Pl. Syst. Evol. 192: 41–48 (1994)

Plant Systematics and Evolution

Chromosome doubling in *Turnera ulmifolia* (*Turneraceae*) induced by regeneration of plants from in vitro cultured leaf explants

GRACIELA LAVIA, AVELIANO FERNANDEZ, and GISELA MARQUEZ

Received September 24, 1993; in revised version March 14, 1994

Key words: *Turneraceae, Turnera ulmifolia.* – Tissue culture, meiotic, pairing, polyploidy.

Abstract: Plants were regenerated from cultured excised leaf segments of *Turnera ulmifolia* (2n = 6x = 30). Cytological studies have demonstrated that chromosome doubling occurred in 100% of the regenerated plants. Probably it was produced by endomitosis, induced by excess of auxins in relation to cytokinins. High bivalent and low quadrivalent frequency, and univalents to octovalents were observed in metaphase I; lagging chromosomes were also found. Probably, the presence of multivalents may be due to the pairing among homoeologous chromosomes because the mother plant is a segmental allohexaploid. The high bivalent frequency may have been caused by preferential pairing of identical chromosomes against the homologous.

Chromosome instability can occur in plant cells cultured as callus or cell suspensions. Early studies in tobacco showed that populations of cultured cells were a heterogenous mixture, often with high incidences of aneuploidy, polyploidy, and structural rearrangements (MURASHIGE & NAKANO 1967, WINFIELD & al. 1993). Further, in vitro chromosome instability was recognized as a general phenomenon of plant cell cultures (JACOBSEN 1981, MCCOY & al. 1982, KARP & al. 1984, LARKIN 1987, ORTON 1987, KARP 1988, STELLY & al. 1989, LISCUM III & HANGARTER 1991, HANG & BREGITZER 1993).

Turnera ulmifolia L. sensu stricto (*Turneraceae*) is a woody neotropic perennial plant with 2n = 6x = 30 (FERNANDEZ 1987).

Tissue culture of *T. ulmifolia* was attempted and the regenerated plants obtained were more vigorous than the mother plant but with a low percentage of pollen viability. Cytogenetic studies were carried out to understand the meiotic behaviour and the causes for low viability. The stomata size variation was also considered.

Material and methods

Turnera ulmifolia, Arbo 2698, was raised from seeds originally collected in Miami and cultivated at the garden of Instituto de Botánica del Nordeste. A voucher specimen is deposited in the Herbarium of the Instituto de Botánica del Nordeste (CTES).

Mature leaves were cut in two halves and the distal ones were cultured in basal medium of MURASHIGE & SKOOG (1962; MS) supplemented with NAA 3 mg/l and KIN 1 mg/l solidified with 0.8% agar for callus induction. Calli were transferred to MS supplemented with NAA 0.01 mg/l and BAP 1 mg/l for shoot regeneration. Obtained shoots were transferred to MS plus NAA 0.1 mg/l for root initiation and finally transplanted in soil.

Cytological studies were carried out using root-tip cells, obtained from shoot cuttings, placed in tap water for root initiation. The roots were pretreated for 3–4 h in 0.002 M 8-hydroxyquinoline at room temperature, fixed in 3 : 1 absolute alcohol : glacial acetic acid at 4 °C and then stored in 70% ethanol. The roots were hydrolysed in 1 N HCl at 60 °C for 8 min, stained with the Feulgen technique, and squashed in 2% acetic orcein. Karyotypes were arranged in groups according to the position of the centromere, two chromosome types are recognized: metacentric = m (centromeric index 50–37.5) and submetacentric = sm (CI 37.5–25).

Meiosis was examined in pollen mother cells (PMC) from buds fixed in 5 : 1 absolute alcohol : lactic acid. Hydrolysis and staining were done as described for mitotic preparations.

Pollen viability was estimated by staining mature pollen grains with 1% acetocarmine-glycerine. For each tested plant about 500 pollen grains were examined.

For stomata observation the DIZEO DE STRITTMATTER (1973) technique was used. The fresh leaves were boiled in 96% ethanol for 10 min, then boiled again in 50% (v/v), 96% alcohol, and 5% sodium hydroxide solution for 4 min. Subsequently, the leaves were rinsed separately several times in running water and twice in distilled water. Then, they were immersed in 50% sodium hypochlorite until they become translucent. The leaves were then washed in distilled water five times and transferred to 5% chloral hydrate solution for 10 min. Finally, small pieces of each leaf were separately mounted in water.

