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Detection of phytochemicals and *in vitro* propagation of Banana (*Musa* variety Gaja Bantal)

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Abstract
The present experiments were conducted to evaluate the phytochemicals present in the sap and stem samples of a *Musa* variety, Gaja Bantal. Attempts has also taken to study various effects of plant hormones such as cytokinin (BAP) and auxins (IAA and NAA) on shoot proliferation and root induction during its *in vitro* micropropagation. Results from various experiments showed that the sap as well as stem extraction contain high phenolic and flavonoid content. Due to heavy phenolic content lethal browning of explants was observed during different stages of *in vitro* propagation Gaja Bantal. While both the samples (Sap and Stem) contain alkaloids, Saponin was slightly detected only in the stem sample. During micropropagation of Gaja Bantal it was observed that media containing high concentration of BAP (8 mg/l) had better respond in initial culture while low concentration of BAP (3 mg/l) had high bud formation in multiplication stage. Gaja Bantal explants grown on multiplication medium supplied with 3 mg/l BAP + 0.5 mg/l IAA had highest numbers of shoot buds. In rooting culture the media containing 1 mg/l IAA as well as media containing 1 mg/l NAA found to be more effective in root induction.

Keywords: *Musa*; *in vitro*; phytochemical; cytokinin; auxins; micropropagation

Introduction
With an annual production of over 100 million tons, banana and plantains are world's second largest cash crops. Banana plants are tall herbs which belong to *Musa* genus of Musacase family. Most cultivated species are triploids or tetraploids which are originated from diploid *Musa acuminate* (AA) and *Musa balbisiana* (BB) (Simmonds and Shepherd, 1955) [16]. Banana and plantains are rich in nutrient contains including minerals like potassium and calcium. It also contains essential vitamins like A, B_6, and C which helps in healing, growth of tissue and bones, body's immune system and many more. Various parts of banana plants such as fruit, stem, sap, roots and bell part of the inflorescence have wide range of medicinal properties. The sap is used to treat a wide variety of ailments, including leprosy, hysteria, fever, digestive disorders, hemorrhage, epilepsy, hemorrhoids and insect bites. Many research findings had also shown that banana fruit peel to have both antifungal and antibiotic components. The neurotransmitters like norepinephrine, serotonin and dopamine have also been identified in the peels of banana fruit (Kumar *et al*., 2012) [14].

India is the highest producer of banana with an annual production of over 30,000 tons and Odisha is one among the largest banana cultivating state of India. Gaja Bantal (ABB) is one of the major local plantain variety of Odisha which have a high market value in local as well as outer states. Traditionally banana plants are propagated through sword suckers but it has certain drawbacks like disease transfer, shortage of good quality planting material, un-uniformity in yield, etc. The problems emerging from conventional breeding process can be solved by propagating banana through tissue culture which offers mass propagation and clean planting material (Ali *et al*., 2011) [1]. Several scientist and researchers had reported various techniques for *in vitro* propagation of banana (Madhulatha *et al*., 2004; Strosse *et al*., 2006; Venkatachalam *et al*., 2006; Munguatosha *et al*., 2014) [9, 18, 19]. In the present study different experiments were conducted to analyze presence of different phytochemical (alkaloids, flavonoids, etc) present in the sap and stem samples along with *in vitro* micropropagation of *Musa* sp. cv. Gaja Bantal.

Material & Methods

The present study was carried out in Banana Tissue Culture Laboratory of Regional Plant Resource Centre, Bhubaneswar in the month of April, 2018. The phytochemical profiling was conducted from samples such as sap and stem collected from the mother plants before doing micropropagation. Fresh samples were collected and homogenized in 80% ethanol solution. Simple procedures were followed to conduct analysis of alkaloids, flavonoids, phenolic compounds and saponins (Obiageli *et al*., 2016).

Disease free sword suckers of 40-45 cm long were collected from the high yielding tissue culture mother plants planted in banana mother block. The suckers were washed under running tap water to remove soil and other particles before cutting them in small cylindrical shapes of 5-6 cm in length and 3-3.5 cm in diameter. Then the explants were cleaned with 1 % labolin solution followed by treatment with 1 % Banvistin (antifungal) for 30 minutes. After that the explants were washed twice in autoclaved double distilled water. Then the explants were treated with 0.1 % mercuric chloride for 30-40 minutes. The explants were then washed 2-3 times in autoclaved double distilled water before cutting into 4 halves. Each explant was transferred into culture vessel containing Murasiuge and Skoog medium (Murashige and Skoog, 1962) [10] supplied with different concentration and combinations of plant hormones such as 6-Benzylaminopurine (BAP), Indole-3-acetic acid (IAA), Naphthaleneacetic acid (NAA). The culture media and tools were sterilized in autoclave at 121°C and 15 PSI for 15 minutes.

