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Study of meta- topolin in multiplication culture of local variety Champa (*Musa* spp.) through *in vitro* culture

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Abstract
The study was conducted to assess the influence of meta-Topolin (mT) and Benzyladenineaminopurine (BAP) on *in vitro* plantlet regeneration of local and exotic banana cultivars. Champa is one of the local Musa variety of Odisha, India. Benzyladenineaminopurine (BAP) is the most commonly used cytokinin in the *in vitro* propagation tissue culture industry due to its effectiveness and affordability. The development and achievement in the field of modern tissue culture techniques have made it possible to discover various micropropagation protocols by using natural and synthetic phytohormone for the banana on a commercial scale. The effect of meta-Topolin (mT) and Benzyladenineaminopurine (BAP) on micropropagation and adventitious shoot regeneration of Champa variety was compared. During the multiplication stage of Champa variety a synergetic effect was observed when meta-Topolin (mT) supplemented in MS medium. In Multiplication culture longest shoot length (4.23 ± 0.45 cm), mean final fresh weight (7.46±0.37) and maximum no. of shoot/ explant (9.40 ± 0.74) was obtained as compared to other BAP.

Keywords: *In vitro*, acclimatization, meta-topolin, micropropagation, Champa variety

Introduction
Banana (*Musa* spp.) belonging to the family Musaceae are the earliest food crop. It being used all over the world and make a major benefaction towards the world's total food production (FAO, 1987) [7]. The Banana fruits contain large amounts of carbohydrates and minerals such as phosphorus, calcium and potassium as well as vitamins A and C with notable amount of several other vitamins (INIBAP, 1987) [9]. It is an important food crop for nearly 400 million people and is an essential source of income for many national economics (Sasson, 1997) [14]. *Musa* spp. are clonally propagated, grown from massive underground corm with highly compressed internodes (Barker and Steward, 1962) [2]. Champa (AAB genome) is one of the hardiest and tallest cultivars grown in the state of Odisha in eastern India. Its cultivation is especially widespread in the east coast of India. It is the local variety of Odisha, India and well known for its delicious taste and fruit used as a dessert. The pulp is creamy in colour and its taste is sub-acid. The fruits turn golden yellow when ripe and keep well.

Plant growth regulators are important for *in vitro* regeneration of crop plants grown in any synthetic medium. Cytokinins (CKs) are plant growth regulators involved in the control of plant cell cycle and development and their exogenous application in plant tissue culture (PTC). In general, cytokinin helps in shoot induction and auxin helps in root formation. But the requirement of cytokinin and auxin depends on the variety of banana and culture conditions (Cronauer and Krikorian, 1984) [4]. The most established banana shoot-tip culture system was achieved by using BAP as a supplement to Murashige and Skoog (MS) basal media (Murashige and Skoog, 1969). The exposure of plant to unsuitable type or concentration of CKs can have negative effects on mass propagation (Van Staden *et al.* 2008) [16]. Meta-topolin [6-(3-hydroxybenzylamino) purine] is an aromatic cytokinin. It was first isolated from poplar leaves. Its name is derived from "topol", the Czech word for pop-lar. The metabolism of meta-topolin is similar to that of other cytokinins. The advantages of *in vitro* propagation include a high multiplication rate, physiological uniformity, availability of disease-free material all the year round and rapid dissemination of new plant materials throughout the world. Compared to conventional materials, they have faster growth in the early growing stages and short harvest interval (Vuylsteke, 1989) [18]. Tissue cultured plants are preferred for new plantations and non-availability of tissue cultured plants of local cultivar like Champa will lead to its replacement by mostly exotic micropropagated cultivars like Grand naine. Thus, development of micropropagation protocol will save this important cultivar from extinction and help its extension in newer areas. To meet the demand of the farmers and for commercialization of Champa variety and increase the total yield, large number of plantlets is needed within a specific time period. Thus *in vitro* clonal propagation could play a role to reduce this scarcity of plantlets and can serve to develop *in vitro* techniques on other cultivars of banana. The aim of this work was to assess the potential of topolins as along with BAP or without BAP in banana tissue culture.

