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SYNERGISTIC EFFECT OF SILVER NITRATE AND PHOTON FLUX DENSITY ON THE *IN-VITRO* MULTIPLICATION OF BANANA (CV. GRAND *NAIN*, AAA)

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ABSTRACT

The present study was aimed to investigate the effect of photon flux density and silver nitrate either alone or in combination on *in-vitro* micro-propagation of banana cv. Grand Nain (AAA). Five different concentrations of silver nitrate (0 to 68 µM) and PFD (0-55 µmol m^{-2} s^{-1}) were assessed. Plantlets were grown in culture room at 24±2°C with 16±8 h photoperiod. Highest number of bud formation was observed in explant cultured in bud establishment medium (MBE) medium supplemented with $AgNO_3$ (34 µM) and placed under dark condition. Additionally maximum bud break was observed in the pre developed buds exposed to PFD of 45 $\mu molm^{-2}s^{-1}$. Maximum shoot and root elongation was observed in shoot elongation medium supplemented with $AgNO_3$ (34 µM) under 50 $\mu molm^{-2}s^{-1}$ of photon flux density. Light quality also affected the total chlorophyll and phenolic content. Incorporation of silver nitrate in the culture medium reduced tissue browning and precocious rooting. Darkness and low light intensity significantly reduce precocious rooting in buds and resulted in albino effect in elongated shoots. However, high light intensity increased shoot proliferation and shoot elongation. The results obtained could be valuable for rapid and mass multiplication of banana to fulfilling the demand of Cavendish sub-group. Simultaneously the substitution of fluorescent lights with light emitting diode (LED) will be cost effective and result in cost saving of annual electricity consumption. The LED lighting system seemed to be the most suitable in terms of propagation efficiency, and plantlet quality.

Keywords **:** Silver nitrate, Photon flux density, Banana, Micro-propagation

INTRODUCTION

The banana and plantains (*Musa spp.*) belong to the family *Musaceae* family and are one of the world's most popular fruit crop. Due to their high nutritional value, they are good source of nutrients for millions of people in tropical and subtropical regions (Oumar *et al.,* 2018). As a cash crop, banana boosts the livelihood and economic sustainability of marginal farmers from developing countries. Due to its high importance in food and nutritional security, its production needs to be improved. However, bananas and plantains are devastatingly threatened by different abiotic and biotic stresses (Rustagi *et al.,* 2019, Shekhar *et al*., 2019). Traditionally, banana multiplication is done by vegetative methods, through suckers, which have several drawbacks such as; slow rate of multiplication, transmission of disease, lack of genetic fidelity, low production rate etc. (Nandhakumar *et al.,* 2018). Hence the conventional multiplication method is apparently inapt to supply the increasing demand for disease free and healthy planting material. Micropropagation is an alternative method for mass propagation of quality planting material. Hence the development of an efficient, fast and reproducible micropropagation method for multiplication of banana is pre-requisite to fulfil the demand of quality planting material in short span of time. However, several constraints still need to be resolved such as, accumulation of phenolic compounds, survival percentage, multiplication potential, long culture cycle, hyperhydricity, tissue browning, and precocious rooting. Plant growth and development under *in-vitro* conditions are regulated by different factors which include phytohormones, plant's growth regulators, light irradiance, humidity, temperature etc. Screening of contemporary research findings suggested that investigation on the effect of different phytohormones on banana micro-propagation has received much attention (Kelta *et al.,* 2018; Deo and Pradhan 2017; Lohidas and Sujin 2015; Shankar *et al.,* 2014; Ngomuo *et al.,* 2013; Buah *et al.,* 2010). Ethylene is one of the plant hormones which influences growth and development of plants. Hence regulation in the production or action of ethylene leads to modulation in the growth and development of plants under tissue culture. However, the influence of ethylene in plant tissue culture is diverse and its responses are variable which depend upon plant species (Fomenkov *et al* 2015; Pimenta *et al.,* 2013; Kumar *et al.,* 2012; Hu *et al.,* 2006). For instance, shoot morphogenesis in rice callus and embryogenesis in anther cultures of *Hordeum vulgare* were positively influenced by ethylene (Adkins *et al.,* 2006). In contrast, ethylene shows inhibitory effect on *in vitro* regeneration of other plant species (Pua and Chi, 1993; Pua,

2007). Different research studies have demonstrated that accumulation of ethylene in *in vitro* tissue culture and supplementation with silver nitrate ($AgNO_3$) in culture medium improves the growth vigour and regeneration frequency of explants. Similarly, light intensity also influences shoot bud multiplication and shoot morphogenesis in many plant species under i*n vitro* culture conditions (Buah 2010; Hung *et al.,* 2015; Waman *et al.,* 2015; Singh and Patel, 2014). However very few scientific reports are available on the impact of light irradiance, and ethylene regulation on mass multiplication of banana.

