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Cytological Changes in Callus Cultures of *Allium commutatum* Guss.

By

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Summary

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Callus tissue was induced on root tips of in vitro cultured seedlings of *Allium commutatum* Guss. cultured on MS medium supplemented with 4.6 μM kinetin and 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Developed calli were transferred to the same basal medium with addition of 1.0, 2.5 and 5.0 μM 2,4-D or without 2,4-D. After three weeks in culture adventitious shoot and root induction was observed in compact greenish coloured callus on medium without 2,4-D. On media supplemented with 1.0 and 2.5 μM 2,4-D the callus was compact and yellowish with adventitious roots. No organogenesis was noticed on medium with 5.0 μM 2,4-D and callus tissue was whitish and slimy. For preliminary cytological analyses samples of callus tissue were collected on the 7. and 14. day after the transfer. Fixed calli fragments were hydrolized in 1 N HCl at 60 °C for 11 minutes and stained in toto with basic fuchsin. The slides were prepared according to standard Feulgen squash technique. In each sample 1000 cells were screened for mitotic activity and for establishing the rate of abnormalities. Relatively low level of genetic alterations was found. Among them micronuclei and polyploid cells were observed.

Introduction

In vitro culture method is widely used for plant breeding and germplasm conservation in many plant species including the species of genus *Allium*. The genus *Allium* which comprises up to 700 species is the largest and the most widely distributed group which belongs to the Mediterranean, Oriental and Caucasian floristic region.

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Allium commutatum Guss. is a typical Mediterranean species which can be found in open habitats on small islands and on the coast by the sea. The species is described as diploid, with exception of some triploid and tetraploid Greek populations. Karyological investigations based on chromosome number and morphology showed the uniformity of chromosome complement with $2n=2x=16$ metacentrics and submetacentrics with characteristic position of secondary constrictions on chromosome pairs VII and VIII (BESENDORFER & al. 1997).

Generally, monocotyledonous plants are not as reactive in in vitro culture as most dicotyledonous species (KELLER 1992). So far, numerous studies reported on various genetic and cytological abnormalities in plant material grown in in vitro conditions (BAYLISS 1980, D'AMATO 1990). During the callus induction and callus tissue cultivation, not only single gene alterations occur, but also a severe cytological and chromosomal alterations could be observed. The natural consequence of such variability and/or abnormality is the genetic mosaicism of callus cells (D'AMATO 1990). Callus culture and its cytological investigations are well developed in some *Allium* species such as garlic (NOVAK & al. 1990).

In the present paper the results on callus induction, callus culture and cytological changes in callus tissue of *Allium commutatum* Guss. are presented.

Materials and Methods

Root tips of in vitro cultured seedlings of *Allium commutatum* were used as initial explants for callus induction.

Sterilization of seeds was successively carried out with 2% water solution of a chlorine product Izosan-G (99% sodium dichloroisocyanurate dihydrate, Pliva, Zagreb) for 5 min and then, after three sterile distilled water rinses (5 min each), with 6% solution of hydrogen peroxide followed by three sterile distilled water washes, each one lasting 5 min.

After 7-14 days, when the roots reached the length of 0.5 cm, root tips were cut off and inoculated in test tubes (30×120 mm) filled with 15 ml of agar nutrient medium. After inoculation, test tubes were capped with cotton plugs and aluminium foil. Basal medium contained MS (MURASHIGE & SKOOG 1962) mineral salts, 100 mg l^{-1} myo-inositol, 0.1 mg l^{-1} thiamine HCl, 0.5 mg l^{-1} pyridoxine HCl, 0.5 mg l^{-1} nicotinic acid, 2.0 mg l^{-1} glycine, 30 g l^{-1} sucrose, 8 g l^{-1} agar, $4.6 \text{ }\mu\text{M}$ kinetin and $4.5 \text{ }\mu\text{M}$ 2,4-dichlorophenoxyacetic acid (2,4-D). Developed calli were transferred to the same basal medium with addition of 1.0, 2.5 and $5.0 \text{ }\mu\text{M}$ 2,4-D or without 2,4-D (control). The pH value of media was adjusted to 5.8 before autoclaving at 118 kPa and 120°C for 15 minutes. The cultures were incubated at $22 \pm 2^\circ\text{C}$ under a 16 hour photoperiod (40 W fluorescent light, $80 \text{ }\mu\text{Em}^{-2}\text{s}^{-1}$).

