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REGENERATION OF PLANTLETS FROM LONG TERM CALLUS CULTURES OF *CUCURBITA PEPO L.*

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The well established compact callus on MS+ 2.0mg/l BAP+1.0mg/l IAA as well granular callus on 2.0mg/l KN+ 1.0mg/l IAA of *Cucurbita pepo* was tested for regeneration. The old callus (180 days old) cultured on MS medium containing high amounts of BAP (10.0-20.0mg/l) + 0.5 mg/l NAA resulted in shoot bud differentiation after two successive subcultures. High frequency regeneration was achieved on MS+ L-Glutamic acid (2.5 mg/l) in combination with kinetin (2.0 mg/l). High amount of sucrose suppressed the regeneration. Low amount of sucrose (1.5 %) enhanced the regeneration. The differentiated buds were transferred to the MS basal medium to obtain shoots. Shoot buds were rooted on MS basal salts containing 3.0 mg/l NAA.

Keywords : *Cucurbita pepo*; Differentiation; Longterm callus; Regeneration; Sub cultue.

Introduction

The procedures of plant tissue culture have developed to such a level that any plant species can be regenerated *in vitro* through several methodologies. The rate of plant regeneration in tissue cultures varies greatly from one species to another[1]. Various cells, tissues and organs from numerous plant species can be cultured successfully to regenerate complete plants [2-4]. Several reviews dealing with plant regeneration have been published in the past few yeas [5-7]. The plants of cucurbitaceae provide a major portion of vegetable and they need to be investigated for maximum utilization. Differentiation leading to the formation of plantlets in callus cultures derived from plants of many familieis have been reported. It has been observed that in short term cultures morphogenesis is readily achieved. In this paper we report the induction of plantlets in longterm callus cultures of *Cucurbita pepo* L.

Material and Methods

The seeds of *Cucurbita pepo* L. were obtained from local field. Seeds were germimated asceptically on paper bridges. After germination cotyledons and hypocotyls were cut into small segments and inoculated on MS medium fortified with 2.0 mg/l BAP+ 0.5 mg/l IAA for induction of callus. After 6 months the callus was tested for regeneration on MS medium supplemented with (10,15,20,25, 30 mg/l) BAP + (0.5 mg/l 1.0mg/l) NAA. The culture tubes were incubated under 2000 lux for 16 hours 25± 2°C.

Results and Discussion

The cotyledons and hypocotyls induced callus on MS medium fortified with (1.0, 2.0, 3.0 mg/l) BAP + 1.0 mg/l IAA (Fig. 1) was removed from the segments and cultured in fresh medium of the same composition. After 3 passages (one passage = 30 days) the callus was transferred to MS basal salts containing 5,10,15,20,25 mg/l BAP + 0.5mg/l NAA (Table 1). In the second passage the callus was turned to green, the subsequent subculture resulted in shoot bud differentiation (Fig.2). However the percent frequency of growth response of cultures were less, but shoot bud differentiation observed at the base of the callus after 30 days of culture. High concentration of BAP (10.0-20.0 mg/l) promoted the differentiation of plants (Fig.3).

In the present study however the callus was 6 months old, it was differentiated into buds and plantlets. The promotion of green spots achieved in *Cucurbita* on MS medium supplemented with 30.0 mg/l BAP + 0.5 mg/l NAA. Similar results were observed from pericarp tissue with IAA treatment[8]. Incorporation of BAP increased the frequency of aerial out growths produced in presence of NAA alone. BAP has generally found to stimulate bud formation [9-10]. The growth and differentiation was observed in callus cultures of cucurbitaceae after few subcultures only. Change in the physiological status of callus was considered as a factor for the loss of ability to form organ[11] may also explain regeneration of buds in old cultures, which failed to do so in its early history. That such change in the physiology of callus is brought about during successive subcultures is clearly indicated from the studies of Syono[12] and Negruitus[13]. High frequency of plantlet regeneration was achieved on MS medium supplemented with 2-5 mg/l L-Glutamic acid and 1.0 mg/l KN (Table 2, Fig.4). According to Krishisagar and Mehta[14] low levels of L-Glutamic acid enhance the growth and differentiation of tissues of plants. Matsubara[15] tested 18 amino acids for the cultures of young *Datura patula* embryos and observed that all except glutamine were inhibiting. In *Luffa* shoot bud differation was observed on MS basal medium fortified with 2.0 mg/l

Table 1. Effect of BAP + NAA on regeneration of plantlets from long term callus cultures of *Cucurbita pepo*.

Hormones (mg/l)	Morphogenetie response
1.0 BAP + 0.5 NAA	Callus growth enhanced.
1.0 BAP + 0.5 NAA	Callus growth enhanced.
1.0 BAP + 0.5 NAA	Greening of callus
10.0 BAP + 0.5 NAA	Callus + small shoot buds.
15.0 BAP + 0.5 NAA	Average No. of shoots
20.0 BAP + 0.5 NAA	Average No. of shoots
25.0 BAP + 0.5 NAA	Greening of callus
30.0 BAP + 0.5 NAA	Callus growth is suppressed
10.0 BAP + 1.0 NAA	2-3 small buds
15.0 BAP + 1.0 NAA	Few shoots formed
20.0 BAP + 1.0 NAA	No shoot formation
25.0 BAP + 1.0 NAA	No shoot formation

Table 2. Effect of L-Glutamic acid + Kinetin on high frequency regeneration of plantlets after two subcultures.