Materials and Methods
Study area
The study was conducted at Banana Tissue Culture Laboratory a unit of Regional Plant Resource Centre, Bhubaneswar, Odisha.

Explant source
Young sword suckers of Champa variety was collected from a healthy true-to-type mother plant from the Banana Mother Block site of Regional Plant Resource Centre. All the *in vitro* experiments were conducted at the Banana Tissue Culture Laboratory. After removing the leaves and the roots,

the suckers were thoroughly washed with running tap water to remove the soil. The older leaves, roots and extraneous corm tissues were carefully removed with the help of a sharp stainless-steel knife and chopped into small pieces. The pieces containing shoot tip with several sheathing leaf bases enclosing the axillary buds and some corm tissues with pseudostem measuring about 5.0-6.5 cm length were trimmed.

Surface sterilization

The prepared shoot tips were taken in a beaker and sterilized by 70% ethanol for 30 seconds and then explants were washed 3 times with double distilled water. Afterwards the explants were sterilized with 1% $HgCl_2$ solution for 20 minutes. Then the $HgCl_2$ solution was poured out and the explants were washed several times with sterile double distilled water to remove $HgCl_2$ from the explants. The outer two to three layers of the rhizome shoot tips were carefully removed aseptically using a scalpel and a cube of tissue of about 1 cm^3 containing the apical meristem was excised inside laminar air flow. The individual explant was inoculated and cultured on Murashige and Skoog medium supplemented with different concentrations and combinations of Plant growth regulators.

Experimental treatment and design

In the first phase of the experiment, the initial culture was established for Champa (AAB) variety. Then a further study was carried out using MS medium (Murashige and Skoog, 1962) [12] for the multiplication stage through *in vitro* culture. The pH of the medium was adjusted 5.7 to 5.8 with 0.1N NaOH or 0.1N HCl. 5.0gms of agar (Plant Tissue Culture grade, Hi-Media, India) was used as the gelling agent and the medium was autoclaved at 15 psi and 121 ℃ for 20 minutes. For multiplication stage BAP and meta-Topolin combination of different concentration were supplemented in MS medium. The cultured bottles were incubated in the thermal insulated tissue culture room with temperature around 25 °C and relative humidity 50-80%. Under 16-h photoperiod with light intensity of 3000 lux was used for illumination.

Data collection and analysis

The data such as the number of the days required for shoot initiation, shoot numbers per explant, shoot length, leaves number per explant and survival rate were recorded after a specific interval of time.

Results

Effect of BAP and MET in Champa variety

From the study it was found that MS medium supplemented with combination of BAP and meta-Topolin (mT) with concentration of (3 mg/l + 0.1 mg/l), showed maximum 7.46 gm average fresh weight of, 4.23 cm average no of shoot length and 9.40 average number of shoot per explants as compared to individual BAP and *m*T From (Table 1) (Fig 1,2) showed that combination of *m*T without BAP treatment having concentrations of (3mg/l) showed maximum proliferation capacity among the other combination of BAP.

Fig 1: 3mg/L BAP

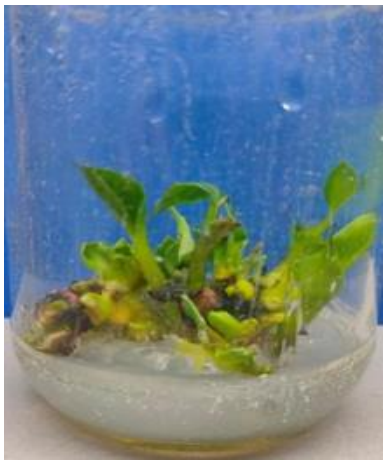

Fig 2: 3mg/L *m*T Effect on multiplication stage

Table 1: Effects of different concentration of BAP and MET on multiplication culture of Champa 1*

