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Karyotype Stability in Long-Term Callus Derived Plants of *Crepis tectorum* L.

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Abstract. The study of *in vitro* growth of *Crepis tectorum* revealed 100 % callusing and 40 % plantlet regeneration. The root and leaf used as explants showed the normal diploid (2n=8) chromosome constitution. In one month old culture 95 % callus cells were diploid. The callus maintained in 2,4-D 1 mg l^{-1} for two years showed 62 % diploid, 5 % tetraploid and 33 % hyperdiploid cells. The differentiation of shoot occurred in two year old calli after subculturing in 2 mg l^{-1} BAP and the potentiality of regeneration was retained for more than one year. The leaf-tips of regenerated plants were homogeneous and identical to the donor plant both in number and morphology of chromosomes.

Karyotype instability in long-term callus following *in vitro* growth has been noted in several species of plants (TORREY 1967, BAYLISS 1980, NOVAK and DOLEZEL 1985). For such a reason shoot multiplication through axillary bud rather than regeneration through callus is often recommended to secure stable regenerants. However, there are a few records of stability in long-term callus culture (CLEMENT 1964, SHERIDAN 1975). But studies on chromosomal constitution in a long-term culture with respect to plantlet regeneration are meagre (TORREY 1967). The species of *Crepis* provided ideal materials for such a study due to the presence of a low chromosome number and apparent lack of somatic endopolyploidy (SINGH 1974, D'AMATO 1977). Thus, the present investigation on long-term callus culture of *C. tectorum* (2n=8) was undertaken to analyse the behaviour of chromosomes in such a culture, their potential for regeneration and genetic constitution of regenerants.

MATERIALS AND METHODS

Sterilized seeds of *Crepis tectorum* L. obtained from Hortus Botanicus, University of Oslo (Norway) were germinated on half strength MS basal medium (MURASHIGE and SKOOG 1962) with 1 % sucrose within 15 days.

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Immature leaves of 0.5—1 cm long plantlets were used as explants. The MS medium was used with different auxins and cytokinins for initiation of callus, its maintenance and regeneration of plantlets. Ten replicates of each set were maintained and repeated twice. The media were solidified with 0.5 % agar and adjusted to pH 5.6—5.8 before autoclaving at 0.1 MPa pressure for 15 min. Cultures were incubated at 22 ± 1 °C with 55—60 % relative humidity under cool, white fluorescent light of 2000 lx at the culture level, and 16 h photoperiod. The callus was formed within 20—25 days on two sets of media viz. (i) MS with 5 mg l^{-1} of NAA and 0.5 mg l^{-1} of kinetin and (ii) MS with 4 mg l^{-1} of IBA and 2 mg l^{-1} of kinetin. After 3 months of growth, calli from both the sets were maintained by regular subculturing at an interval of 28—30 days for two years on MS medium supplemented with 1 mg l^{-1} of 2,4-dichlorophenoxy acetic acid. Eighteen months old callus was transferred to MS medium without auxin containing 1, 2 and 5 mg l^{-1} of BAP for shoot regeneration. Green leafy structures that appeared on a callus grown on MS with 5 mg l^{-1} of BAP within 15—20 d were developed into leafy shoots (30—50 per culture) within 40 d when transferred to an unsupplemented MS basal medium. Leafy shoots when excised from the base developed roots in the same medium within 20 days. The regenerative ability (no. of leaves/culture) was studied with the culture age.

To study the chromosome constitution, the calli were cut into small bits 4—5 days after subculturing, fixed in 1 : 3 acetic acid-ethanol overnight, followed by a change in 45 % acetic acid for 10—15 min and stained with 2 % aceto-orcein: 1M HCl (9 : 1). Tips of leaves regenerated from callus were examined every month. Leaf-tips were pretreated in 0.05 % colchicine for 3 h at 12—15 °C, fixed in 1 : 3 acetic acid-ethanol overnight and stained with 9 : 1 aceto-orcein : 1M HCl and squashed in 45 % acetic acid. Five cells with well spread chromosomes from each leaf-tip were randomly selected for comparison between plants.

RESULTS

Cytology

The donor plant revealed 99 % diploid cells in leaf-tip (Fig. 1a). The normal karyotype included one pair of chromosomes with secondary constrictions (A), one pair with submedian constriction (B) and two pairs of comparatively short acrocentric chromosomes (C) (Fig. 1b).

