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In vitro stable regeneration of onion and garlic from suspension culture and chromosomal instability in solid callus culture

Madhumita J. Mukhopadhyay, Papiya Sengupta, Sandip Mukhopadhyay*, Sumitra Sen

Department of Botany, Centre of Advanced Study in Cell and Chromosome Research, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700019, India

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Abstract

An efficient regeneration system from bulb-derived callus tissues in suspension of onion (*Allium cepa* L.) and garlic (*A. sativum* L.) was established. Callus culture was induced in Gelrite®-solidified Murashige and Skoog's (MS) modified basal medium with 9.05 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.93 μM kinetin supplements. After 4 weeks of induction, the callus tissue was partly transferred to liquid MS media containing different levels of α-naphthaleneacetic acid (NAA) and kinetin for plant regeneration and the rest was maintained in the same medium for chromosome analysis and nuclear DNA quantification following in situ microspectrophotometry. The cultures in suspension, maintained in agitated condition for 8 weeks, showed a high frequency of rapidly regenerated plants after transferring to Gelrite®-solidified one half strength of MS basal medium. Chromosome analysis of the regenerated plants, transferred to the field with 90% survival rate, revealed stable chromosome number ($2n = 16$) in both species. On the other hand, callus tissues maintained in solid induction medium for long period showed abnormality in chromosome behavior leading to the formation of both hypo- and hyper-diploid cells along with the diploid cells. The frequency of aneuploid cells (2.2–48.9%) increased with callus age in both species with high and statistically significant number of hyperdiploid cells. The role of endoreduplication as well as non-

* Corresponding author. Fax: +91 33 2474 1042.
E-mail addresses: sanmukh@vsnl.net, sandip135@yahoo.com (S. Mukhopadhyay).

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disjunction of chromosomes resulting in instability in chromosome number has been suggested. This was also supported by the nuclear DNA value in successive passages with statistically significant increase.

Keywords: Alliaceae; *Allium*; Callus culture; Chromosome; Nuclear DNA; Plant regeneration

1. Introduction

The species of *Allium*, particularly *A. cepa* and *A. sativum* of Alliaceae are important vegetable crops worldwide. They also have medicinal value as a possible cancer preventive (Krest and Keusgen, 1999; Steinmetz and Potter, 1996). As these crops are propagated through vegetative means, micropropagation through organ culture and regeneration from callus tissue would be viable alternative for raising elite clones with the objective of enhancing the rate of multiplication (Mukhopadhyay and Desjardins, 1994a; Benmoussa et al., 1996; Chakraborty and Sen, 1988; Mukhopadhyay et al., 2002). There are a few reports on callus regeneration of *Allium* species (Ayabe et al., 1995; Barandiaran et al., 1999; Zheng et al., 1998), though gynogenesis in onion has been possible earlier (Bohanec et al., 1995). However, regeneration from suspension culture in species of *Allium* has yet to be established.

Callus tissue is considered to be the most useful source of genetic variation in plant species. Chromosome structure changes in cells in culture under different conditions have been well documented (Bayliss, 1980; Mukhopadhyay et al., 2000) despite the fact that genetic stability has also been reported in several plant species (Mukhopadhyay et al., 1989, 1991; Mukhopadhyay and Desjardins, 1994b; Benmoussa et al., 1997). Different factors thought to be responsible for such variation in culture include choice of explant, type of growth regulator and duration of culture that resulted in endoreduplication, non-disjunction and fragmentation of chromosomes (D'Amato, 1985; Mukhopadhyay and Sharma, 1990; Mukhopadhyay et al., 2000).

In the present investigation, we have studied the influences of growth regulators, state of culture and light intensity on regeneration potential and chromosomal status of callus cells of two species of *Allium*. In situ 4C nuclear DNA amount has also been estimated to indicate correlation, if any, with the changes in chromosome number.

2. Materials and methods

2.1. Materials

Bulbs of two common species of *Allium*, *A. cepa* var. *rosette* and *A. sativum* var. *rosette* were collected from local markets and grown in an incubator at 18–20 °C. The inner fleshy scale leaves of the bulbs containing basal disc regions were chosen as explants.