Code	Treatment	Concentration	Mean Fresh Weight (gm)	Mean Shoot Length (cm)	Mean no. of shoots/explants
Z1	*m*T	1mg	3.90±0.54	2.14±0.54	6.10±1.19
Z2	*m*T	2mg	4.25±0.47	2.75±0.18	6.78±1.15
Z3	*m*T	3mg	6.75±0.28	3.79±0.60	7.80±1.13
Z4	*m*T	4mg	3.65±0.64	2.79±0.69	6.4±1.30
Z5	BAP+*m*T	1mg + 0.1mg	4.75±1.05	3.12±0.67	6.09±0.87
Z6	BAP+ *m*T	2mg + 0.1mg	6.96±0.79	2.89±0.89	8.10±0.69
Z7	BAP+ *m*T	3mg + 0.1mg	7.46±0.37	4.23±0.45	9.40±0.74
Z8	BAP+ *m*T	4mg + 0.1mg	5.90±0.53	3.14±0.54	8.10±1.19
Z9	BAP	1mg	2.96±0.79	2.89±0.15	5.10±0.69
Z10	BAP	2mg	3.16±0.69	2.23±0.45	5.40±1.71
Z11	BAP	3mg	3.80±0.53	3.14±0.78	5.95±1.05
Z12	BAP	4mg	5.76±0.46	3.98±0.57	7.10±0.69

Data presented of 21 days old culture showed the different effect of PGR in term of growth parameter. *Mean value within a column followed by Standard Deviation.

Discussion

In the present study the effect of meta-Topolin and Benzyladenineaminopurine (BAP) was compared during multiplication stage of Champa variety. Previous reports (Cronauer and Krikorian 1984; Jarret *et al*. 1985; Vuylsteke and De Langhe 1985) [4, 10, 19] indicated that 5 mg/L is the optimum cytokinin concentration for most banana tissue cultures.The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of bananas (Rahman et al., 2006; Farahani *et al.*, 2008; Buah *et al.*, 2010) [13, 8, 3]. BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures (Buah *et al.*, 2010) [3]. But when Murashige& Skoog Basal medium when supplemented with BAP and meta-Topolin for multiplication medium showed significant growth. Result indicated that MS media enriched with BAP and meta-Topolin at certain concentration (3 mg/l + 0.1 mg/l) showed maximum growth rate in term of 7.46 average fresh weight, 9.40 average shoots per explant. But at an optimum concentration of BAP (4 mg/L), Champa explants responded for shoot induction and produced mean 7.10 shoots per explant. Whereas, explants from meta-Topolin (3 mg/L) supplemented medium induced mean 7.80 shoots per explant. Bairu *et al.* (2008) reported the influence of BA and mT in the micropropagation of two important banana cultivars (Williams and Grand Naine). They observed a high frequency of shoot induction and a higher number of shoots from pseudostem explants cultured on mT containing medium than for medium supplemented with BA. Similarly, Ahmad and Anis (2019) [1] and Singh and Kumaria (2019) [15] also observed the dominance of *m*T over BA in shoot bud induction and proliferation. Vijayakumar *et al*. (2017) [17] evaluated the influence of different cytokinins (BA, Zeatin, kinetin, TDZ, and *m*T) individually on micropropagation and organogenesis of safflower and concluded that compared to other cytokinins tested, *m*T at a concentration of 3.5 mg/L induced higher number shoots from shoot-tip and nodal explants. The superiority of *m*T over BA was reported in *Malus domestica* (Dobranszki *et al*., 2002) [6], and *Beta vulgaris* (Kubalakova and Strnad, 1992). Similar to these reports, we also observed a high frequency of shoot induction and a maximum number of shoots per explant cultured on meta-Topolin compare to BAP.

Conclusion

In present study an efficient regeneration protocol for Champa variety by augmenting *m*T in the MS media during multiplication stage was marked. Meta-Topolin positively influenced the shoot regeneration capacity and other growth factor as compared to BAP in cultured plantlets of Champa variety. In future it needs more research to increase regeneration capacity through *in vitro* culture by implementing suitable field performance for improvement of growth and development of Champa variety in Odisha, India.

Conflicts of interest

The authors declare that they have no conflict of interest

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