Results

From the explants 100% callus induction was obtained with an adequate growth rate. The calli were green with white dots, compact, and rough. Nine regenerated plants were obtained, but only five survived and were designated as U 17–20, U 22.

These five plants show more vigour, with larger leaves and flowers (Fig. 1), than the hexaploid progenitor (2n = 6x = 30). A similar result was reported by KUMAR & WALTON (1992) with a hybrid of *Elymus canadensis* × *E. trachycaulus*.

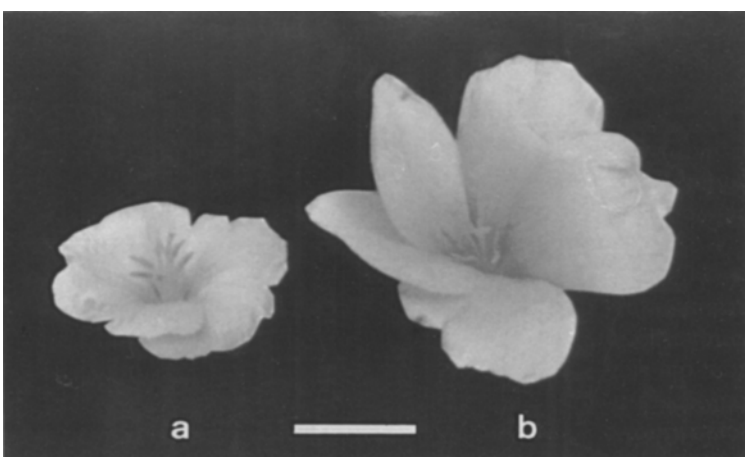

Fig. 1. *Turnera ulmifolia*. Flowers. *a* Mother plant, *b* regenerated plant. – Bar: 2 cm