The present study aimed to identify the best suitable conditions for an efficient mass micro-propagation through organogenesis in banana cv. Grand *Nain*. We evaluated the effect of different concentrations of $AgNO_3$ and different levels of photon flux density (PFD) either alone or in combination on *in vitro* multiplication of shoot tips of banana and their impact on phenolic content, and precocious rooting.

MATERIAL AND METHODS

Plant material, culture condition and medium composition

Disease-free plants of banana [*Musa acuminata* cv. Grand *Nain* (AAA)] were obtained from National Bureau of Plant Genetic Resources, New Delhi, India and propagated under *in vitro* condition. The cultures were maintained under cool white fluorescent light (45 µmol m^{-2} s^{-1}), 16+8 h photoperiod at 25 ± 2°C. MS medium supplemented with sucrose 3% (w/v), ascorbic acid (140µM), 6-benzylaminopurine (BAP) 36µM, kinetin (9 µM), adenine sulphate (54 µM), indole acetic acid (IAA) 2 µM, pH 5.8 was used as bud establishment medium (MBI). MS medium supplemented with sucrose 3% (w/v), ascorbic acid (140µM), 6-benzylaminopurine (BAP) 9µM, indole acetic acid (IAA) 2 µM, adenine sulphate (54 µM) gelled with 8% agar, pH 5.8 was used as shoot induction medium (MSI) for shoot induction.

Light source and arrangement

LED white cool florescent tubes (Phillips, India) of 18 watt and 22 watt were arranged in parallel orientation along shelves. The light arrangements of LED tubes comprised of horizontal orientations to achieve the uniform light distribution within the shelves. Light treatment was varied by placing culture vessels at different PFD (0, 40, 45, 50, and 55 µmol m^{-2} s^{-1}) supplied by LED tubes fitted with a timer set at 16±8 h light/dark photoperiod at 24 ± 2°C. PFD was measured (on top of the culture vessel) with a photosynthetic light quantum meter, Taiwan.

Effect of silver nitrate on bud establishment and shoot proliferation

Shoot tip explants with the apical dome fully covered with 4-5 leaf primordia were harvested from *in vitro* grown plantlets and placed in MBI medium for bud establishment. For the proliferation of shoots, pre-developed buds were transferred to the MSI medium. Both media were supplemented with/without $AgNO_3$ (0, 17, 34, 51, and 68 µM) as mentioned in previous section to investigate the effect of $AgNO_3$ on *in vitro* banana multiplication.

In Vitro Root Induction and Acclimatization

In vitro raised shoots were transferred to rooting medium as described previously along with/without $AgNO_3$ (0-68 µM). Plantlets were carefully taken out of the medium after successful root establishment and washed to remove any traces of medium. *In vitro* raised rooted plantlets were transferred to plastic pots containing sterile agropeat, which were further transferred to greenhouse at 24±2°C. To maintain high relative humidity, plantlets were covered with polythene bags and irrigated with Hoagland solution once in two days. Subsequently humidity was reduced by making small perforations in the polythene bags.

Measurement of Plantlet Growth

To investigate the influence of different combinations of $AgNO_3$ and photon flux density on the *in vitro* multiplication of banana (cv. Grand *Nain*), the morphological developmental pattern of root and shoot were recorded. The explants were inoculated on agar solidified MS medium supplemented with/without silver nitrate for bud formation. Total number of developed buds was recorded up to the end of the first culture cycle of 20 days. Simultaneously the pre-developed buds were cultured on MS medium (mentioned above) and the time taken for bud break was recorded up to the end of the culture cycle of 20 days. Further the sprouted buds were cultured on shoot elongation medium with/without silver nitrate and shoot and root length was measured after 20 days of sub-culture.

