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Multiple shoot induction from Stem node explants of Cucurbita maxima (L). – A medicinal plant

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ABSTRACT: The MS medium supplemented with different concentrations of Cytokinins alone and in combinations with various auxins were used for high frequency shoot regeneration from nodal explants. In vitro shoot and multiple shoot induction was achieved in one of the important medicinal plants of cucurbitacae family Cucurbita maxima (L) which has been historically been used to treat a wide assortment of diseases. MS medium supplemented with 1.0 mg/l BAP was found to be optimum to induce shoots (100%) directly from the node explants. Significant increase in the number of shoots per explant was found in MS medium supplemented with 1.0 mg/l BAP and 15 mg/l Adenine Sulphate. All the tested combinations have little effect on increasing the number of shoots. The present study established reliable and reproducible protocol for rapid multiple shoot induction from node explants of Cucurbita maxima (L) using different concentrations and combinations of cytokinins. Micro shoots developed in the above culture rooting was also induced by BAP, NAA+Kn 0.5mg/l to 5.0 mg/l from callus. Maximum frequency of callus with small shoots induction was observed with different normal concentrations with MS Medium.

Keywords: Multiple Shoots, Cucurbita maxima, BAP Kn L-Glutamic acid

I. INTRODUCTION

Plant tissue culture organogenesis is a process of differentiation by which plant organs simultaneously adventurous development of callus with plant lets. The existence of genetic variability in the form of wild relatives of domestic crops is the source for continued improvement in yield and resistance to disease or stressful changes in environmental conditions. The primary aim of this study has been to gain some knowledge about the genotypic differences for callus initiation and high frequency plant regeneration from long term callus cultures of Cucurbita maxima (L). This variety resembles cucumber and is used as a vegetable. The fruits are slender and elongated, the length varying from a few inches to about 3ft. They are pale or dark green in colour, smooth or ridged, with soft downy hairs covering the skin when tender. Seeds are smaller than those of the musk melon. This variety is cultivated both as a hot weather crop and as a rainy season crop. It grows on any kind of soil, but thrives best on well – manured rich loamy soils with abundant water supply. The seeds are small and edible, and are used in confectionery. Several workers in past have micropropagated some of the important Asclepiadaceae members such as Ceropegia bulbosa (Patil, 1998; Britto et al., 2003), Venkateshwarlu (2020) & Thoyajalosa & Rai (2016). Hemidesmus indicus (Misra et al., 2003; Patnaik and Kishore, 1996) Venkateshwarlu et al (2018) & Venkateshwarlu (2017). and Holostemma ada-kodien (Martin, 2002, 2003). Since very scarce information is available about micropropagation about this important medicinal plant, an attempt was made to develop a reproducible protocol for shoot and multiple shoot induction from nodal explants of one of the tissue culture recalcitrant medicinal plants of cucurbitaceae family, Cucumis melo var. Utilissimus. Using various concentrations of Benzyl Amino Purine and Adenine Sulphate. In the recent years there has been a major crop plant development Application In Vitro culture an important field crop improvement.

II. MATERIALS AND METHODS

The isolated in vitro raised explants segments observations were recorded on yield and various yield traits on normal loading plants selected randomly. MS medium supplemented with different concentrations of Benzyl Amino Purine (BAP) (0.1-1.2mg/l were used for shoot induction. For multiple shoot induction MS medium supplemented with 1.0 mg/l BAP and 5-20 mg/l Adenine Sulphate were used. The pH of all media was adjusted to 5.75 before adding 0.8% agar and autoclaved at 151b and 121°C for18 min. All the media were kept at 26±2°C for 3 days before use. The shoot segments after removing the leaves were cut into 2cm pieces, each containing a single node region and washed under running tap water for 15

min, followed by brief washing with sterile distilled water. Node explants (1.25 cm) were surface sterilized in 70% (v/v) ethanol for 60 sec followed by 0.1% (w/v) mercuric chloride for 6 min. explants were thoroughly washed in sterile distilled water and blot dried on sterile Whatmann 1 mm filter paper. For shoot induction, nodal explants were again trimmed into 1.0 cm and transferred to MS medium supplemented with 0.1 – 1.0 mg/l BAP. Cultures were incubated at 26±2° under a 16/8 h photoperiod for 26-28 days at a relative humidity of 65%. Node explants (1.0 cm long) were used as explants for multiple shoot induction on MS medium fortified with 1.0 mg/l Benzyl Amino Purine and 5-20 mg/l Adenine Sulphate. After two weeks of culturing at 26± 2° under a 16/8 photoperiod shoots were subcultured onto fresh medium for proliferation. All the experiments were repeated thrice (each with 15 explants) and the response was scored after 26-28 days of culture initiation. The data pertaining to mean percentage of culture showing response, number of shoots per explants and shoot length were scored and presented in mean ±SE. The perusal of clustering pattern indicated that the grouping was not influenced by the place of organ.

