Effect of different growth hormones on the in vitro development of shoot and root in Turmeric (Curcumalonga L.)

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Abstract- *The present investigation was carried out to study the effect of different growth hormones, namely auxins and cytokinins and their different concentrations on in vitro development of shoot and root in Turmeric local variety Erode. Sprouted rhizome tips were used as explants and they were surface sterilized with tween-20 and 0.1% mercuric chloride. For culture, MS medium was used alone and in combination with different concentrations of NAA, IBA and BAP. The pH of medium was adjusted to 5.8 and the culture was maintained at a temperature of 25 ^{0}C with a photoperiod of 16 hours light. Subculture was done after 30 days of inoculation on the same fresh medium. There was a significant influence of BAP alone or in combination with NAA and BAP at 2.0 mg/l in producing the highest number of shoots. IBA at 0.5 mg/l gave a highest rooting success than NAA. Thus, turmeric plants could be micro propagated by using MS medium with 2.0mg/l BAP + 0.2mg/l NAA for shoot multiplication and MS medium with 0.5 mg/l IBA for rooting.*

Keywords- In vitro, Turmeric, MS medium, NAA, IBA.

I. INTRODUCTION

Turmeric (Curcuma longa L.) is one of the important spice crops. It is a rhizomatous perennial crop belonging to the family Zingiberaceae. The rhizomes are processed and used for various purposes. It is called 'Indian saffron' because of its orange yellow colour. Turmeric has the valuable properties of spice, colorant, cosmetic and drug.

As a spice, turmeric is used for its coloring property and aroma. It is an important ingredient of curries and curry powder. Important constituents of turmeric rhizomes are curcumin (2 – 8%) and volatile oil (5 – 6%). Turmeric is used for dying wool and cotton fabrics. It is also employed as a coloring material in pharmacy, confectionary and food industry, as well as in paints and varnishes. In cosmetics, turmeric is an inexpensive and indigenous beauty aid.

Traditionally turmeric is being used in Indian system of medicine as stomachic, carminative, blood purifier, vermicide and antiseptic. Wound healing antiseptic property of turmeric is well know to Indians since long. Curcumin is the main biologically active phytochemical compound of turmeric with wide range of therapeutic effects. In recent years, pharmacological properties and actions of curcumin have been widely researched and its beneficial effects have been well established. Curcumin is known to have a marked anti inflammatory effect. Curcuma also protects liver from a number of toxic compounds. Clinical and laboratory research have indicated that the diet that include turmeric or curcumin stabilize and protect biomolecules in the body at the molecular level which is shown in its anti mutagenicand anti carcinogenic properties. Turmeric organated in South East Asia and India has the predominant position as the largest producer in the world. Though India is the leading producer of turmeric, average productivity and quality are not satisfactory. Few cultivars can yield 30 – 35 t/ha of a turmeric but actual realized yeild is much less. This is attributed to very little work carried out in crop improvement. However, effects on crop improvement have been made in recent years through clonal selection for exploiting naturally occurring variation. Several varieties have been released through this method. Other method of crop improvement such as hybridization have not been possible due to reproductive sterility [1].

The major production constraints in turmeric are long duration, low rhizome yield, low curcumin, content of popular varieties and incidence of foliar diseases. Advent of in vitro techniques has opened new avenues in important of crop plants. Tissue culture technique have been standardized for many horticultural crop. The in vitro propagation of turmeric was first attempted by Nadagouda et al., 1982. However, commercial application of this technique for propagation has limitation on account of smaller size of rhizomes in the first generation of plantlets and high cost [2]. Still, this technique can be used for crop improvement studies. There is need to standardize the requirements of in vitro techniques for local varieties.

The development of efficient in vitro culture methods has facilitated the improvement of both seed and vegetativly propagated plants. In many vegetativly propagated crops in vitro culture technique is the only effective method for crop improvement [3].

Considering the above facts, the present investigation was carried out to study the effect of different growth hormones and concentrations on the in vitro development of shoot and root in turmeric local variety "Erode".

