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Cytological and Cytophotometric Analysis of Direct Explant and Callus Derived Plants of *Ornithogalum thyrsoides* Jacq.

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Genetic uniformity and stability of cell in tissue culture are prerequisites for their use in plant propagation. However, plant tissue and cell cultures often exhibit genetic instability including changes in chromosome number and structure (Murata and Orton 1983, D'Amato 1985, Jha and Sen 1987, Lavania and Srivastava 1988, Lee and Phillips 1988). Such instability in chromosome complement also leads to differences in DNA content (Delezel and Novak 1985, Gavallini *et al.* 1986, Gavallini and Natali 1987). On the other hand, reports are also available on genetic stability of cultured tissues and regenerated plants (Sheridan 1975, Hanna *et al.* 1984, Nayak and Sen 1987, Jha *et al.* 1989).

Ornithogalum thyrsoides Jacq., a monocotyledonous plant belonging to family Liliaceae, characterised by low chromosome number (2n=12) and simple karyotype, is a favourable material for the study of chromosome behaviour in callus and regenerants. The previous report shows that, this species can easily be cultured and regenerated *in vitro* (Hussey 1976). An attempt has been given to understand the extent to which stability and instability of chromosome behaviour in culture are affected by the ploidy level, nature of the explant and age of culture.

Materials and methods

The plant *O. thyrsoides* was collected from a nursery in Eastern Himalayas. Leaf and bulb scale explants of about 1 cm length were used to generate callus culture. Surface sterilized explants were grown on Murashige and Skoog's (MS) (1962) basal medium modified by 1 mg/l thiamine hydrochloride, 2% sucrose and 8 mg/l napthalene acetic acid (NAA). Sufficient calli became available within 2 months. The calli were regularly subcultured on fresh medium at monthly intervals. Leaf calli and bulb calli could be maintained on same medium upto 300 days and 390 days respectively. Regeneration of shoots directly from explants and from calli could be obtained by growing them on hormone free MS basal medium. Rooting occurred in half strength of same basal medium giving rise to complete plant. Plants could be regenerated from both 60 days and 300 days old leaf callus as well as 60 days and 390 days old bulb callus.

For cytological analysis, the leaf and bulb scale explants, calli, root tips as well shoot tips of regenerated plants were pretreated with saturated solution of paradichlorobenzene: 0.002 M hydroxyquinoline (2 : 1) for 3 hr. Fixative used was acetic-ethanol (1 : 3), but for callus tissues, it was Carnoy's fluid containing ethanol: chloroform and acetic acid in 6: 3: 1 ratio. The aceto-orcein staining technique was used. Chromosome morphology was determined following Battaglia's classification (Battaglia 1955).

For *in situ* estimation of DNA content in root tips, shoot tips, calli and explants the tissues were hydrolysed with 1 N HCl at 58°C and stained with Feulgen reagent. The DNA content was measured with Leitz Wetzler microspectrophotometer following single wavelength

(550 nm) method (Mc Leish and Sunderland 1961). The DNA content in arbitrary units was transformed to C value by taking the mean DNA content of root apex metaphase (=4C) as a standard. The values in arbitrary units were converted to picograms (pgs) by using Van't Hof's (1965) 4C nuclear DNA value for *Allium cepa* as a standard.

Results

Cytology of source plant

Cells were all diploid with 12 chromosomes and distribution of nuclear DNA content showed presence of one peak corresponding to 4C DNA content.