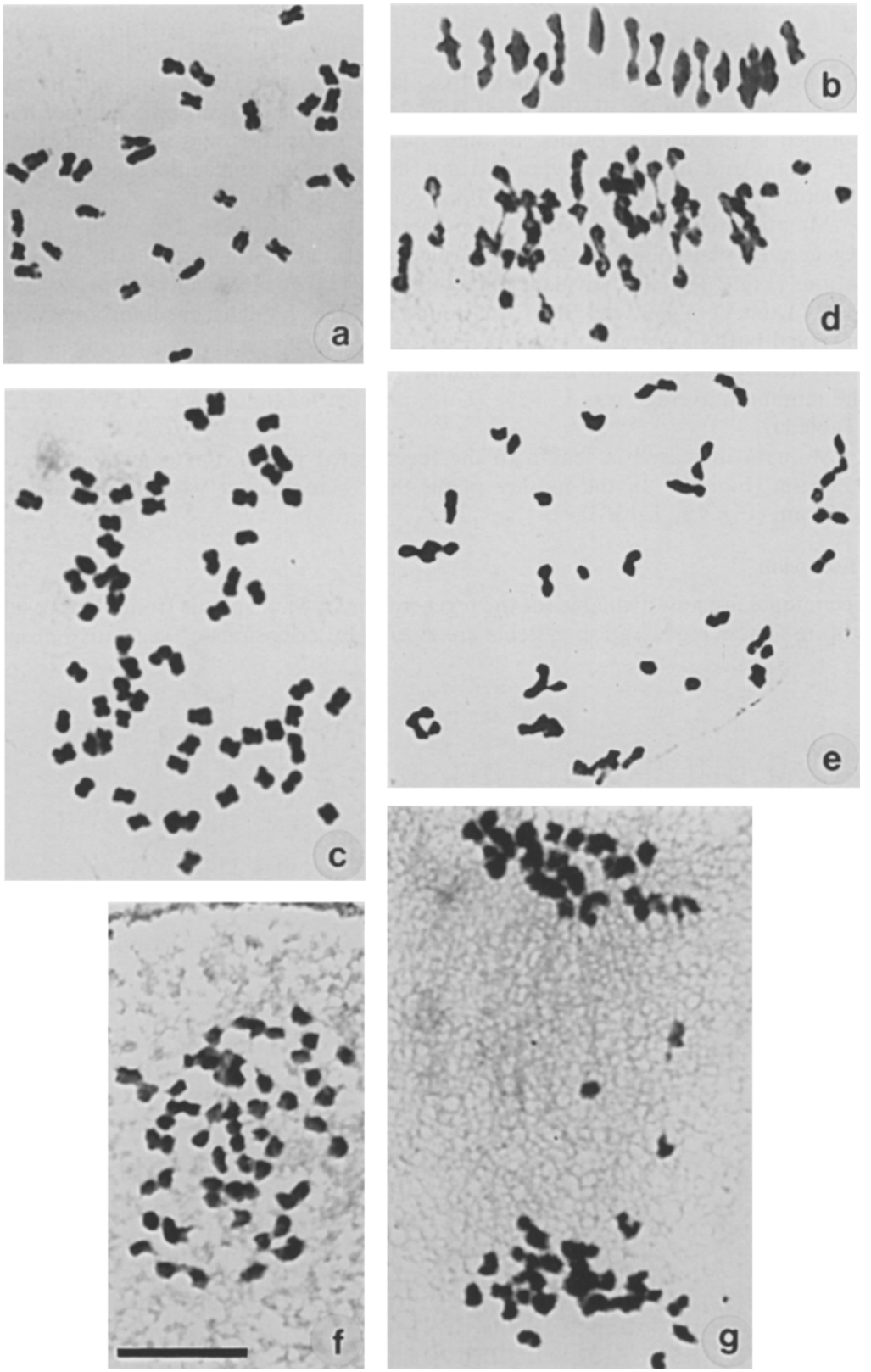

Fig. 2. *Turnera ulmifolia*. *a–b* Mother plant, *a* mitosis (2n = 30), *b* meiosis (15 II). *c–g* Regenerated plants, *c* mitosis (2n = 60); *d–g* meiosis, *d* first metaphase, 8 I + 19 II + 2 III + 2 IV, *e* diakinesis, 7 I + 16 II + 4 III + 2 IV, *f* mostly univalents, *g* anaphase I, bridge and lagging chromosomes. – Bar: 10 µm

Mitoses had 2n = 12x = 60 in all five plants (Fig. 2 c). This means, in contrast to 2n = 6x = 30 in the mother plant (Fig. 2 a), that the chromosome number had doubled in regenerated plants. In these plants, aneuploids and mixoploids were not found and their karyotypes did not show considerable differences in basic structure compared with the 2n = 30 karyotype (Fig. 3).

Meiotic analysis revealed 15 II in the mother plant (Fig. 2 b), while in the regenerated plants univalents to octovalents were observed in different configurations (Fig. 2 d–f). The bivalent frequency ranged from 18.42 in U 17 to 22.67 in U 22 (Table 1); a cell with 30 II was found in U 19. Lagging chromosomes were observed both in anaphase I and II (Fig. 2 g, Table 2).

Pollen viability was 90% in the mother plant, whilst in the regenerated plants the minimum average was 12.82% (U 18) and the maximum was 29.89% (U 22) (Table 1).

Stomata increased in length in the regenerated plants, up to an average of 35.06 µm (Fig. 4 b). In the mother plant, they were smaller with an average of 25.86 µm (Fig. 4 a, Table 3).

Discussion

Techniques are now available for the regeneration of whole plants from plant tissue culture. These regeneration systems are essentially extensions of vegetative prop-

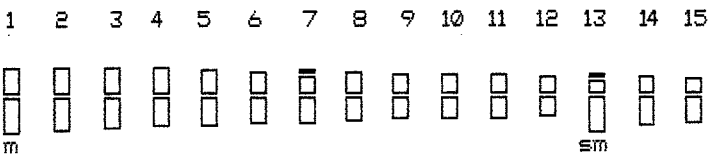

Fig. 3. *Turnera ulmifolia.* Idiogram of the mother plant. – Bar: 10 µm

Table 1. Mean and variation range of chromosomic pairing per cell at first meiotic metaphase in *Turnera ulmifolia*

Plants		2n	No. of cells	Mean and variation range per cell of								Staining pollen
				I	II	III	IV	V	VI	VII	VIII	
6x		30	77	–	15.00	–	–	–	–	–	–	90.00
12x	U 17	60	17	7.14	18.42	1.71	2.57	0.14	–	–	–	15.63
				3–13	14–26	0–5	0–7	0–1				
	U 18	60	16	3.44	22.50	0.31	2.56	–	0.06	–	–	12.82
				0–10	18–26	0–2	0–5	0–1				
	U 19	60	17	5.58	18.53	1.29	2.17	0.23	0.35	0.05	0.11	20.46
				0–13	10–30	0–4	0–5	0–2	0–2	0–1	0–1	
	U 20	60	14	7.36	18.64	1.29	2.57	0.07	0.14	–	–	25.84
				2–19	12–24	0–5	0–6	0–1	0–1			
	U 22	60	20	5.40	22.10	1.00	1.80	–	–	–	–	29.89
				2–16	17–27	0–4	0–4					
Average				5.78	20.04	1.12	2.33	0.088	0.098	0.01	0.022	