For initial culture the explants were grown on media containing 2 mg/l BAP + 0.25 mg/l IAA (B1), 4 mg/l BAP + 0.25 mg/l IAA (B2), 6 mg/l BAP + 0.25 mg/l IAA (B3) and 8 mg/l BAP + 0.25 mg/l IAA (B4). In multiplication stage the explants were grown on 1 mg/l BAP + 0.5 mg/l IAA (M1), 2 mg/l BAP + 0.5 mg/l IAA (M2), 3 mg/l BAP + 0.5 mg/l IAA (M3), 4 mg/l BAP + 0.5 mg/l IAA (M4) and 5 mg/l BAP + 0.5 mg/l IAA (M5). For root induction the explants were grown on media containing 0.5 mg/l IAA (R1), 1.0 mg/l IAA (R2), 2.0 mg/l IAA (R3), 0.5 mg/l NAA (R4), 1.0 mg/l NAA (R5) and 2.0 mg/l NAA (R6).

The culture vessels after inoculation were kept in culture room with temperature 24°-25° C and 3000 Lux light. During initial culture the explants were grown on initial medium for 15 days. After initial culture the explants were cultured up to five subcultures before transferring them into rooting medium. Each explant grows for 21 days in all five subcultures as well as in rooting culture.

After the root formation the banana plantlets were transferred to nursery for primary hardening. The plantlets were taken out of culture vessel and the MS medium was removed by washing in tap water. The plantlets were planted in cocopit inside the primary hardening chamber and watered regularly. After primary hardening for 2 weeks the plantlets were transferred to polybags containing soil and low cost minerals for secondary hardening. The polybags contained river bed soil, sand and farm yard manure in the ratio 1:1:1. The data observed during the present experiments were analysis by using MS Excel to find average and standard deviation, taking 20 explants from initial, multiplication and rooting culture into consideration.

Results

The quantity of secondary metabolites (alkaloids, flavonoids, phenolic compounds (tannins) and saponins) varied from plant to plant mostly due to stress conditions. The extracted samples from sap and stem portion of Gaja Bantal were found to turn reddish in colour due to oxidation reactions. A white colour sticky mass was also marked in the sap samples. After conducting the phytochemical analysis it was observed that both the sap and stem samples were having alkaloids, flavonoids and high amount of phenolic compounds (Table 1). Slight trace of saponin was noted in stem sample only.

Table 1: Phytochemical analysis of sap and stem samples of Gaja Bantal plant.

Plant Samples	Alkaloids	Phenoloic	Saponins	Flavonoids
Sap	+	++	-	+
Stem	+	++	+	++

'+' means present; '++' means present in high quantity; '-' means absent

Obiageli *et al*., (2016) while conducting phytochemical compositions of fruits of three *Musa* species (Banana, Plantain and Saba banana) at three stages of development observed that the fruit peel of plantain species contained high quantity of phenols. Phenols in the three *Musa* species revealed no significant difference between them but rather between their stages of development. The phenol contents of the fruits were higher at the immature and green mature stages than at the ripe stages. This implies that the fruits at the immature and green mature stage are rich source of antioxidants because studies have shown that antioxidants capacity of plants is correlated with phenol compounds (Chun *et al*., 2003; Stintzing *et al*., 2002) [4, 17].

During conducting micropropagation of Gaja Bantal it was observed that contamination rate remained a little bit higher (15 %) during initial culture but it gradually decreased in multiplication and rooting culture. During initial culture the explants cultured on high concentration of BAP had better growth and proliferation in comparison to explants grown on low BAP concentration. Optimum results were obtained in explants grown on MS medium supplied with 8 mg/L BAP along with 0.25 mg/l IAA (B4). The lowest growth was marked in explants cultured on MS medium supplied with 2 mg/l BAP and 0.25mg/l IAA (B1).

Cytokinin such as benzyl amino purine (BAP) and Kinetin are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana (Shirani *et al*., 2009) [15]. The most established banana shoot-tip culture system was achieved by using BAP as a supplement to basal media (Devendrakumar *et al.*, 2013) [5]. In case of multiplication culture the explant cultured on MS medium supplied with 3 mg/l BAP + 0.5 mg/l IAA (M3) had highest number of shoot buds as well as individual shoots. The other concentration of BAP had induced bud proliferation but comparatively low rate in comparison to M3 (Fig. 1).

