Induction of mitosis in polytene nuclei and hormonal effect on nuclear changes during callus initiation in diploid Urginea indica Kunth. (Liliaceae)

S. Jha & S. Sen
Centre of Advanced Study, Dept. of Botany, University of Calcutta, 35, B.C. Road, Calcutta-700 019, India

Received 23.9.1987 Accepted in revised form 29.7.1989

Abstract

Bulb scale and inflorescence explants of Urginea indica Kunth. (2n = 20) were cultured in vitro on modified Murashige & Skoog’s medium with different hormonal composition. Media containing 2,4-dichlorophenoxy-acetic acid (2,4-D) (2 and 4 mg l⁻¹) and α-naphthalene-acetic acid (NAA) (2 mg l⁻¹) could induce callus in inflorescence explants. Combination of 2,4-D (4 mg l⁻¹) + NAA (2 mg l⁻¹) + Kinetin (2 mg l⁻¹) only could induce callus formation in scale explants. The bulb scale explants contained mostly diploid cells while the inflorescence explants contained cells with nuclear DNA content ranging from 2C to 64C. The lowest karyological heterogeneity was recorded in callus derived from bulb scale and in callus derived from inflorescence induced with NAA. The highest variability was recorded on media with 2,4-D alone. Induction of division, probably of the pre-existing polytenic nuclei in the inflorescence explant, has been suggested to be the cause of origin of polyploid cells in such cases.

Introduction

Chromosomal instability in vitro is a common phenomenon and has been extensively reviewed by D’Amato (1978), Bayliss (1980) and Constantin (1981). However, the factors controlling the origin of numerical variation of chromosomes are not fully understood (Constantin, 1981). Polyploidization during callus growth has been described in a variety of systems. Torrey (1965) suggested that hormonal composition of the medium plays an important role in determining the type of cells of the explant which would undergo division. In cultured explants and calli of potato, a high degree of chromosomal variation has been reported and possible mechanisms responsible for chromosomal variation elucidated (Pijnacker et al., 1986; Sree Ramulu, 1986).

Previous studies on callus cultures derived from bulb scale explants of Urginea indica Kunth. show karyological instability (Jha & Sen, 1987a) which leads to regenerants with changes in structure and number of chromosomes (Jha & Sen, 1987b). The present work deals with nuclear changes during the initiation of callus, derived from inflorescence and bulb scales. The influence of hormones on these changes has been studied as well.

Material and methods

The bulbs and inflorescence stalks of a diploid (2n = 20) cytotype of U. indica collected from Almora were used as explants. The procedure followed for sterilization of bulb explants has
been reported earlier (Jha et al., 1984). Of young inflorescences (6–8 cm high), segments (0.5 cm) were cut and sterilized with 0.1% mercuric chloride for 10 min. and washed in 5 changes of sterile water. The basal medium (BM) for induction of callus was Murashige and Skoog’s (1962) medium supplemented with 10 mg{l}^{-1} thiamine-HCl, 5 mg{l}^{-1} nicotinic acid, 1 mg{l}^{-1} pyridoxine-HCl, and 3% sucrose and with different variants of growth regulators as follows:

1. BM without growth regulators (Control)
2. BM + 4 mg{l}^{-1} 2,4-dichlorophenoxy-acetic acid (2,4-D)
3. BM + 2 mg{l}^{-1} 2,4-D
4. BM + 2 mg{l}^{-1} α-naphthalene-acetic acid (NAA)
5. BM + 2 mg{l}^{-1} Kinetin (Kn)
6. BM + 4 mg{l}^{-1} 2,4-D + 2 mg{l}^{-1} NAA + 2 mg{l}^{-1} Kn.

The media were adjusted at pH 5.6, solidified with 0.6% agar and sterilized in an autoclave for 15 min at 1.05 kg/cm² pressure (120 °C). Explants were placed in culture tubes containing 25 ml of medium and grown at 22–25 °C and a maximum relative humidity of 55–60% under Philips fluorescent day light tubes emitting 3200 lux for a 16/8 h light/dark period. The culture tubes were capped with cotton plugs.

For cytological analysis, samples of cultured material at different growth stages such as 6, 15 and 30 days of culture were fixed in chilled Carnoy’s fixative (glacial acetic acid: chloroform:ethanol 1:3:6), hydrolysed and stained with basic fuchsin.

In situ quantitative measurement of DNA content in explants before culture was carried out through single wavelength method (550 nm) following standard Feulgen staining technique (McCleish & Sunderland, 1961). For each observation, measurements from at least 50 nuclei were taken in a Reichert Zetopan with a microphotometer and the average DNA content was calculated in arbitrary units. These values were transferred to C values by taking as root apex prophases of control plants as a standard (Jha & Sen, 1987a).

The nuclei observed are diploid showing 2C DNA value, polyploid having more than 2C value, polytopic in a multistranded prophase stage and endomitotic polyploids where the metaphase chromosomes are multistranded in a polyploid cell. These multistranded chromosomes on separation give rise to endopolyploid cells.

**Results**

**Nuclear condition of explants before culture**

Initially, in the explant, the inflorescence tissue was found to contain non-dividing cells with nuclei of various sizes. The nuclear DNA ranged from 2C to values higher than 64C. The bulb scale tissue on the other hand, consisted of cells with nuclear DNA distributed in one peak corresponding to the 2C value. Cells with intermediate DNA values were observed in low frequency in the tissue of bulb scale and in high frequency in that of the inflorescence. In the inflorescence tissue polytenic nuclei were noted in the tapetal cells.

**Nuclear condition in vitro**

Induction of callus (Table 1) in tissue of bulb scales was secured only in BM supplemented with suitable combinations of 2,4-D + NAA + Kinetin (Jha et al., 1984). This combination has been found to be also effective in inflorescence explants. These hormones applied individually could not induce mitotic division in the scale explants. On the other hand, swelling and callusing was initiated in inflorescence explant with 2 and 4 mg{l}^{-1} of 2,4-D and 2 mg{l}^{-1} NAA. With kinetin, there was induction of division but no callusing in the inflorescence explant (Table 1).

Very little mitotic activity was noted in inflorescence explants cultured on basal medium without growth regulators. The mitotic activity of the explants in BM with 2 mg{l}^{-1} kinetin was initially very low, followed by a rise and complete inhibition after 20 days. The mitoses observed were mostly diploid. The percentage of polytenic nuclei undergoing division was also high – nearly