II. MATERIALS AND METHODS

Ex plant: Healthy rhizomes of Erode variety of turmeric were placed in moist sand for sprouting. When rhizomes just sprouted, the sprouted tips were scooped and used as explants.

Surface sterilization: The scooped shoot tips were first washed with running tap water. Two drops pf tween-20 were added and the shoot tips were washed with distilled water. Washed explants were then treated with 0.1 per cent mercuric chloride for 5 minutes. Explants were then washed with sterile distitted water.

Preparation of media: The basal medium used for the study was MS medium suggested by [4]. Growth regulators used for the study were NAA, IBA and BAP. pH of the medium was adjusted to 5.8 using 0.1N NaOH or HCL. After making up the volume, agar was added 90.6%.

Inoculation: The laminar flow cabinet was prepared for inoculation by swabbing the table with 80% ethanol and then switching on the UV lamp for 20 minutes. The surface sterilized shoot tips were placed in the autoclaved tubes in the laminar flow cabinet.

Subculture: The inoculated explants and cultures were transferred to new fresh medium after 30 days of inoculation by separating them into individual shoots.

Culture conditions: The inoculated cultures were incubated in the culture room at a temperature of 25^0C with a photoperiod of 16 hours light.

Effect of different levels of auxins and cytokiniens on the multiplication of shoot

MS + 0.5 mg BAP + 0.1 mg/l NAA
MS + 0.5 mg BAP + 0.2 mg/l NAA
MS + 0.5 mg BAP + 0.5 mg/l NAA
MS + 1.0 mg BAP + 0.1 mg/l NAA
MS + 1.0 mg BAP + 0.2 mg/l NAA
MS + 1.0 mg BAP + 0.5 mg/l NAA
MS + 2.0 mg BAP + 0.1 mg/l NAA
MS + 2.0 mg BAP + 0.2 mg/l NAA
MS + 2.0 mg BAP + 0.5 mg/l NAA
MS + 3.0 mg BAP + 0.1 mg/l NAA
MS + 3.0 mg BAP + 0.2 mg/l NAA
MS + 3.0 mg BAP + 0.5 mg/l NAA

The design employed was factorial completely randomized design (CRD).

Observation recorded: Observations were recorded after 30 days of culture on the following attributes.

a. **Number of shoot per explant:** While sub-culturing, multiple shoots were separated, counted from five explants and expressed as shoot per explant.

b. **Length of shoots:** Multiple shoots of five explants were measured with a scale during subculture and mean expressed in centimeter.

Effect of different levels of auxins on rooting:

There were eight treatment in factorial CRD and each treatment repeated three times. The treatments details are as follows:

MS + 0.2 mg/l NAA
MS + 0.5 mg/l NAA
MS + 1.0 mg/l NAA
MS + 2.0 mg/l NAA
MS + 0.2 mg/l IBA
MS + 0.5 mg/l IBA
MS + 1.0 mg/l IBA
MS + 2.0 mg/l IBA

Observations recorded:

a. **Per cent rooting:** Number of shoots producing roots were counted and expressed as per cent.
b. Number of roots produced per shoot from five cultures was mean was calculated.
c. **Length of roots:** From each shoot, the length of longest root was measured with a scale and expressed in centimeter.

Hardening: The rooted plantlets were transferred to coir peat medium in pots after washing off the adhering agar. The plantlets were first kept in moist chamber for 15 days. Then these were planted in a potting mixture of 1:1:1 proportion of soil, sand and compost filled in poly bags and kept in shade

house. Two and half months old plants were finally planted in field.

The results of experiments conducted to standardize the protocol for tissue culture in turmeric are presented below.

Effect of BAP and NAA on shoot multiplication

The results on shoot multiplication of turmeric cv. Erode on MS medium with different treatments of BAP and NAA are as follows.

III. RESULTS

Number of shoot per explants

There was a significant influence of BAP alone or in combination with NAA, BAP at 2.0 mg/l in producing the highest number of shoots. There was a progressive increase in number of shoots from BAP 0.5 mg/l up to treatment with 2.0 mg/l. However, when medium was supplemented with 3.0 mg/l BAP, the shoot production decreased significantly.