Dedifferentiating Calli

One month old cultured tissue on MS + 6 mg l^{-1} of NAA and 0.5 mg l^{-1} of kinetin, was composed predominantly of diploid cells (95 %) with a few polyploid cells (5 %) (Table 1) whereas 2 year old unorganised calli maintained on 1 mg l^{-1} of 2,4-D contained 62 % diploid and 38 % polyploid cells (Table 1).

Irregular nuclear processes *e.g.* lagging, multipolarity and anaphasic abnormalities were rarely observed in cultured cells.

Differentiating Calli

The green meristemoids differentiating from one and half year old culture were composed of small cells which were predominantly diploid (81.6 %)

TABLE 1
Morphology and cytology of one month and two year old cultured cells grown on different media for various periods of time

Age of callus [months]	Morphology	Culture medium	No. of metaphases studied	No. of cells with 2n = 8 chromosome (s. e.)	Diploid cells [%]	No. of metaphases with hyperdiploid chromosome no.					
						> 2n (chr. No.)	3n (3n = 12)	4n (4n = 16)	> 4n (chr. No.)	Total no. of 2n cells (s. e.)	Hyperdiploid cells [%]
1	Compact, unorganised	Initiation: MS + NAA (6 mg l^{-1}) + kinetin (0.5 mg l^{-1})	100	95 (1.23)	95	1(10)	0	4	0	5 (1.26)	5
18	Differentiating culture with green leafy structure	Organogenetic: MS + BAP (5 mg l^{-1})	71	58 (0.90)	81.7	6(10) 1(9)	3	0	3(24)	13 (0.90)	18.3
24	Nodular, unorganised	Maintenance: MS + 2,4-D (1 mg l^{-1})	84	52 (1.17)	61.9	8(10) 2(11)	10	4	8(24)	32 (1.27)	38.1

TABLE 2

Chromosome analysis of leaf tip cells differentiating from one and half year old callus. All diploid cells revealed the normal 2A + 2B + 4C karyotype

No. of differentiating culture	No. of leaf-tip studied	No. of metaphase studied per leaf-tip	No. of cells per leaf-tip with diploid chr. no. (2n=8)	any other chr. no. (variant no.)
1	5	4–6	All	—
2	6	6–8	All	—
3	4	5–6	All	—
4	6	8–12	All	—
5	6	8–10	All	—
6	5	6–8	All	—
7	8	10–12	All	—
	1	10	8	2(12)
8	10	8–10	All	—
9	4	10–12	All	—
	1	12	10	2(16)
10	5	10–12	All	—

(Fig. 1c). Nearly 18.4 % cells were hyperdiploid with 9, 10, 11, 12, 16, 24 chromosomes. Formation of binucleate cells, sticky bridge and unequal separation of chromosomes were recorded in a low frequency (Fig. 1d).

Regenerated Plants

Sixty regenerants except two obtained from 10 differentiating cultures showed only diploid cells with 2n=8 chromosomes. The other two plants revealed a few triploid and tetraploid cells in addition to the modal diploid number (Table 2) (Fig. 1e, f).

The chromosome type was noted to be identical with the karyotype of the parent plant (2A + 2B + 4C) that was used as initial explant.

DISCUSSION

The results indicate that the long-term callus culture of *Crepis tectorum* showed a relative cytological stability. The previous reports on the culture of *C. capillaris* by different authors revealed that polyploidisation and karyotype alteration could be noted even in short-term culture (SACRISTAN 1971, BAYLISS 1980). However, the frequency of such cells may vary. In the present study even after one and half year of culture the meristematic region contained a high frequency (82 %) of diploid cells which exclusively participated in plantlet regeneration. Thus the polyploid cell population, though present in culture did not disturb the chromosomal constitution of the regenerants and the regenerants were of diploid constitution with no apparent morphological variation. It is presumably because in the absence of somatic endopolyploidy the rate of induction of polyploid cells in culture was low. In such a system the diploid cell line remained dominant throughout the period of culture. As a result, organogenesis was not affected even in one and half year old cultures of *C. tectorum* because diploids appear to have a selective advantage during regeneration.

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Fig. 1 at the end of the issue