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National Academy of Agricultural Science (NAAS) Rating : 3.03

Effect of L-Tyrosine & Adenine Sulphate on the in Vitro Shoot Development of the Large Cardamom *(Amomum subulatum* Roxb).

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ABSTRACT: *The effect of L-Tyrosine & Adenine Sulphate on the in vitro shoot development of large cardamom was evaluated. The well-established cultures were taken for the consideration. The cultures have been transferred to the basal MS media + 1 mg/l BAP with different concentration of L Tyrosine & Adenine sulphate, both singly and in combination. The cultures were kept in 25 degree Centigrade temperature, 60% relative humidity for the 12 hours of light duration. The observation was recorded after four weeks. Result showed that a combination of both Chemicals L – Tyrosine& adenine sulphate together with concentration of 50 mg/l L-tyrosine & 40 mg /l Adenine sulphate showed the highest percentage 98 % of healthy shoots with good morphological Characteristics.*

Key Words: *Amomum subulatum roxb, micro propagation, Growth Promoters, Abbreviations- BAP – Benzyl Amino Purine, MS- Murashige & Skoog etc.*

INTRODUCTION

Large cardamom (Amomum *subulatum* roxb) is one of the important spices which are also known for its peculiar aroma & medicinal values. It a member of Zingiberaceae family under the order Scitaminane .it is also popular in India as Black cardamom because of its color. It is one of the main cash crop in India, mainly cultivated in the sub Himalayan state of Sikkim and Darjeeling dist. of west Bengal. It is also cultivated in Uttaranchal and in some other north eastern states. Nepal & Bhutan are the the other countries where the large cardamom is cultivated. It mainly grown in the sub Himalayan region of India & Nepal between elevations of 700 to 1800m. It is used as a spice and in several Ayurvedic preparations. It is shade loving plant & mainly cultivated in forests land on step hills. The plant is perennial herb having rhizomes which give rise to leafy shoots & panicles. The plant height ranges from 1.8 to 3 meters. On average of leafy shoots per healthy clumps varies from 20 to 30. The leaves are dark green, with both surfaces. But from last few years the cultivation of large cardamom has been badly affected because of the viral disease mainly viz; Chirkey and foorkey. They caused considerable crop loss in almost all the area of Sikkim & Darjeeling Dist. of west Bengal .Chirkey is characterized by mosaic appearance on the tender leaves, which causes the gradual decline of the plant. Foorkey is characterized by small tillers appears at the base of the affected plant which become stunted and fall to give any yield. Even influences also noticed to produce unproductive spikes. To fulfill the huge demand of the disease free planting material it is only best option is the micro propagation of elite high yielding verities & which less susptible to the disease is. In the present study effort was made to establish a protocol for the in vitro development of healthy planting material for supply the huge demand of the planting material for the socio economic delovepment of the state farmers & cultivators.

MATERIALS & METHODS

The fresh rhizomatous buds from disease free planting material given by the spices board Sikkim, were collected, washed properly in running tap water for one hour to remove the traces of the soil dipped in Trichoderma + 1% antifungal solution for one day. On next day the explants has been washed properly in detergent solution & then was taken to the laminar flow bench for finally wash with 10 %sodium

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hypochlorite solution for 10 minutes for the decontamination of the soil born disease, after many washes with sterilize water finally culture was inoculated in fresh medium. Initially the cultures had been kept in 18 degree centigrade for few days to avoid contamination. The well-established initiated culture had been taken for the examination. The fresh medium was made with basal MS medium with 1.mg/l BAP+ with different concentration varies from 10mg, 40mg, 80mg, 100 mg of L tyrosine & Adenine sul phat either individually or in combination. The ph of the medium was adjusted to 5.8,before autoclaving. The molten medium was dispensed in jam bottle before autoclaving.

The 6 g /l agar added to solidify the media. The medium was autoclaved for 20 minutes with 121 degree centigrade and 15 psi pressure. After three days of the observation the medium had been used for fresh subcultring the pre initiated culture. One culture / bottle have inoculated; each treatment has 30 culture bottles. all the cultures has been kept in 25 degree cartridge temperature with relative humidity of 60 to 70% for the 12 hour light/12 hours dark period.

RESULTS & DISCUSSION

The different combinations were used for the mass multiplication of the in vitro shoots. In all combination the Basal MS medium with 1 mg/l BAP were used. The evaluation was made on the basis of the growth of the shoot, length of the shoot & no of the leaves & healthy Growth of the roots after four weeks of the culture growth.

In first evaluation (Table 1) five trials was done with single use of adenine sulphate, in all trail the Basal combination MS basal medium with 1mg/l BAP with various concentration of the adenine sulfate were used which is ranging from 10 mg/l, 40mg/l, 50mg/l, 80mg/l, 100mg/l . In all the combination the trail T5 (MS medium +1mg/l BAP) with 100 mg/l adenine sulphate showed the maximum percentage 65% of the healthy shoots & 10% of the healthy roots).