Measurement of Physiochemical Attributes

To investigate the effect of different photon flux density and in combination with varying concentrations of silver nitrate on physiochemical attributes of *in vitro* growing plants, total phenolic content, percentage of tissue browning, total chlorophyll content and percentage of precocious rooting were recorded. Total phenolic content (TPC) was estimated according to Vinothani *et al.* (2017). The leaves and root tissue of *in vitro* grown plants were separately ground in 2 ml of pre-chilled solution of acetone: water (1:1, v/v). Further 100 µl of extract was mixed with 1 ml of Folin Ciocalteu (FC) reagent and incubate at room temperature for 5 min. Thereafter, 1 ml of Na_2CO_3 solution (7.5 % w/v) was added to the extract and mixed thoroughly and kept at room temperature for 10 min. The absorbance of solution was measured at 760 nm. The TPC was calculated by preparing a standard plot of gallic acid and expressed as mg g^{-1} gallic acid equivalent (GAE). Total chlorophyll was measured according to the method described by Arnon (1949).

Statistical analysis

All the experiments were conducted with completely randomized design (CRD) and repeated thrice. Each treatment was given to 5 replicates and each replicate consisted of one jam bottle containing three organogenic cultures. Data were analyzed by ANOVA using a completely randomized design at 5 % significance level, and the comparison of means was performed with the Student t-test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Regeneration of shoots from meristem explants

In vitro mass multiplication of banana through meristem culture offers a viable solution in the mass production of disease free planting materials. Present study

was conducted to scrutinize the effect of $AgNO_3$ and PFD on banana micro-propagation. Initiation of shoot buds in banana shoot tip explants was observed after one week of inoculation in MBI medium under control condition. However, use of $AgNO_3$ reduced the time of bud initiation by 2 to 4 days as compared to the control explants (Figs. 1 and 2). The highest frequency of bud initiation (9.7 ± 1.4) (mean value) was recorded in the medium supplemented with 34µM of $AgNO_3$ and placed under dark condition (Table 1). Simultaneously bud break was favoured by combination of 34µM of $AgNO_3$ and PFD of 50µmol$m^{-2}s^{-1}$ (Fig. 1). The timing of bud break was reduced by 9 to 16 days as compared to the control explants (Table 1). Different research studies suggest that supplementation of $AgNO_3$ in plant tissue culture media greatly improves the plant regeneration under tissue culture condition (Giridhar *et al*., 2004). Array of physical and chemical agents have been investigated towards development of efficient and reproducible protocol for banana micro-propagation, such as plant growth regulators, silver nitrate, cobalt chloride, amino oxyacetic acid, different light intensities etc. (Oumar *et al.,* 2018; Buah, 2010; Kumar *et al.,* 2009). $AgNO_3$ has been employed in tissue culture studies for inhibition of ethylene action. Silver ions inhibit ethylene action by reducing the binding capacity of ethylene receptor (Beyer 1979; Yang and Hoffman 1984). Ethylene is a ubiquitous plant hormone that influences growth and development of plants. Hence regulation of ethylene activity could be a useful tool towards manipulation of growth and development of plants in plant tissue culture. The findings of the present study corroborate the results obtained by Giridhar *et al.* (2004) and Oumar *et al.* (2018), where inhibition of ethylene action significantly enhanced the bud development and bud break. At the bud initiation stage, precocious rooting decreases the rate of bud establishment and multiplication by diverting most tissue nutrients to root formation rather than shoot formation (Al- Khateeb, 2008). Several research groups report that the high auxin concentration in the culture medium is a major factor for this problem. Anjarne and Zaid (1993) reported that root initiation requires high auxin concentrations. In the present investigation higher root elongation was favoured in culture media with low auxin concentrations. In the present study it has been observed that the combined effect of $AgNO_3$ (51 µM) and