Table 2. Meiotic analysis in anaphase I and II of *Turnera ulmifolia*

Plants		Anaphase I		Anaphase II	
		No. of cells	Laggards %	No. of cells	Laggards %
6x		50	–	40	–
12x	U 17	9	78	20	62
	U 18	20	55	12	25
	U 19	30	60	34	68
	U 20	46	33	6	67
	U 22	59	56	35	54

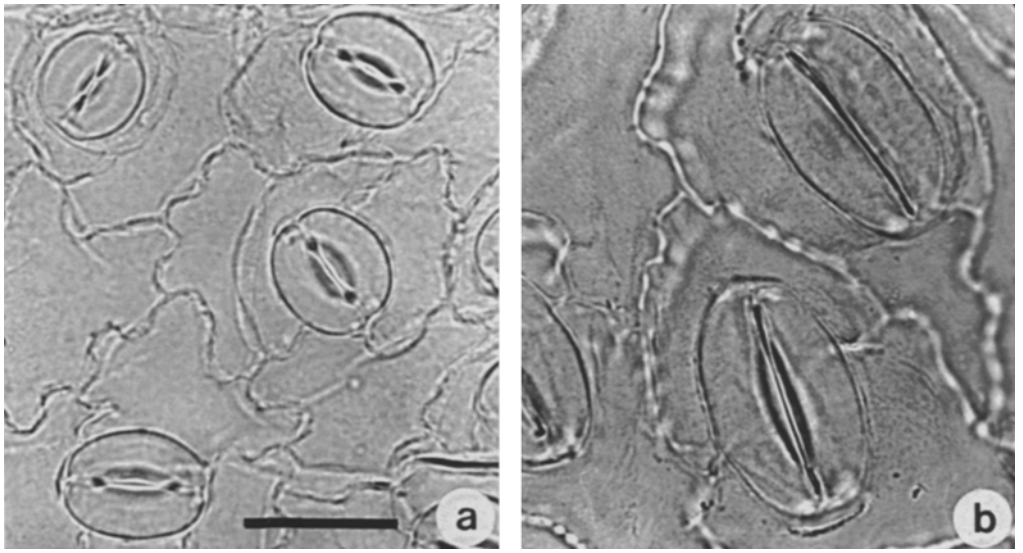

Fig. 4. *Turnera ulmifolia*. Stomata. *a* Mother plant, *b* regenerated plant. – Bar: 25 µm

Table 3. Stomata length in 6x compared with 12x *Turnera ulmifolia*

Plants		2n	Stomata	Mean ± SE
6x		30	50	25.86 ± 0.30
12x	U 17	60	50	34.08 ± 0.66
	U 18	60	50	38.20 ± 0.06
	U 19	60	50	31.68 ± 0.36
	U 20	60	50	30.54 ± 0.42
	U 22	60	50	40.80 ± 0.42

agation and as such it would be expected to give rise to clonal uniformity (KARP 1988). Nevertheless, there are many cases where this does not occur, because changes in chromosome number and structure, meiotic pairing, and pollen viability happen (SUNDERLAND 1977, D'AMATO 1978, BAYLISS 1980, CONSTANTIN 1981, LARKIN & SCOWCROFT 1981, ORTON 1983, LEE & PHILLIPS 1988, KARP 1988, ZIAUDDIN & KASHA 1990).

In *Turnera ulmifolia* chromosome doubling occurred in 100% of the regenerated plants. It could be explained by the following factors: type of tissue used as explant, ploidy level, callus phase, growth regulators, genotype stability; and age of the culture (KARP 1988). Chromosome doubling occurs at higher frequencies in plants regenerated from mature leaves than from immature leaves, e.g., in tobacco (KASPERBAUER & COLLINS 1972) and potato (KARP & al. 1984).

Auxins and cytokinins were used for the regeneration. Auxins stimulate DNA synthesis, and cytokinins are required for completion of mitosis and cytokinesis (JOUANNEAU & TANDEAU DE MARSAC 1973). As the auxin-cytokinin ratio consistently changes during culture due to degradation by cellular metabolism and the chemistry of the medium, hormonal regulation of the mitotic cell cycle may be disrupted in such a way that DNA synthesis and cytokinesis become non-synchronized. Excess of auxins in relation to cytokinins can induce endomitosis, produced by DNA synthesis without cellular divisions, thus raising polyploid cells. On the other hand, excess of cytokinins in relation to auxins could cause cells to divide prematurely and have their chromosomes broken or lost during cell division, thus inducing aneuploidy (EVANS 1984, LISCUM III & HANGARTER 1991).