Table 1. Frequency of diploid and tetraploid cells of leaf and bulb callus line at different days of culture

Leaf Callus Line				Bulb Callus Line			
No. of days in culture	No. of cells examined	Frequency of diploid cells (%)	Frequency of tetraploid cells (%)	No. of days in culture	No. of cells examined	Frequency of diploid cells (%)	Frequency of tetraploid cells (%)
4	25	96.0	4.0	8	33	97.0	3.0
7	40	92.5	7.5	16	53	96.3	3.7
20	67	86.5	13.5	30	65	95.5	4.5
30	61	77.0	23.0	60	70	94.5	5.5
60	69	73.9	26.1	90	66	94.2	5.8
90	70	67.1	32.9	120	71	93.4	6.6
120	76	67.1	32.9	150	62	92.7	7.3
150	73	65.7	34.3	180	72	92.3	7.7
180	74	64.8	35.2	210	76	91.5	8.5
210	68	63.2	36.8	240	68	89.4	10.6
240	76	61.8	38.2	270	63	87.5	12.5
270	74	60.0	40.0	300	73	85.8	14.2
300	70	58.6	41.4	330	66	83.5	16.5
				360	67	82.7	17.3
				390	60	80.0	20.0

Cytology of cultured cells

Explant: Dividing cells were noticed in leaf and bulb explants after 4th and 8th day of explantation and mitotic index was 1 and 1.2% respectively. Chromosome count revealed that most of the cells were diploid excepting a few tetraploids.

Callus: There was predominance of diploidy during initiation of callus. The predominance was retained in both leaf and bulb callus lines throughout the culture period (Table 1), along with gradual increase in rate of polyploidization with days in culture. Variation beyond tetraploidy was not observed. Aneuploid cells or mitotic abnormalities were absent. The frequency of diploid mitosis was high in bulb callus than the callus of leaf origin.

Regenerated plants: The total number of plants analysed from different sources (Table 2) were 117. Of these 101 plants were exclusively diploid with $2n=12$ chromosomes. The remaining 16 plants were predominantly diploid (60–90 per cent) with a few cells showing 24 chromosomes in the roots. Shoot tip analysis revealed only diploid chromosome number (Table 2).

Detailed karyotype analysis

On the basis of length, position of centromere and secondary constriction, 6 pairs of chro-

Table 2. Chromosome number and DNA content in plants regenerated from different sources

	Total No. of plants analysed from different sources	Total No. of cells analysed and chromosome No. ( ) in		Range of mean DNA content in root tip nuclei (pg)±Range of standard error	Range of DNA content in shoot tip nuclei (pg)±Range of standard error
		Root tips	Shoot tips		
Control	From field 10	550 (12)	—	16.2–16.4±(0 1–0.3)	—
Exclusively diploid regenerants	Directly from leaf explant 15	715 (12)	275 (12)	16.2–16.4±(0.1–0.2)	16.1–16.2±(0.3–0.5)
	From short-term (60 days) leaf callus 25	700 (12)	250 (12)	16.0–16.3±(0.2–0.3)	16.1–16.3±(0.1–0.3)
	From long-term (300 days) leaf callus 9	297 (12)	116 (12)	16.0–16.3±(0.2–0.4)	16.0–16.2±(0.1–0.2)
	Directly from bulb explant 15	405 (12)	180 (12)	16.2–16.3±(0.2–0.5)	16.2–16.4±(0.1–0.4)
	From short-term (60 days) bulb callus 20	496 (12)	166 (12)	16.2–16.3±(0.1–0.4)	16 0–16.4±(0.2–0.4)
	From long-term (390 days) bulb callus 7	319 (12)	133 (12)	16.1–16.4±(0.2–0.3)	16.2–16.4±(0.2–0.3)
Predominantly diploid regenerants	From long-term (300 days) leaf callus 11	245 (12) 43 (24)	154 (12)	16.3–17.5±(0 3–0.5)	16.2–16.4±(0.2–0.4)
	Plants from long-term (390 days) bulb callus 5	137 (12) 18 (24)	72 (12)	16.2–17.3±(0 2–0.4)	16.1–16.4±(0.1–0.3)

Table 3. Comparative data showing mean length (L) and F% of homologous pairs of chromosomes from different sources (Data based on averages of 5 metaphase plates in each case)