PFD significantly suppressed precocious rooting during bud development and bud breaking stage in MBI medium (Table 1) and root elongation in MSI medium (Table 2). The highest root length (4.2 ± 0.3 cm) could also be explained by the degradation of auxin due to increasing concentration of $AgNO_3$ in MSI medium and presence of high PFD (Fig. 3). The result of present study reported that under the presence of $AgNO_3$, high light intensity promotes root formation and root elongation, while total darkness or low light intensity restricts root formation. Our results indicated that the root length increases significantly by increasing the PFD from dark to 50µmol$m^{-2}s^{-1}$. This indicates that the combination of $AgNO_3$ and PFD might be a causal factor for the rooting in regenerated shoots. Combined effect of $AgNO_3$ and PFD significantly increased the shoot length. Concerning shoot length, highest shoot length (10.5 ± 0.3) (Figure 3) was recorded in sprouted buds under the combined effect of $AgNO_3$ (51 µM) and 50µmol$m^{-2}s^{-1}$ of PFD (Table 2). The combined effect of $AgNO_3$ and PFD influenced total chlorophyll content and frequency of greening of shoots (Figure 3). However, albinism was also evidenced (Figure 1) in the cultures which were placed under dark condition with or without the supplementation of $AgNO_3$. Highest chlorophyll content (138 ± 4.4 µg g^{-1} FW) was observed in those plantlets, which were placed in the combination of $AgNO_3$ (51 µM) and PFD of 50 µmol$m^{-2}s^{-1}$ (Table 2). There are several reports indicating that appropriate concentration of silver nitrate plays an important role in improving photosynthetic quantum efficiency (Sadak 2019; Sharma *et al.,* 2012). Excessive production of phenolic compounds in banana tissue culture is a major constraint. This leads to tissue browning, growth retardation and increases the chances of infection. Aliyu (2009) reported that leached secondary metabolites from the cut surfaces of explants oxidize and turn the media brown and cause toxicity to the explants. In a normal tissue, no browning occurs due to membranous separation of polyphenol oxidase and phenolic compounds (Mager and Harel, 1979). Therefore, the disruption of the membrane leads to tissue browning. The propitious contribution of $AgNO_3$ in *in vitro* micro-propagation has been proclaimed in different research findings. From these studies it has been inferred that $AgNO_3$ has a fostering effect in various crops such as tomato, cassava, cabbage, cauliflower (Batista *et al*., 2018; Shah *et al.,* 2014; Yordanov *et al.,* 2002; Mohamed *et al.,* 2006). Our results show that synergistic effect of silver nitrate and PFD reduced the production of total phenolic content by 38% to 58% (Table 2) and it may be attributed to the lower level of ethylene. As higher level of ethylene disrupts auxin translocation, induces hyperhydricity, reduces chlorophyll content and leads to tissue mortality (Lentini *et al.,* 1988). Roustan *et al.* (1990) reported that Ag^+ interferes with ethylene metabolism and reduces ethylene production by promotion of polyamine biosynthesis. In the light of these reports, results in this study indicate that specific binding of Ag^+ to certain ethylene receptors was most likely responsible for reduction of total phenolic content. Our results also showed that high light intensities enhance shoot greening during the multiplication as well as shoot elongation phase. Outcome of the present study is also synonymous with previous findings where use of $AgNO_3$ lead to enhancement in total chlorophyll content. The results of present investigation corroborate those of other authors (Zhang *et al.,* 2014; *Skribanek et al.,* 2012; Anjarne *et al.,* 2005) who reported the effect of light intensity on total chlorophyll content. The beneficial effect of synergistic impact of $AgNO_3$ and PFD was recorded in terms of all the morphological and physiochemical growth parameters. The results obtained in the present study indicate that $AgNO_3$ (51µM) alone was well suited for bud establishment. However, combined effect of $AgNO_3$ (51 µM) and PFD of 50 µmol$m^{-2}s^{-1}$ showed a higher rate of shoot proliferation, and chlorophyll content with low levels of total phenolic content and precocious rooting.