The levels of NAA also had significant influence on the shoot production. Absence of NAA in the medium produced only 5.41 shoots. Whereas, addition of NAA increased the shoot production (2.0 mg/l). However, with different levels of NAA viz., 1.0, 2.0 or 0.5 mg/l were on par.

Interaction of effects of BAP and NAA was significant. Combination of 2.0 mg/l BAP with 0.2 mg/l NAA recording the highest number of shoots followed by 2.0 mg/l BAP with 0.1 mg/l NAA. The lowest number of shoots was observed in 0.5 mg/l BAP with out NAA.

Shoot length (cm) and number of leaves

The observation on the length of shoots as influenced by the concentrations of BAPand NAA indicated that both the levels of individual growth regulators and their interaction had significant influence. The lowest concentration of 0.5 mg/l BAP and non addition of NAA gave the higher number of leaves. As the concentration increased, the number of leaves reduced. The reduction in number of leaves was significant between the levels of BAP. Whereas, only the highest level of NAA 0.2 and 0.5 mg/l showing significant reduction in leaves. Interaction of the levels of BAP and NAA indicated the highest number of leaves at BAP 0.5 mg/l with out NAA. This treatment was on par with 0.5 mg/l and 1.0 mg/l in combination with NAA 1, 1.0, 2.0 and 0.5 mg/l and BAP 0.5 mg/l with NAA 0.2 and 1.0 mg/l.

Effect of auxin levels on rooting of microshoots of turmeric

Rooting percentage

There was a significant influence of type of auxins, their concentrations and the interaction of type and concentration on the rooting percentage. Indole butyric acid (95.50%) gave a higher rooting success than Naphthalene acetic acid. With regard to concentrations of auxins, 1.0 mg/l gave the highest rooting (100.00%), followed by 0.5 mg/l (91.17%) which were on par. Both the auxins at 1.0 mg/l produced rooted shoots. This was followed by IBA at 0.5 and 0.2 mg/l.

Number of root per shoot

Type of auxin did not influence the number of roots. Whereas, leaves of auxins and their interaction with type gave significant results. The highest number of roots was produced in medium containing 0.5 mg/l of auxin which was par with 1.0 mg/l. The lowest number roots was seen in 2.0 mg/l of auxins. Perusal of interaction effect reveals that IBA at 0.5 mg/l produced the maximum number of roots per shoot followed by NAA at 2.0 mg/l. NAA at 0.5 mg/l recorded the lowest number of roots (5.85%)

Length of roots.

Length of roots also behaved similar to number of roots with regard to significance of the effects. Auxin level of 0.5 mg/l had the highest root length, which was on par with 0.2 and 1.0 mg/l, but significantly higher than 2.0 mg/l. With respect to interaction effects, IBA at 0.5 mg/l recorded the highest root length followed by IBA at 1.0 mg/l and 0.2 mg/l which were on par. The shortest root were recorded in medium supplemented with IBA at 2.0 mg/l.

IV. DISCUSSION

Like several other monocotyledons, in herbaceous plants, new shoots are thought to be of adventitious organ and their development requires inclusion of cytokinin in the medium [5]. Often cytokinin and auxin are used for shoot multiplication. Synergistic effects of auxin with cytokinin in shoot multiplication have been reported by several workers [6,7]. In the present study, the hiest number of adventitious shoots were obtained in MS medium supplemented with BAP 2.0 mg/l and NAA 0.2 mg/l. This results confirms to the findings of [7]. However, several workers have repoted good multiplication with BAP alone [8,5].

Rooting of micro shoots require addition of auxins to the medium. Both IBA and NAA have been used for rooting. In the present study, IBA was found to be better for inducing of shoots at lower concentrations than NAA, [7].also observed that IBA at 0.2 mg/l was found to be best.

These findings also agree with the results obtained by [9] in Ginger and [10] in Turmeric.

Thus turmeric plants could be micro propagated by using MS medium supplemented with 2.0 mg/l BAP + 0.2mg/l NAA for shoot multiplication and MS medium with 0.5 mg/l IBA for rooting.

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