Chromosome pair	Source plant (Control)		Leaf callus		Bulb callus		Regenerants from leaf explant		Regenerants from bulb explant		Regenerants from short term (60 days) leaf callus		Regenerants from short term (60 days) bulb callus		Regenerants from long term (300 days) leaf callus		Regenerants from long term (390 days) bulb callus		Mean chromosome length from different sources *in vitro*
	L±SE	F%	L	F%	L	F%	L	F%	L	F%	L	F%	L	F%	L	F%	L	F%	L±SE
1	7.5 ±(0.09)	9.3 21.8	7.1	9.0 21.2	7.5	9.2 20.8	6.8	8.5 21.1	7.0	9.1 20.2	7.3	8.3 20.1	7.7	9.3 21.3	6.9	8.3 21.0	7.5	9.1 22.3	7.2±(0.07)
2	6.3 ±(0.07)	3.7 14.8	6.8	3.5 19.6	6.5	4.0 14.2	6.1	3.9 14.7	6.2	4.2 15.2	7.0	3.7 14.2	6.1	3.6 14.7	6.0	3.6 15.1	6.6	4.1 15.5	6.3±(0.09)
3	6.2 ±(0.08)	4.0	6.2	4.2	6.2	4.3	5.8	4.0	5.9	4.1	6.1	4.1	5.8	3.9	6.0	3.9	6.4	4.5	6.0±(0.08)
4	6.0 ±(0.1)	4.0	6.1	3.8	5.8	4.1	6.0	3.7	5.8	4.0	5.9	4.8	5.6	3.6	6.0	3.8	6.2	4.2	5.8±(0.1)
5	3.2 ±(0.08)	7.6	3.2	7.1	3.0	7.2	3.5	7.2	3.1	7.5	3.1	7.1	3.0	7.6	3.0	7.1	3.3	7.2	3.1±(0.07)
6	5.3 ±(0.1)	18.1	5.3	18.1	4.7	18.3	5.0	18.1	5.6	18.6	4.9	18.7	5.2	18.1	5.5	18.6	4.8	18.1	5.1±(0.1)

mosome could be identified. A comparison of mean length of chromosomes as well as position of centromeres as determined by F%, has been presented in Table 3 on the basis of measurement of five well spread metaphase plates each from cultured and non-cultured (source) materials reveals no significant differences in karyotypes (Fig. 1). The differences are well within the limits of variability within a clone.

Cytophotometric study

The nuclear status of explant, callus and regenerated plants determined by chromosome analysis was also confirmed by distribution of DNA values determined through *in situ* cytophotometry.