In *T. ulmifolia* changes in the hormonal ratios may have favoured auxins, thus inducing chromosome doubling by endomitosis, resulting in the segregation of the sister chromatids within the nuclear membrane and without mitotic apparatus formation, as shown in barley by ZIAUDDIN & KASHA (1990).

Meiotic analysis of *T. ulmifolia* hybrids with other species showed high frequency of multivalents, suggesting it is segmental allohexaploid (FERNANDEZ, unpubl.), as other polyploid species of *Turnera* (FERNANDEZ & ARBO 1993). The mother plant has always 15 II (Fig. 2 b), probably due to the Ph gene action that inhibits the pairing of homoeologous chromosomes, as in wheat (RILEY & CHAPMAN 1958) and barley (GUPTA & FEDAK 1985).

To the contrary of what may be expected, a high frequency of bivalents was observed after chromosome doubling (Fig. 2 d), reaching a maximum of 30 II in U 19, and a low quadrivalent frequency (Table 1). Penta-, hexa-, hepta-, and octavalents were observed, probably due to the pairing among homoeologous chromosomes, because the mother plant is segmental allohexaploid. The high bivalent frequency may have been caused by preferential pairing of identical chromosomes against the homologous, as observed in autotetraploids of rye (ORELLANA & SANTOS 1985).

Numerous crossings were carried out between the regenerated plants and the mother plant with the purpose of studying their compatibility. When the mother plant was used as the female parent the flowers faded and dropped two days after pollination; in the reciprocal crosses, fruits were apparently produced but 5 or 6 days after pollination they were aborted. Seeds were never obtained. This means that pre-zygotic barriers exist in $2n = 6x = 30 \times 2n = 12x = 60$ crosses, probably

because pollen grains did not germinate. When 2n = 12x = 60 × 2n = 6x = 30 crosses were carried out, the barriers have been post-zygotic.

KARP & al. (1984) found that chromosome doubling by tissue culture in potato is better than doubling by colchicine, because regeneration techniques have the advantage of obtaining a large number of plants simply and rapidly, and only a very small percentage of these are mixoploid or aneuploid.

Thus, it can be concluded that chromosome doubling by regeneration from cultured leaf explants is an efficient way of obtaining polyploids.

References

BAYLISS, M. W., 1980: Chromosomal variation in plant tissues in culture. – In VASIL, I. K., (Ed.): Perspectives in plant cell and tissue culture, pp. 113–144. – Int. Rev. Cytol. Suppl. **11A**. – New York: Academic Press.

CONSTANTIN, M. J., 1981: Chromosome instability in cell and tissue cultures and regenerated plants. – Environ. Exper. Bot. **21**: 359–368.

D'AMATO, F., 1978: Chromosome number variation in cultured cells and regenerated plants. – In THORPE, T. A., (Ed.): Frontiers of plant tissue culture, pp. 287–295. – Calgary: University of Calgary.

DIZEO DE STRITTMATTER, C. G., 1973: Nueva técnica de diafanización. – Bol. Soc. Argent. Bot. **15**: 126–129.

EVANS, M. L., 1984: Functions of hormones at the cellular level of organization. – In SCOTT, T. K., (Ed.): Hormonal regulation of development II, pp. 23–79. – Encyclopedia of plant physiology, n.s. **10**. – Berlin, Heidelberg, New York, Tokio: Springer.

FERNANDEZ, A., 1987: Estudios chromosómicos en *Turnera* y *Piriqueta* (*Turneraceae*). – Bonplandia **6**: 1–21.

– ARBO, M. M., 1993: Relaciones genómicas entre seis especies de *Turnera* (Serie *Canaligerae*) del Paraguay. – Candollea **48**: 305–318.

GUPTA, P. K., FEDAK, G., 1985: Genetic control of meiotic chromosome pairing in polyploids in the genus *Hordeum*. – Canad. J. Genet. Cytol. **27**: 515–530.

HANG, A., BREGITZER, P., 1993: Chromosomal variations in immature embryo-derived calli from six barley cultivars. – J. Heredity **84**: 105–108.