In second evaluation (Table 2) five trails was done, As the previous trails the same Basal medium with 1mg/l BAP with different concentration of L-Tyrosine were used. The best response came at the concentration of 100mg/l L- Tyrosine that is 75% healthy shoots with 65% healthy roots. In the third evaluation (Table 3) the combined effect of both the chemicals was evaluated, with six trails with various concentration of the Adenine sulphate & L Tyrosine with the same basal MS medium in combination with the 1mg/l BAP. The best result came with trail T4. The combination of 40mg/l adenine sulphate and 50mg/l L-Tyrosine, this gave 98% healthy shoot and equally the healthy leaf and root development with excellent morphological characteristics. The previous work showed that the adenine sulphate with BAP in low dose (1mg/l) stimulated a good regeneration percentage (80%), The higher dose of BAP associated with adenine sul phate had been obserebed earlier for stimulation of all the parameters but also the formation of callus at the base of the plants of the *Trifoliumrepens* L by G.Vicas (2011). The 5mg/l BAP with 40mg/l adenine sulphate resulted the highest no of shoots in vitro multiplication of *Phaseolus vulgaris* by Gatica Arias M.A *et al.* (2010).Adenine sulphate initiated shoot formation from the callus but multiple shoot formation comes from in addition with BAP in the ornamental plant *Thevetiaperuviana* (Z. Garima & Batra. A, 2010). Adenine sulphate was found most suitable growth regulator require for achieving 1.2 folds increase in shoot multiplication rate in *Meliaazedarachl* L. by Husain M. K. & Mohammad A (2009). Beegum *et al.* (2007) in *Ophiorrhizaprostrata*.

The adenine sulphate at higher condition stimulated both axillary shoot development & rooting, in *Fuchsia hybrida*by K. Wroblewska (2012). The experimental data showed that the callus formation was 100% with L-Tyrosine concentration of 50mg/l. The maximum no multiple shoots per

Table 1
Effect of Adenine Sulphate Alone on in Vitro Shoot Development of Large Cardamom

Trails	*Combination*	*Conc of Adenine sulphate Mg/l*	*% of healthy shoots & leaf*	*% of healthy roots*
T1	MS+1mg/l Bap	10	5	5
T2	MS+1mg/l Bap	40	15	10
T3	MS+1mg/l Bap	50	20	10
T4	MS+1mg/l Bap	80	50	10
T5	MS+1mg/1 Bap	100	65	10

Table 2
Effect of L- Tyrosine alone in Vitro Shoot Development of Large Cardamom

Trails	*Combination*	*Conc of L Tyrosine Mg/l*	*% of healthy shoots & leaf*	*% of healthy roots*
T1	MS+1mg/l Bap	10	2	1
T2	MS+1mg/l Bap	40	55	10
T3	MS+1mg/l Bap	50	65	25
T4	MS+1mg/l Bap	80	70	65
T5	MS+1mg/l Bap	100	75	65

Table 3
Combined Effect of Adenine Sulphate & L tyrosine on in Vitro Shoot Development of Large Cardamom

Trails	*Combination*	*Conc of adenine sulphate (mg/l)*	*Conc of L Tyro-sine (mg/l)*	*% of healthy shoots & leaf*	*% of healthy roots*
T1	MS+1mg/l Bap	10 mg/l	40	35	25
T2	MS+1mg/l Bap	40	10	55	35
T3	MS+1mg/l Bap	50	40	70	60
T4	MS+1mg/l Bap	40	50	98	98
T5	MS+1mg/l Bap	80	100	85	75
T6	MS+1mg/l Bap	100	80	75	65

explants was reported with use of BAP, TDZ & Tyrosine in vitro culture of *Artemisia vulgaris L*, by Kumar Pradeep S & Kumari Ranjitha B. D. *(*2010). L – form of amino acids are commonly used, L -Tyrosine contribute to shoot initiation as stated by Singh M.P & Kumar Sunil. (2009). Smith. H. Roberta, (2012). The high frequency shoot development was reported by the use of adenine sulphate, L Tyrosine with BAP by Kumar. G. K *et al*. (2011). In another work by Afsan Nazz *et al*., (2014) reported that the shoot necrosis was considerably reduced by the use of adenine sulphate. The callus formation was reported 100% by 50 mg/l use of L Tyrosine in *GloriosaSuperba* Linn by Muangsan. N (2008) The present study shows that the multiple shoot induction is govern by the proper use of the Growth regulating substances, which varies from species to species & depends on the nature of the plant. Here study was emphasized to establish the synergistic effect of both adenine sulphate & L Tyrosine on the multiplication & shoot & root development of large cardamom. From our study it revealed that the 40 mg/l Adenine Sulphate & 50 mg/l L- Tyrosine is the best combination for the efficient & healthy shoo & root development of plant. The healthy Plantlets transferred to the green house where it showed 98% survival Rate. The study provides an efficient in vitro propagation of method for the production of the healthy production of the shoots, which could be commercially feasible for the large cardamom. This works reports the highest number of the micro propagated shoots within four weeks' time period of growth by the above said concentration of both the chemicals. By judicious use of this technique the huge demand of the Planting material can be fulfilling in limited time period.

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