Hormones (mg/l)	Morphogenetic response
0.5 L.Glutamic acid + 0.5 KN	Callus growth promoted
1.0 L.Glutamic acid + 1.5 KN	Greening of callus
1.5 L.Glutamic acid + 0.5 KN	2-3 shoots + callus
2.0 L.Glutamic acid + 0.5 KN	Few shoots developed
2.5 L.Glutamic acid + 0.5 KN	No shoot formation
3.0 L.Glutamic acid + 0.5 KN	No shoot formation
1.0 L.Glutamic acid + 1.0 KN	Greening of callus
1.5 L.Glutamic acid + 1.0 KN	Shoot buds formed
2.0 L.Glutamic acid + 1.0 KN	More number of shoots formed
2.5 L.Glutamic acid + 1.0 KN	High frequency of regeneration
3.0 L.Glutamic acid + 1.0 KN	High frequency of regeneration
3.5 L.Glutamic acid + 1.0 KN	Average No. of shoot buds

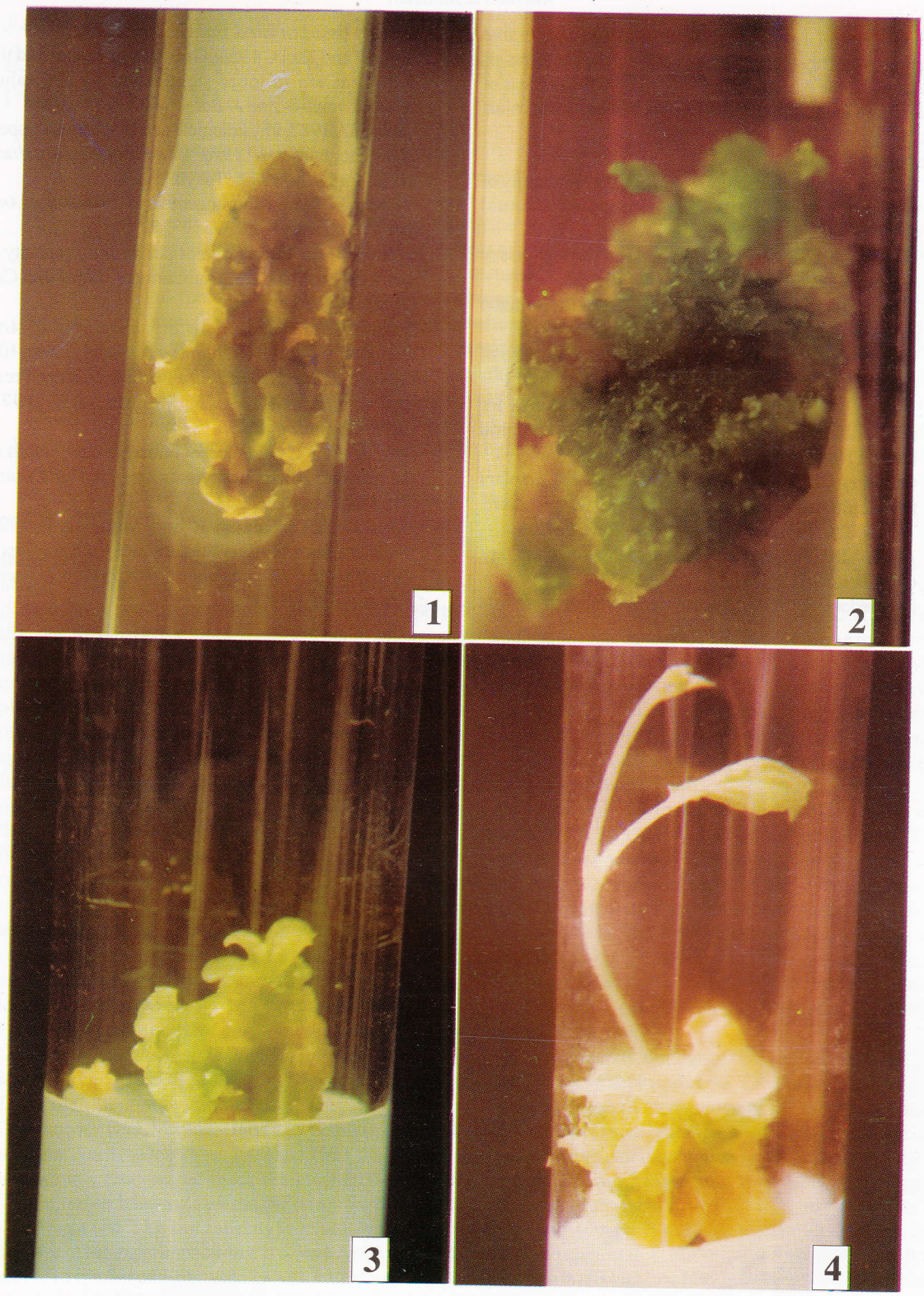

FIG.1. Induction of callus from hypocotyls on MS-medium with 2.0 mg/l BAP+ 1.0 mg/l IAA.
2. Differentiation of shoot buds on MS+10.0 -20.0 mg/l BAP + 0.5 mg/l NAA.
3. Differentiation of plantlets on MS + 20.0 mg/l BAP.
4. High frequency of plantlets regeneration on MS-medium +3.0 mg/l L-Glutamic acid+ 1.0 mg/l KN.

BAP + 0.5 mg/l L-Glutamic acid after the successive subcultures[16]. The change in physiological characters of callus may be the determining factor in both the process of loss and gain in morphogenetic response of longterm callus cultures.

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