For cytological analysis samples of callus tissue were collected seven days after the transfer. Calli fragments were fixed in ethanol-acetic acid (3:1). Fixed calli fragments were hydrolyzed in 1N HCl at 60°C for 11 minutes and stained in toto with basic fuchsin. The slides were prepared according to standard Feulgen squash technique (SHARMA & SHARMA 1972).

The slides were examined under the light microscope, and in each sample at least 1000 cells were screened for mitotic activity and chromosome aberrations.

Also, analysis of nucleoli number and morphology in interphase callus tissue cells was performed according to silver-staining procedure by HIZUME & al. 1980 with minor modifications.

Results and Discussion

The sterilization procedure for seeds was satisfactory. After 2-week incubation the percentage of sterile cultures was 97.9 with 85.4% germinated seeds. Primary roots were separated from seedlings and transferred to the callus induction medium. Developed calli were transferred to media supplemented with three different 2,4-D concentrations or without 2,4-D after 4-6 weeks.

On all media tested callus development started within 7 days after transfer. After three weeks in culture different types of callus with varying morphogenetic potential were obtained. On medium without 2,4-D, the callus tissue was compact and greenish coloured with adventitious shoot and root induction. The calli on media supplemented with 1.0 and 2.5 μM 2,4-D were compact and yellowish coloured. There was no adventitious shoot induction, but adventitious roots were present. On medium with 2.5 μM 2,4-D somatic embryogenesis was noticed. Callus tissue developed on medium with 5.0 μM 2,4-D was soft, slimy and whitish coloured and there was no organogenesis.

Mitotic activity analyses of callus tissue grown on MS medium supplemented with three different 2,4-D concentrations (1.0, 2.5, 5.0 μM) or without 2,4-D showed a significant difference between samples. The highest level of mitotic activity (4.44%) was noticed in callus cells grown on medium with addition of 1.0 μM 2,4-D, while in other samples mitotic activity decreased. The lowest value of mitotic activity was observed in callus cells grown on medium supplemented with 5.0 μM 2,4-D (2.22%).

As far as chromosome aberrations are concerned, relatively low level was observed (the highest value of 1.2% was observed at 1 and 2.5 μM 2,4-D). Among them micronuclei were of significant appearance (1.1% for 1 μM 2,4-D; 1.2% for 2.5 μM 2,4-D), while other abnormalities like polyploidy and bridges in anaphase and chromosome fragments were rarely found (<0.1%). In in vitro cultures polyploidy and aneuploidy are very often as well as various mitotic irregularities like lagging chromosomes and multipolar anaphases (BAYLISS 1980), but this depend on many factors (species, growth regulators and time of culture). Structural chromosome changes are less common, but in some species they were observed with high frequency (JOACHIMIAK & al. 1993).

The number and morphology of nucleoli were analysed in interphase callus tissue cells grown for a month on MS medium with addition of 1.0, 2.5 and 5.0 μM 2,4-D or without 2,4-D. The number of nucleoli was analysed in the population of 1000 interphase cells per sample. Results on nucleoli number distribution are shown in Fig. 1.

The number of nucleoli in meristematic cells of *A. commutatum* range from 1-4 (BESENDORFER & al. 1997). In callus tissue, interphase cells with different number of nucleoli (1-9) of equal or different size were observed. In the majority of callus cells grown on basal medium 2 or 3 nucleoli were observed, while the number of cells with 4 or more nucleoli increased with the concentration of 2,4-D as can be seen in Fig. 1. The variability in nucleoli number and size increased with the concentration of 2,4-D probably due to polyploidy observed in

some callus cells, but we expect a detailed and more precise explanation from our future investigations.

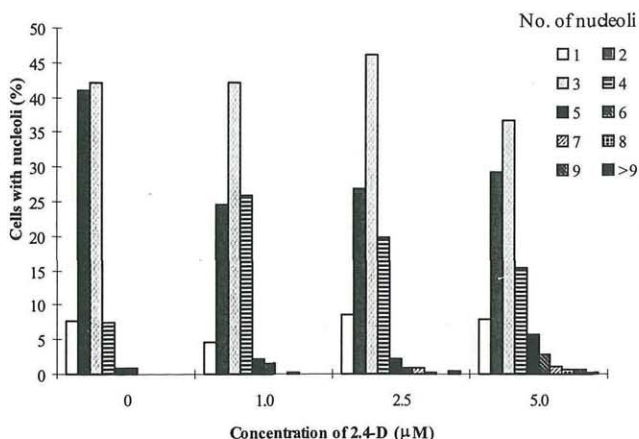


Fig. 1. The number of nucleoli (1-9) in callus tissue cells grown on MS medium supplemented with different concentrations of 2,4-D.

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