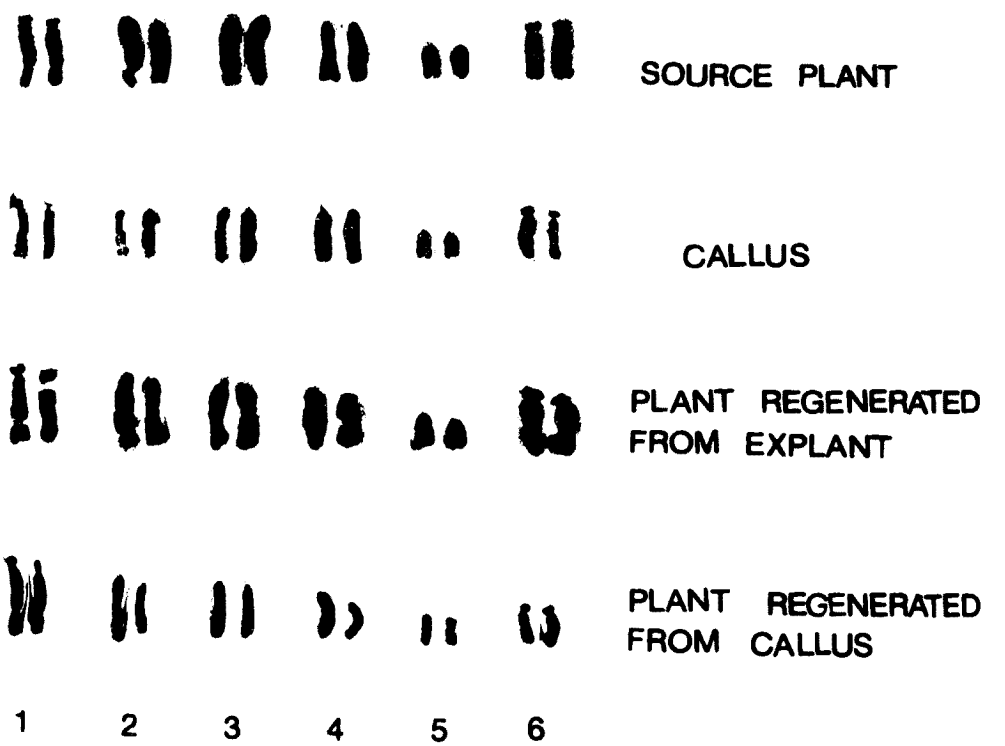

Fig. 1. Karyotypes of *Ornithogalum thyrsoides* (2n=12) in source plant, in leaf callus, in root tip of plant regenerated from leaf explant and from leaf callus (×1350).

After explantation, dividing cell could be noted in cultured leaf explant from 4th day onwards and in bulb explant after 8th day onwards. In leaf callus line, the mean DNA values which was 16.3 pg in 4th day showed a rise up to 23.2 pg in 300 days and in bulb callus line, the values which was 16.1 pg in 8th day raised to 19.32 pg in 390 days indicating chromosome variation. The increase in leaf callus line may be due to more hyperdiploid cells. However, conspicuously in both the lines, the diploid cells containing 4C DNA content was predominant throughout the culture period.

Out of 117 plants, 101 plants were diploid revealing 4C value as in control (Table 2). The mean DNA content of root tip and shoot tip of the regenerants varied from 16.1 pg to 16.4 pg (Table 2) which was close to the diploid mean (16.2 pg) of root tips of control plants. In other 16 plants obtained from long-term culture of 300 days onwards, root tip analysis revealed

several nuclei with 8C DNA value. The mean DNA content in root tips of these plants varied from 16.2 pg to 17.5 pg (Table 2). In shoot tip, no 8C value was recorded and the range was from 16.1 pg to 16.4 pg.

Discussion

Plant cells grown *in vitro*, exhibit cytological variability, which have often been traced to the mixoploid nature of the primary explant (Swedlund and Vasil 1985).

The original explant of leaf and bulb scale used in the present study, was predominantly diploid comprising only a few tetraploid cells which apparently divided in culture. The ploidy level was confined to tetraploidy both in leaf and bulb callus line after 300 and 390 days in culture respectively. Mitotic abnormalities were not encountered during the course of this investigations.

Despite gradual increase of precentage of tetraploid cells in culture, diploidy predominated in both the callus lines. Regeneration of exclusively normal green diploid plants directly from explant, from short term (60 days) culture and predominately diploid plants from long-term (300 and 390 days) culture, indicate a selective mechanism operating in favour of normal diploid cells to regenerate. The genetic stability of karytypes in culture is thus maintained.

The influence of organogenesis on karyotype stability is distinct. Callus tissue with low degree of organization, yielded more instability as compared to root or shoot tip cells of regenerants, as reported by Kovacs (1985) as well.

The data on chromosome counts was also confirmed from *in situ* nuclear DNA estimation. The stability of chromosome number, karyotype and DNA content reflect that, in this species, *in vitro* growth does not induce instability. The slight instability noted can be traced to the cells of the explant.

Summary

The chromosome analysis and *in situ* DNA estimation of *Ornithogalum thyrsoides* (2n= 12) *in vitro* revealed the maintenance of normal karyotype in leaf and bulb explants, in 300 days old calli and in roots and shoots of regenerated plants.

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