CONCLUSION

Results of the present investigation suggest that additive effect of $AgNO_3$ and PFD greatly fasten the regeneration of banana cultures in terms of number and frequency. The appropriate conditions for *in vitro* micro-propagation of banana (cv. Grand *Nain*, AAA) have been established. From this study we can conclude that the developed protocol provides rapid and reproducible micro-propagation technique that enables to minimize the time required for large-scale production of high yielding banana varieties. Simultaneously use of LED light could minimize the production cost.

Table 1: Effect of MBI+ Silver nitrate and Photon flux density on different parameters.

MBI + $AgNO_3$ (µM)	Photon flux density (µmol m^{-2} s^{-1})	Average no. of Buds/explant	Days of bud breaking	Precocious rooting
0	0	2.9 ± 0.3^{b}	27.4 ± 0.7^{ab}	2.3 ± 1.2^{a}
	40	2.4 ± 0.4^{ab}	21.0 ± 0.7^{a}	61.7 ± 19.8^{a}
	45	1.8 ± 0.3^{ab}	18.0 ± 1.1^{b}	74.0 ± 21.5^{ab}
	50	1.5 ± 0.5^{a}	17.4 ± 0.6^{ab}	82.1 ± 15.3^{ab}
	55	1.2 ± 0.5^{a}	19.6 ± 1.2^{b}	87.4 ± 9.9^{b}
17	0	3.2 ± 0.5^{ab}	23.8 ± 1.7^{ab}	1.8 ± 3.0^{ab}
	40	2.6 ± 0.4^{b}	21.2 ± 1.0^{ab}	56.0 ± 13.8^{ab}
	45	2.1 ± 0.2^{ab}	20.3 ± 1.0^{a}	50.0 ± 11.3^{a}
	50	1.7 ± 0.3^{ab}	13.2 ± 0.6^{ab}	40.0 ± 12.2^{b}
	55	1.3 ± 0.2^{a}	16.3 ± 0.9^{b}	34.5 ± 8.5^{ab}
34	0	9.7 ± 1.4^{ab}	20.8 ± 1.2^{ab}	1.6 ± 1.4^{b}
	40	8.7 ± 1.1^{ab}	17.2 ± 0.9^{b}	44.6 ± 17.2^{b}
	45	6.8 ± 1.0^{a}	12.9 ± 0.5^{b}	39.2 ± 15.0^{a}
	50	5.3 ± 0.8^{a}	10.3 ± 0.4^{ab}	32.0 ± 15.3^{a}
	55	6.4 ± 1.1^{ab}	11.4 ± 0.3^{ab}	30.2 ± 10.3^{ab}
51	0	4.4 ± 0.9^{a}	11.6 ± 1.0^{ab}	1.2 ± 0.8ab
	40	3.2 ± 0.8^{b}	9.2 ± 1.8^{a}	28.2 ± 9.8^{a}
	45	3.0 ± 0.6^{ab}	9.0 ± 1.1^{b}	32.0 ± 5.8^{b}
	50	2.4 ± 0.5^{b}	7.8 ± 1.5^{ab}	36.3 ± 6.1^{ab}
	55	2.3 ± 0.5^{ab}	13.5 ± 1.4^{a}	41.8 ± 4.3^{b}
68	0	2.2 ± 0.4^{ab}	38.7 ± 2.4^{ab}	1.2 ± 0.5^{ab}
	40	1.8 ± 0.4^{ab}	29.4 ± 3.2^{b}	8.4 ± 3.7^{a}
	45	1.8 ± 0.3^{b}	24.2 ± 3.2^{a}	12.2 ± 4.8^{b}
	50	1.4 ± 0.3^{ab}	22.4 ± 2.4^{a}	16.0 ± 7.3^{ab}
	55	1.2 ± 0.4^{a}	23.1 ± 2.8^{ab}	21.3 ± 8.2^{ab}

Data are means of 5 replicates with 3 explants per replicate. Values are mean ± standard deviation of 5 repetitions. Values with the same uppercase letter are not statistically different (P>0.05).

Table 2: Effect of MSI+ Silver nitrate and Photon flux density on different parameters