III. RESULTS AND DISCUSSION

Different combination of MS Media is used for organogenesis high concentrations of nitrogen as ammonium unlike media. The cultivation of suspensions in liquid Agar medium growth regulators concentration in the culture medium in critical for morphogenesis callus tissue comprises a wide range of cell types. The results scored on the above mentioned aspects (shoot and multiple shoot induction) are summarized in the following order. In order to assess the effect of different concentrations of Benzyl Amino Purine (0.1-1.2 mg/l) on shoot induction from Cucurbit maxima nodal explants were surface sterilized and inoculated onto MS media supplemented with various concentrations of Benzyl Amino Purine. Shoot induction was monitored after 24-28 days of inoculation by counting the number of shoots induced from each explant. Shoot induction was observed in all the concentrations of Benzyl Amino Purine tested with variation in per cent response of shoot induction. The highest per cent of shoot induction was observed in MS with 1.0 mg/l Benzyl Amino Purine followed by 80.4 and 80.2 in the medium containing 0.8 and 0.7 mg/l Benzyl Amino Purine respectively (Tabe 1). The number of shoots produced from nodal explants on medium with 1.0 mg/l BAP was 3.8 with an average height of 2.5 cm (Figure 1.). We found an increase in the per cent response of shoot induction and number of shoots with an increase in the concentration of Benzyl Amino Purine from 0.1 mg/l to 1.2. The percentage of explants exhibiting shoot induction was found to be between 40-80 is most of the concentrations of Benzyl Amino Purine tested except MS medium supplemented with 1.0 mg/l Benzyl Amino Purine. After 26-28 days of culture, nodal explants derived shoot cultures were subcultured to MS medium fortified with same concentration of hormone for shoot elongation. Significant elongation has been achieved in medium with 0.8 and 1.0 mg/l Benzyl Amino Purine. There was no significant variation in shoot length between the different concentrations of Benzyl Amino Purine except in the case of medium with 0.2 mg/l producing average shoot length of 2.74 cm (Table 1, Plate 1). The shoots sub cultured to fresh medium with same concentration of Benzyl Amino Purine proliferated additional 3-4 shoots after 26 days of culture.

In general, the nodal explants cultured on medium with Benzyl Amino Purine developed pale yellow intermediate callus at the basal portions due to the accumulation of auxins at the basal cut ends (Figure 1). The effect of Benzyl Amino Purine in inducing shoot induction was already reported in some of the important medicinal plants of Asclepiadaceae family members such as Ceropegia bulbosa (Patil, 1998; Britto et al., 2003), Gymneme elegans (Komalavalli and Rao, 2000) and in Holostemma ada-kodien (Martin, 2002). The promotive effect of Benzyl Amino Purine on shoot induction and multiplication was well understood in various plants like Phytolocca decanta (Demeke and Huges, 1990), Saussuriea lappa, Clerodendran colebrookianum (Mao et al., 1995), Trichopus zeylanicus (Krishnan et al., 1995) and in Woodfordia fruticosa (Krishnan and Seeni, 1994). To analyse the shoot induction ability of nodal explants from in vitro multiplied plants, nodal explants were used as an ideal source of explants for reculturing. Additional 2-3 shoots per node explants on MS medium fortified with 1.0 mg/l indicate the effectiveness of explants on multiple shoot induction without surface sterilization (Figure 2). A similar effect of the hormone in enhancing shoot induction has been reported in one of the Asclepiadaceae family members, Ceropegia candelabrum (Beena et al., 2003). As expected, contamination rate has been drastically reduced in recultured nodal explants. The auxin and increasing the cytokinin combinations is traditionally performed to induce multiple shoots organogenesis from green callus.

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Table 1: Multiple shoot induction from stem node explants of Cucurbita maxima (L)

S.No.	BAP+KN+L-Glutamic acid concentration (mg/l)	Shoot length (cm) (Mean ± SE)	No of shoots produced per explant (Mean ± SE)
1	0.1	1.20±0.42	1.12±0.44
2	0.2	2.50±0.07	1.20±0.42
3	0.3	1.2±0.44	1.22±0.46
4	0.4	1.70±0.09	24±0.42
5	0.5	1.40±0.06	1.50±0.40
6	0.6	1.82±0.05	1.6±0.47
7	0.7	1.8±0.05	2.1±0.36
8	0.8	2.04±0.06	2.1±0.38
9	0.9	2.08±0.08	1.4±0.40
10	1.0	2.9±0.08	3.6±2.6

Callus may be serially sub cultured and grown for extended periods but its composition and structure may change with tissue as certain cells are favored growth by MS Medium. In the present study, Adenine Sulphate when used in combination with Benzyl Amino Purine induced multiple shoots. Among the combinations tested, Benzyl Amino Purine (1.0) with 5.0 mg/l Adenine Sulphate produced maximum number of shoots with intermittent callus at the basal cut end. Of the various concentrations of Adenine Sulphate tested, 15 mg resulted in maximum number of shoots followed by 20mg/l (9.8), 10mg/l (6.5) and 5mg/l (6.8). Average number of shoots generated per explant on medium with 1.0 mg/l Benzyl Amino Purine and 15 mg/l Adenine Sulphate is an improvement of almost 3 fold in the multiplication rate as compared with shoots induced on MS medium with 1.0 mg/l Benzyl Amino Purine alone. Addition of 5 and 10mg/l Adenine Sulphate had no significant effect on number of shoots produced per explant. The presence of Adenine Sulphate initiated friable callus and suppress the formation of new shoots. A similar observation was reported in Hemidesmus indicus using 5-20 mg/l Adenine Sulphate with Benzyl Amino Purine and naphthalene acetic acid (Misra et al., 2003). The present study identified the concentrations of cytokinins like Benzyl Amino Purine and Adenine

Plate 1: Multiple shoots from stem node explants Cucurbita maxima (L)

IV. CONCLUSION

The great potential of In vitro methods for large scale plant production multiplication can be tapped by cutting down the cost of production per plant by applying low cost tissue culture methods. Explants from both mature and immature organs can be induced to from callus and then plant regeneration.

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