mitotic chromosome numbers of of *Agropyron repens* cv. Purdue (Agr.) and in the vegetative progenies of its somaclones (SC7-1-6)

Chr. No.	Agr.		SC_1		SC_2		SC_3		SC_4		SC_5		SC_6	
	No.	%	No.	%	No.	%	No	%	No	%	No.	%	No.	%
2n=42	17	85	7	70	3	23	8	73	5	71	10	77	6	75
mixoploid	3	15	3	30	9	69	3	27	2	29	3	23	2	25
2n=38	-	-	-	-	1	8	-	-	-	-	-	-	-	-
Plants total	20	100	10	100	13	100	11	100	7	100	13	100	8	100

Chi^2=15.6 $P_{(2.5\%)}$ =14.4

Only the vegetative progenies of the SC2 somaclone showed significant differences from the rest of the entries. Moreover, among the SC2 progenies, a hipoploid clone with chromosome numbers of 2n = 38 was selected.

Table 3. Meiotic chromosome pairing in the new genotype of *Agropyron repens* cv. Purdue (Agr.) and in the vegetative progenies of its somaclones (SC7-11)

Plants	I	II	III	IV	VI	VII	VIII	X	XII	XIV	Cells
Agr.	1.3	16.3	0.6	0.4	0.7	-	-	-	-	-	7
SC_7	0.06	6.2	0.06	0.67	0.20	2.02	0.08	0.10	0.06	0.02	49
SC_8	0.7	14.3	0.14	1.14	0.71	-	-	-	-	-	7
SC_9	-	8.42	-	0.58	0.19	0.08	0.15	0.04	0.04	0.08	26
SC_{10}	0.03	7.77	0.03	0.83	0.20	-	0.13	0.07	0.03	-	30
SC_{11}	0.13	12.3	0.5	1.5	0.38	-	0.63	-	0.25	-	8

Meiotic chromosome association in the vegetative progenies: The frequencies of univalents (I) and trivalents (III) in all of the examined vegetative progenies were lower than in the control of *A. repens cv.* Purdue plants (Table 3). Furthermore, the appearance of quadrivalents (IV), hexavalents (VI), and oktavalents (VII) was observed in the SC7-11 progenies (Fig. 1. B -D). Low examined cell number of certain plants (Agr., SC_8, SC_{11}) pointed to their sterility.

DISCUSSION

Numerical and structural changes in the chromosome constitutions of tissue culture regenerants are the main sources of somaclonal variation in higher plants D'Amato 1985; Fehér et al.1989; Larkin et al. 1989; Geier 1991). 306 papers were studied by Geier (1991), of these 295 mentioned numerical- (polyploidy, aneuploidy), 95 structural changes.

er cells with abnormal chro-
Progenies of the SC2 so-
nal (2n = 42) chromosome
e constitution (2n = 42) of

2.) was performed after the

Agropyron repens cv.
ones (SC7-1-6)

	SC_5		SC_6	
%	No.	%	No.	%
1	10	77	6	75
9	3	23	2	25
	-	-	-	-
0	13	100	8	100

$P_{(2.5\%)}$ =14.4

ed significant differences
genies, a hipoploid clone

Agropyron repens
clones (SC7-11)

XII	XIV	Cells
-	-	7
0.06	0.02	49
-	-	7
0.04	0.08	26
0.03	-	30
0.25	-	8

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In our experiment, progenies of *A. repens cv.* Purdue somaclones also showed deviations from the original plant. Unlike these results, variation among barley somaclones (Singh 1986) and among the hybrid somaclones of *Triticum aestivum* x *Agropyron desertorum* (Li et al. 1994) has not been found. It has been concluded that somaclonal variation primarily depends on the genetic backround of the donor plants which can be classified in two groups: (1) the polysomatic (aneusomatic), and (2) the non-polysomatic plant species (D'Amato 1985). As the present results show, *A. repens cv.* Purdue belongs to the polysomatic group.

The study of Eizenga and Dahleen (1990) also suggests the importance of genotype. Nature and extent of chromosome variability depends on a complex network of interacting exogenous and endogenous factors: pre-existing variability, callus initiation and subculture, and plant regeneration (Fehér et al. 1989). Nevertheless, the mixoploidy of somaclones might be temporary and can go on a terminal stage of a stable chromosome set (Geier 1991).

At plant level, the genetic instability of apical meristems results in chromosome mosaicism which is manifested in the variance of vegetative clones, as was also observed in the present study. Moreover, this chromosome instability can be transmitted into meiotic abnormalities due to the transition of the shoot meristem from vegetative to generative phase (D'Amato 1985). In cases of monocot plants, the role of environmental factors (either *in vitro* or *in vivo*) on chromosome instability should also be considered, as was proved by Creemers-Molenaar et al. (1988). In addition, certain biological phenomena are actually dependent upon programmed or rapid genomic changes in the genome (Chen 1993).

In the present study, a stable aneuploid clone with 2n=38 was selected from the SC2 progenies. Similar cases of the stabilization of aneuploid chromosome sets were also reported in tobacco (Ogura 1978) and in alfalfa (Groose and Bingham 1984; Fehér et al. 1989). The genetic reason for these kinds of stabilization was suggested by the effect of variator genes (Ogura 1978).

Deviations were revealed in rice somaclones during meiosis (Ling 1987). The appearance of multivalents like tetravalents, hexavalents, octovalents and decavalents was frequent. Barley tetraploid somaclones showed 3 to 7 quadrivalents at metaphase I. and imbalanced chromosome segregation at anaphase (Ahloowalia 1987). Genomic rearrangements were reported in maize induced in tissue culture (Lee and Phillips 1986). Enviromental factors might also play an important role in triggering genomic rearrangement. An increase in meiotic instability somaclones compared to the initial plant of tall fescue was also observed (Eizenga 1989; Dahleen and Eisenga 1990). In our experiments, the types of meiotic chromosome configurations showed a shift towards multivalents (quadrivalents, hexavalents, and oktavalents). The higher level of multivalents suggests a higher frequency of chiasma and crossing-overs, which can obviously result in the further development of new *A. repens* genotypes.

To conclude, all of the observations presented prove the usage of subsequent selection work for new genotypes among either the vegetative (see Table 1-2) or generative (Table 3) progenies of *A. repens cv.* somaclones.

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