MSI + $AgNO_3$ (µM)	Photon flux density (µmol m^{-2} s^{-1})	Shoot length (cm)	Root length (cm)	Total chlorophyll content (µg g^{-1} FW)	Total phenolic content (mg g^{-1}GAE)
0	0	4.0 ± 0.4^{a}	0.5 ± 0.03^{ab}	0	1.8 ± 0.2^{ab}
	40	4.8 ± 0.4^{ab}	1.1 ± 0.4^{b}	80 ± 5.6^{a}	8.5 ± 1.4^{a}
	45	5.2 ± 0.5^{ab}	1.7 ± 0.3^{b}	92 ± 6.4^{ab}	6.2 ± 1.5^{ab}
	50	6.5 ± 0.4^{b}	2.1 ± 0.5^{b}	102 ± 5.2^{b}	4.8 ± 0.4^{ab}
	55	5.0 ± 0.7^{a}	1.2 ± 0.6^{ab}	87 ± 3.8^{a}	10.7 ± 1.7^{b}
17	0	4.6 ± 0.6^{ab}	0.7 ± 0.4^{a}	0	1.2 ± 0.3^{ab}
	40	6.1 ± 0.4^{ab}	1.8 ± 0.05^{ab}	100 ± 6.2^{ab}	6.4 ± 1.5^{ab}
	45	6.8 ± 0.7^{ab}	2.5 ± 0.06^{ab}	108 ± 5.4^{a}	5.3 ± 1.5^{b}
	50	7.3 ± 0.5^{b}	3.1 ± 0.04^{b}	114 ± 6.0^{ab}	4.1 ± 1.4^{ab}
	55	6.0 ± 0.4^{a}	2.0 ± 0.07^{ab}	96 ± 5.7^{b}	7.0 ± 1.0^{ab}
34	0	6.7 ± 0.5^{ab}	0.6 ± 0.07^{ab}	0	1.2 ± 0.3^{ab}
	40	7.7 ± 0.4^{ab}	2.0 ± 0.05^{b}	110 ± 5.8^{ab}	5.6 ± 1.1^{a}
	45	9.3 ± 0.3^{a}	2.6 ± 0.06^{b}	118 ± 4.1^{ab}	4.5 ± 0.7^{a}
	50	10.5 ± 0.3^{ab}	3.3 ± 0.6^{a}	128 ± 3.7^{ab}	$3.6 \pm 0.\ 8^{ab}$
	55	6.3 ± 0.2^{ab}	2.3 ± 0.5^{ab}	99 ± 5.2^{ab}	7.2 ± 1.2^{ab}
51	0	5.4 ± 0.7^{ab}	0.8 ± 0.04^{ab}	0	0.6 ± 0.04^{b}
	40	7.1 ± 0.4^{ab}	2.7 ± 0.4^{b}	118 ± 4.6^{a}	4.2 ± 0.8^{ab}
	45	7.8 ± 0.6^{ab}	3.8 ± 0.3^{ab}	127 ± 5.7^{ab}	3.3 ± 0.5^{ab}
	50	8.3 ± 0.7^{ab}	4.2 ± 0.3^{ab}	138 ± 4.4^{b}	2.4 ± 0.5^{a}
	55	6.1 ± 0.5^{b}	3.5 ± 0.2^{b}	102 ± 6.0^{ab}	6.2 ± 0.7^{ab}
68	0	4.0 ± 0.2^{ab}	0.5 ± 0.07^{ab}	0	1.3 ± 0.3^{ab}
	40	4.8 ± 0.3^{a}	1.8 ± 0.3^{ab}	92 ± 4.4^{ab}	6.3 ± 1.4^{a}
	45	5.7 ± 0.6^{ab}	2.1 ± 0.6^{ab}	103 ± 5.1^{a}	5.5 ± 0.6^{b}
	50	6.3 ± 0.4^{ab}	3.2 ± 0.4^{ab}	130 ± 3.2^{ab}	$3.8 \pm\ 1.0^{ab}$
	55	4.2 ± 0.5^{a}	1.4 ± 0.5^{a}	113 ± 4.8^{ab}	7.1 ± 1.1^{b}

Data are means of 5 replicates with 3 explants per replicate. Values are mean ± standard deviation of 5 repetitions. Values with the same uppercase letter are not statistically different (P>0.05).

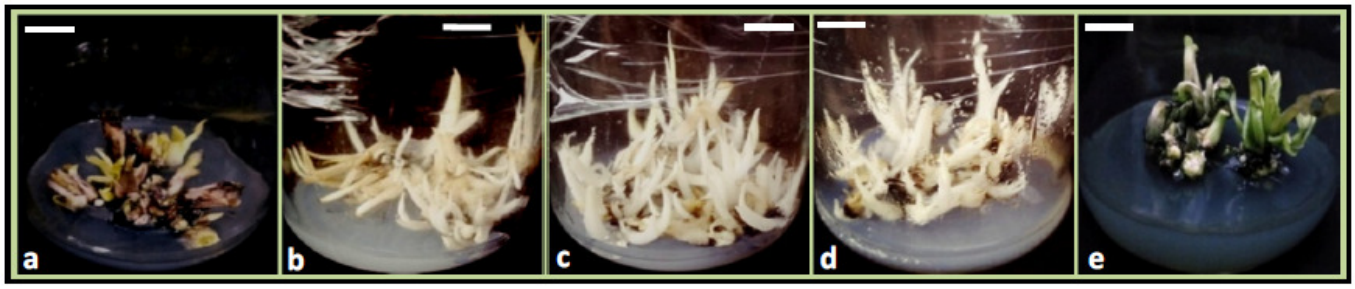

Fig. 1 : Bud induction in the meristem explants of banana placed on bud induction medium (MBI) supplemented with five different concentration of $AgNO_3$ after two culture cycle. (Bar 1 cm).

a. Bud induction on MBI medium without $AgNO_3$ (Control).
b. Bud induction on MBI medium supplemented with $AgNO_3$ (17µM).
c. Bud induction on MBI medium supplemented with $AgNO_3$ (34µM).
d. Bud induction on MBI medium supplemented with $AgNO_3$ (51µM).
e. Bud induction on MBI medium supplemented with $AgNO_3$ (68µM).

