Journal of Biotechnology
and Crop Science
5(7): 82-84, 2016

Short Communication

Standardiazation of regeneration protocol of '*Canna indica*' in different nutrient media

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Received: 12 October 2016 Revised Accepted: 05 December 2016

ABSTRACT

Different nutrient media were used to test the totipotency of *Canna indica.* The *Canna* responded by exehibiting growth after the 7-12 days in presence of MS media. It was also observed that Canna grew fast through tissued cultured rasied bulbs and it was best, when at least 6-8 hours of sunlight each day is provided during the growing season. This protocol for plant tissue culture raised clone was standardized at National Botanical Research Institute (NBRI), Lucknow laboratories.

Key Words: *Canna, Callus regeneration, Field condition, Growth regulators, MS media*

Canna (*Canna indica*) is one of the most colorful summer bulbs which have flamboyant appearance, as they look like tropical American ancestry with ruffled spikes tapering to refined buds. Usually these perennials are popular for vast variety of color and boast immense, which are often veined, paddle-shaped leaves sheathing leafstalks in shades of green or bronze. *Canna* is also known as *canna* lily as exactly it is not a true lily. *Canna* genus has 19 species of flowering plants which belongs to *Cannaceae* (Tanka *et al* 2001). *Canna* grow best in full sun with moderate water in well-drained rich or sandy soil. They grow from perennial rhizomes, but are frequently grown as annuals in temperate zones for an exotic or tropical look in the garden. In arid regions, cannas are often grown in the water garden, with the lower inch of pot submerged. In all areas, high winds tear the leaves.The rhizomes are frost tender and will rot if left unprotected in freezing conditions.The areas where about −10 °C temperature in the winter, the rhizomes can be dug up before freezing and stored in a protected area (above 7 °C) for replanting in the spring, otherwise, they should be protected by a thick layer of mulch over winter. *Canna* seeds have a very hard seed coat, which contributes to their dormancy. Germination is facilitated by scarification of the seed coat, which can be accomplished by several techniques (Organic gardening). The species are known for its huge, attractive foliage and horticulturist who turn it into the large-flowered and brighten garden plant. It is famous for its world's richest starch source in an agricultural plant. Plants of tropic region have mostly developed in the temperature climate and are easy to grow in most of the countries of the world. Generally they receive at least 6-8 hours average sunlight during the summer season and require temperature between 23-25^{o}C. The flowers of *Canna* are found in different typical colors such as red, orange, yellow or any combination of those colors which are aggregated in inflorescences that are spikes or panicles. They seem to be so beautiful and they attract pollinators to collect nectar and pollen such as, bees, humming birds, sun birds and bats. The mechanism of pollination is conspicuously specialized when the pollen is shed on the style the bud and the species and the early hybrids are found on the stigma because they are present on the high position of anther, which indicates that they are self-pollinating. *Canna* lacks much of research work. Kromer and Kukulczanka (1985) found that meristem tips of *Canna indica*

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survived better on a mixture of 25g/l glucose plus 5g/l fructose, than on 30g/l sucrose. It has been reported that few of canna cultivars are edible perennial plants with high starch content in the rhizome.

Two set of media were used in present study have been listed in Table 2 and 3. Regeneration of callus was done by the help of explant we used. The very basis of regeneration in tissue cultures is the recognition that somatic plant cells are totipotent (i.e. capable of giving rise to whole plant) and can be stimulated to regenerate into whole plants in-vitro via organogenesis (shoot formation) or somatic embryogenesis. They are given the optimum hormonal and nutritional conditions (Skoog and Miller 1957) for regeneration. Adventitious shoots or embryos are supposed to differentiate giving rise to non-chimeric transformed plants after gene transfer.

Table 1 Used different growth regulators for Canna.