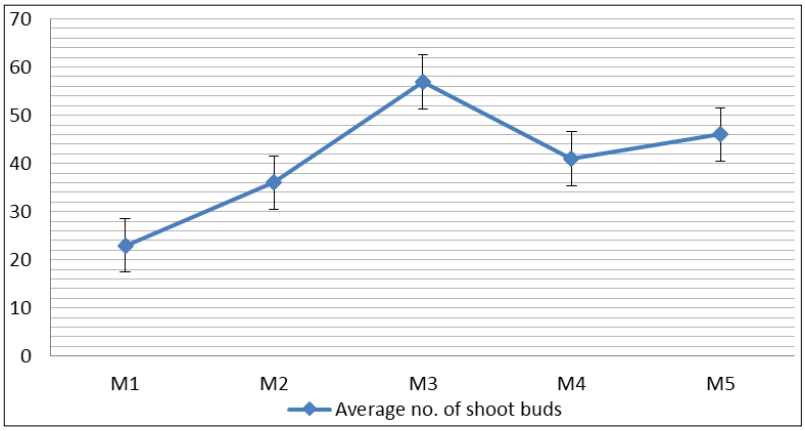

Fig 1: Effects of different plant growth hormones on shoot bud formation during *in vitro* multiplication culture of Gaja Bantal.

In high concentration of BAP few explants developed callus like mass instead of individual shoot. Where as in low concentration of BAP (M1) the number of shoot buds reduced drastically. The effectiveness of BAP over other cytokinin in inducing multiplication shoot buds during *in vitro* tissue culture has been reported in various cultivating species of bananas (Buah *et al*., 2010; Farahani *et al*., 2008) [2, 6]. BAP has an outstanding effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures (Rahman *et al*., 2006) [13]. However, if BAP is applied at higher concentrations it inhibits elongation of adventitious shoots and there conversion into complete plants (Buising *et al*., 1994) [3].

In rooting phase the Gaja Bantal plantlets were cultured on MS medium supplied with different concentration of IAA and NAA. For root growth and proliferation both IAA and NAA at 1 mg/l were found to be better in comparison to other concentrations. With the increase in concentration of IAA and NAA in the rooting media the numbers of root increased but at higher concentration (2 mg/l) the root lengths reduced (Fig. 2).

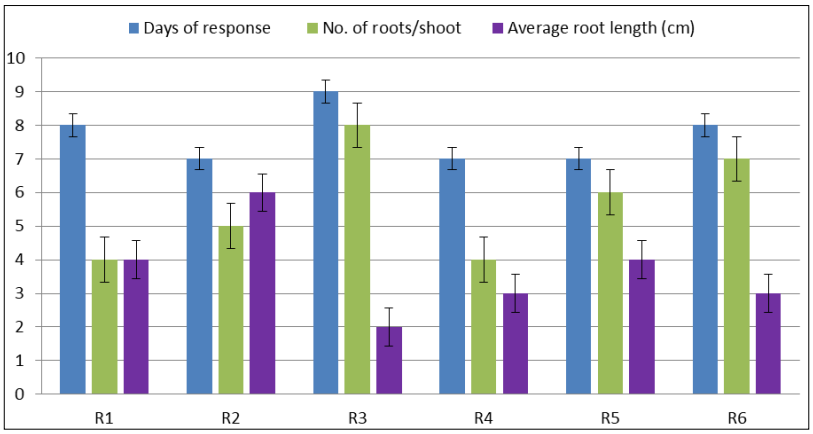

Fig 2: Root induction and growth of Gaja Bantal plantlets in rooting culture.

Hussein, (2012) [8] reported that Naphtalene acetic acid (NAA) promoted plant rooting during *in vitro* propagation of *Musa* spp. Through micropropagation process of Gaja Bantal it was marked that due to stress condition high phenolic secretion occurs which causes lethal browning. Due to this the survival rate of explants in each stage differs. It also affects the shoot bud proliferation during different sub-cultures and root elongation during rooting culture. For prevention of lethal browning different absorbent like activate charcoal or ascorbic acid can be used.

From the above observations it is concluded that BAP is suitable for micropropagation of Gaja Bantal. For initial culture the explants required high concentration of BAP but its application increases the amount of phenolic secretion. In multiplication stage as the explants started to form shoot buds BAP was required at moderate concentration. In rooting culture auxins played important role in root initiation and growth. Both IAA and NAA were found to be effective for root formation in Gaja Bantal plantlets.

High concentration of phenolic compounds was observed in both the sap and stem extraction from Gaja Bantal plant. The plant phenols may influence with all stages of the cancers processes, potentially resulting in a reduction of cancer risk (Hollman, 2001) [7]. Further studies will be conducted in future to do detail analysis on phytochemical content of the plant as well as development of standardized protocol for mass propagation of *Musa* sp. cv Gaja Bantal.

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