JACOBSEN, E., 1981: Polyploidization in leaf callus tissue and in regenerated plants of dihaploid potato. – Pl. Cell Tissue Organ Culture **1**: 77–84.

JOUANNEAU, J. P., TANDEAU DE MARSAC, N., 1973: Stepwise effects of cytokinin activity and DNA synthesis upon mitotic cycle events in partially synchronized tobacco cells. – Exp. Cell Res. **77**: 167–174.

KARP, A., 1988: Origins and causes of chromosome instability in plant tissue culture and regeneration. – In BRANDHAM, P. E., (Ed.): Kew Chromosome Conference III, pp. 185–192. – London: HMSO.

– RISIOTT, R., JONES, M. G. K., BRIGHT, S. W. J., 1984: Chromosome doubling in monohaploid and dihaploid potatoes by regeneration from cultured leaf explants. – Pl. Cell Tissue Organ Culture **3**: 363–373.

KASPERBAUER, M. J., COLLINS, G. B., 1972: Reconstitution of diploids from leaf tissue of anther-derived haploids in tobacco. – Crop Sci. **12**: 98–101.

KUMAR, P. S., WALTON, P. D., 1992: Plant regeneration and chromosome instability in tissue culture of *Elymus canadensis* × *E. trachycaulus* F_1 hybrid. – Genome **35**: 88–91.

LARKIN, P. J., 1987: Somaclonal variation: history, method, and meaning. – Iowa State J. Res. **61**: 393–434.

– SCOWCROFT, W. R., 1981: Somaclonal variation: a novel source of genetic variability from cell cultures for plant improvement. – Theor. Appl. Genet. **60**: 197–214.

LEE, M., PHILLIPS, R. L., 1988: The chromosomal basis of somaclonal variation. – Annu. Rev. Pl. Physiol. Pl. Mol. Biol. **39**: 413–437.

LISCUM III, E., HANGARTER, R. P., 1991: Manipulation of ploidy level in cultured haploid *Petunia* tissue by phytohormone treatments. – J. Pl. Physiol. **138**: 33–38.

MCCOY, T. J., PHILLIPS, R. L., RINES, H. W., 1982: Cytogenetic analysis of plants regenerated from oat (*Avena sativa*) tissue cultures; high frequency of partial chromosome loss. – Canad. J. Genet. Cytol. **24**: 37–50.

MURASHIGE, T., NAKANO, R., 1967: Chromosome complement as a determinant of the morphogenic potential of tobacco cells. – Amer. J. Bot. **54**: 963–970.

– SKOOG, F., 1962: A revised medium for rapid growth and bioassays with tobacco tissue culture. – Physiol. Pl. **15**: 473–497.

ORELLANA, J., SANTOS, J. L., 1985: Pairing competition between identical and homologous chromosomes in autotetraploid rye. I. Submetacentric chromosomes. – Genetics **111**: 933–944.

ORTON, T. J., 1983: Spontaneous electrophoretic and chromosomal variability in callus cultures and regenerated plants of celery. – Theor. Appl. Genet. **67**: 17–24.

– 1987: Genetic instability in celery tissue and cell cultures. – Iowa State J. Res. **61**: 481–498.

RILEY, R., CHAPMAN, V., 1958: Genetic control of cytologically diploid behaviour in hexaploid wheat. – Nature **182**: 713–715.

STELLY, D. M., ALTMAN, D. W., KOHEL, R. J., RANGAN, T. S., COMMISKEY, E., 1989: Cytogenetic abnormalities of cotton somaclones from callus cultures. – Genome **32**: 762–770.

SUNDERLAND, N., 1977: Nuclear cytology. – In STREET, H. E., (Ed.): Plant tissue and cell culture, pp. 177–205. – Berkeley: University of California Press.

WINFIELD, M., DAVEY, M. R., KARP, A., 1993: A comparison of chromosome instability in cell suspensions of diploid, tetraploid, and hexaploid wheats. – Heredity **70**: 187–194.

ZIAUDDIN, A., KASHA, K. J., 1990: Long-term callus cultures of diploid barley (*Hordeum vulgare*). II. Effect of auxins on chromosomal status of cultures and regeneration of plants. – Euphytica **48**: 279–286.

Address of the authors: Lic. G. I. LAVIA (correspondence), Ing. Agr. A FERNÁNDEZ, and Lic. G. MÁRQUEZ, Instituto de Botánica del Nordeste, C.C. 209, 3400 Corrientes, Argentina.

Accepted March 29, 1994 by F. EHRENDORFER