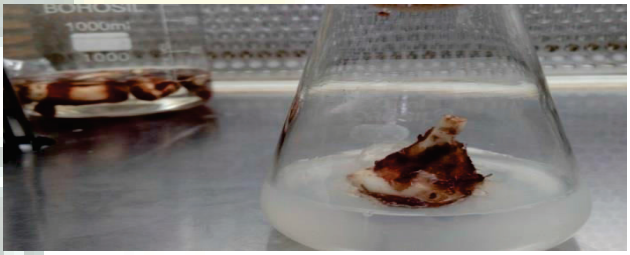
Growth regulators(mg/ml)	Required concentration (mg/l)
TDZ (thidiazuron) (1mg/ml)	1.0mg/l
2,4-D (2, 4-dichlorophenoxyacetic-acid)(1mg/ml)	5.0 mg/l
NAA(napthaleneacetic acid) (1mg/ml)	1.0 mg/l
Kinetin (1mg/ml)	1.0 mg/l
TDZ (thidiazuron) (1mg/ml)	2.0mg/l
GA (gibberellic acid) (1mg/ml)	1.0 mg/l
IAA (Indole-3-acetic acid) (1mg/ml)	1.0 mg/l
BAP (benzyl-amino purine) (1mg/ml)	5.0 mg/l
TDZ	1mg/l
2,4-D (2, 4-dichlorophenoxyaceticacid) (1mg/ml)	5 mg/l
NAA(napthaleneacetic acid) (1mg/ml)	1.0 mg/l

Table 2 Used different growth regulators for Rhizome.

Growth regulators(mg/ml)	**Required concentration (mg/l)**
BAP (benzyl-amino purine) (1mg/ml)	5.0 mg/l
Kinetin (1mg/ml)	2 mg/l
Ascorbic acid (20mg/l)	100mg/l
Ads	10mg/l

1a Surface sterilization of Canna Rhizome.

1b Removing outer dead layer of Rhizome.



1c RhizomeInoculation in growth hormone medium.

1d Transfer of developed shoot in MS Growth medium.

1e Shoot development in MS liquid growth medium.

1f Group of Inflorescence stalk from Canna field.

Figure 1 Different steps of Canna shoot development in MS medium.

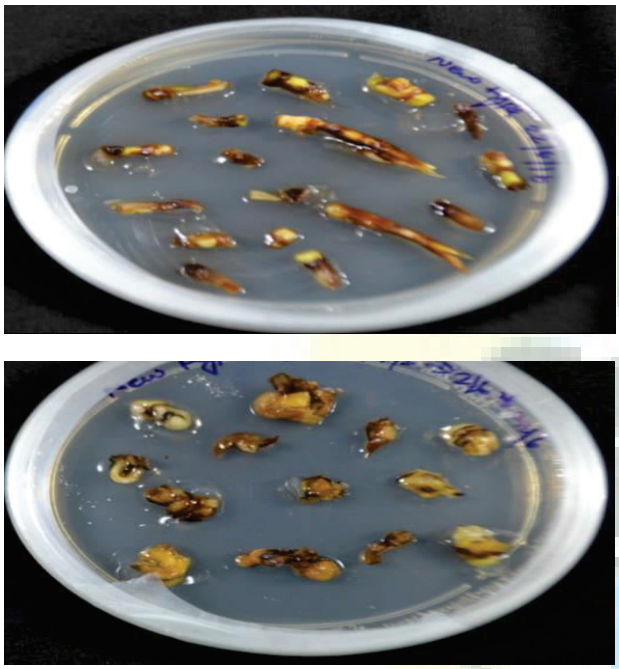


Figure 2 Inoculation and induction of inflorscence stalk (Induction of callus) in MS Growth medium

Phytohormones or growth regulators were used to induce callus tissue and the growth of many cell lines. As an auxin, 2, 4- dichlorophenoxyacetic acid (2, 4-D) or naphthalene acetic acid (NAA), were the two growth harmones were frequently used. The concentration of auxins in the medium was generally between 0.1 to 50l. Kinetin or benzylaminopurine (BAP) as cytokinin were occasionally used together with auxins for callus induction at concentrations of 0.1 to 10l. Other derivatives of auxin and kinetin were also used in some cases. Since each plant species requires different kinds and levels of phytohormones for callus induction for its growth and metabolite production, it is important to select the most appropriate growth and regulators and to determine their concentration. Gibberellic acid was also added to the medium if necessary. The *Canna* responded by exehibiting growth after the 7-12 days in presence of MS media. It was observed that *Canna* grew fast through tissued cultured rasied bulbs and it was best, when at least 6-8 hours of sunlight each day is provided during the growing season. This protocol for plant tissue culture raised clone was standardized at National Botanical Research Institute (NBRI), Lucknow laboratories. The MS Media is the best media for regeneration **'***Canna indica'*.

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