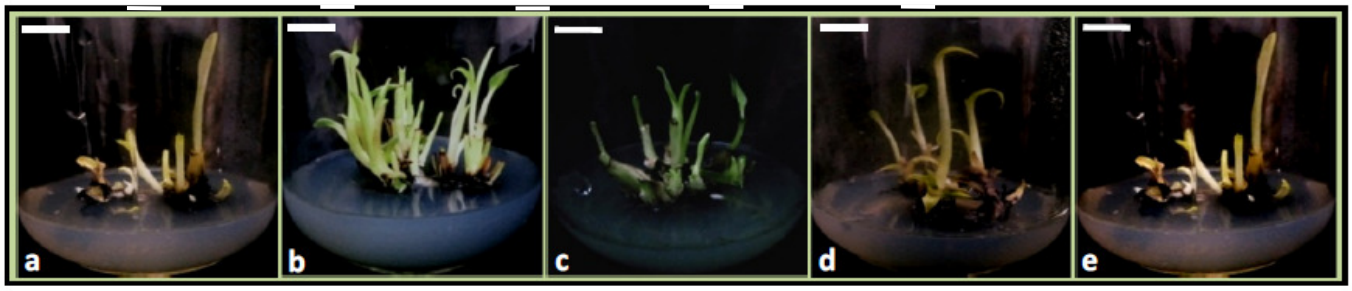

Fig. 2 : Bud induction in the meristem explants of banana placed on MBI medium and placed under five different levels of photon flux density after two culture cycle. (Bar 1 cm):

a. Bud induction on MBI medium without PFD (Control).
b. Bud induction on MBI medium placed under PFD of 40µmol$m^{-2}s^{-1}$.
c. Bud induction on MBI medium placed under PFD of 45µmol$m^{-2}s^{-1}$.
d. Bud induction on MBI medium placed under PFD of 50µmol$m^{-2}s^{-1}$.
e. Bud induction on MBI medium placed under PFD of 55µmol$m^{-2}s^{-1}$.

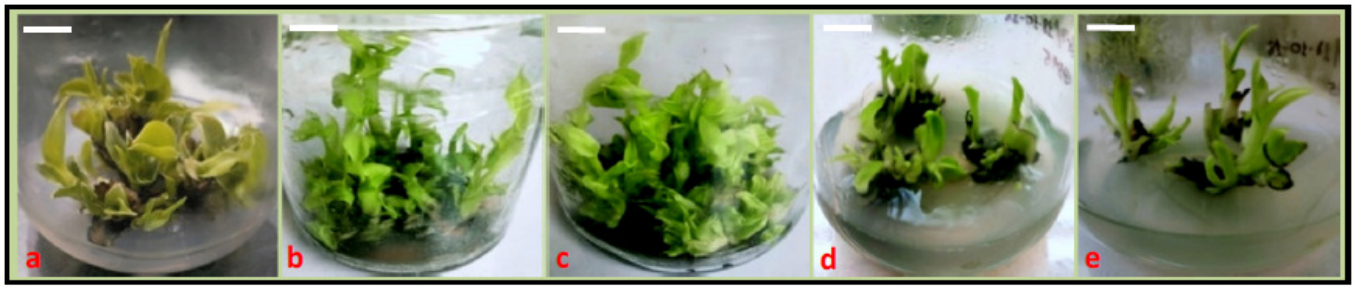

Fig. 3 : Shoot Proliferation in the pre developed buds of banana placed on shoot induction medium (MSI), supplemented with five different concentrations of $AgNO_3$ and placed under PFD of 50µmol$m^{-2}s^{-1}$ after two culture cycle. (Bar 1 cm):

a. Shoot proliferation on MSI medium without $AgNO_3$ (Control).
b. Bud induction on MSI medium supplemented with $AgNO_3$ (17µM).
c. Bud induction on MSI medium supplemented with $AgNO_3$ (34µM).
d. Bud induction on MSI medium supplemented with $AgNO_3$ (51µM).
e. Bud induction on MSI medium supplemented with $AgNO_3$ (68µM).

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Conflict of interest

The authors declare no conflict of interest.

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