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EFFECT OF AUXINS SUPPLEMENTATION ON CALLUS INDUCTION AND ROOT REGENERATION IN BANANA (*Musa paradisiaca* L.) cv.

UDHAYAM UNDER *in vitro* CULTURES

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KEYWORDS

Banana

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ABSTRACT

Present study was conducted to study the effect of different auxins concentration on callus induction and root regeneration in *Musa paradisiaca* L. cv. Udhayam. MS media supplemented with 2, 4-D alone (2.00, 3.00, 4.00, 5.00, 6.00, mg l⁻¹) was used for induction of callus whereas MS media was supplemented with nine combinations of IBA and NAA were used for regeneration of roots from induced callus. Result of study revealed that minimum time (24.41 days) of callus induction was observed in 2,4-D (4.00 mg l⁻¹); whereas maximum time (39.42 days) was noted in control. Further, combination of IBA (2.50 mg l⁻¹) and NAA, (2.00 mg l⁻¹) also have significant effect on the root proliferation in banana shoots. Minimum days taken for root initiation (11.73 days) were found in IBA (2.50 mg l⁻¹) and NAA (2.00 mg l⁻¹) combination. Similarly, maximum number of roots (7.03) and maximum rooting percent (87.30%) per plantlet was recorded under the treatment of IBA (2.5mg l⁻¹) and NAA (2.00 mg l⁻¹). Therefore from the results of current study it can be concluded that 2,4-D with concentration of 4.00 mg l⁻¹ has good response for callus induction whereas the combination of IBA (2.50 mg l⁻¹) and NAA (2.00 mg l⁻¹) was found better for regeneration of roots.

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1 Introduction

Banana (“Adam fig”, “Apple of Paradise”), is one of the oldest fruit of the world which belongs to Musaceae family. Botanically it is classified as *Musa paradisiaca*. edible banana has been reported to be originated in warm and moist part of South East Asia, possibly in the hilly regions of Assam, Burma, Thailand or Indo-China. In addition to being staple food crop for millions of people, it is also most commercial fruit crop of tropical areas all around the world (Singh & Saxena 2008).

Fruit of banana is popular for its aroma and luscious texture. It is considered as nutritious ‘gold mine’ as it is rich in carbohydrates and vitamins particularly vitamin B6. It contains carbohydrate, crude fibre, protein, ash, potassium, phosphorus, calcium, iron, magnesium, riboflavin, niacin and ascorbic acid (FAO, 2014). Regular consumption of banana fruit helps in reducing the risk of heart diseases and is recommended for patients suffering from high blood pressure, arthritis, ulcer, gastroenteritis and kidney disorders (Dayarani et al., 2013).

In India banana fruit covers an area of 8.58 million hectare, producing 29.16 million tonnes with a productivity of 33.98 MT/ha during the year 2016-17 (Indian Horticulture N H B Data base 2016-17). In India, Gujarat ranks first in production followed by Andhra Pradesh, Tamil Nadu and Uttar Pradesh. However, productivity was recorded highest in Madhya Pradesh followed by Gujarat and Punjab (Indian Horticulture N H B Data base 2016-17).

Most commonly propagated banana species are triploid and derived from two diploid species *M. acuminata* (Malaysia) and *M. balbisiana* (India) (Ali et al., 2011) and it is propagated vegetative through sword suckers and other planting materials like bits, butts and peepers (Uma et al., 2010); However, quality planting material is the key factor responsible for successful production of banana. Development of tissue culture technology has been proved as one of the foundation for production of high quality, disease free planting materials on a large scale, particularly in vegetatively propagated crops like banana. *In vitro* propagation is a magnificent approach for the production of good planting material of banana at low price with the potential of producing 50-60 shoots per sucker in 4-5 months (Georget et al., 2000). Tissue cultured plants have been reported to produce 39 per cent higher yield than plants from sword suckers (Farahani et al., 2008). Every year, approximately 2500 million plants are requisite for cultivation of banana in India, but only 2.5 per cent of the total requirements are fulfilled. Therefore, keeping this in view, present study was conducted to assess the effect of auxins on callus induction and root regeneration in banana (*M. paradisiaca* L.) cv. Udhayam under *in vitro* cultures.

2 Material and Methods:

2.1 Preparation of MS media

All chemicals were purchased from Himedia and all solution was prepared in double distilled water. Murashige & Skoog 1962 (MS) media was prepared with growth regulators viz., auxins (2,4-D, IBA and NAA) according to the treatment requirement. Firstly, the stock solution of MS media and plant growth regulators (2,4-D, IBA and NAA) were prepared by weighing the required amount of the chemicals using digital balance and dissolved them in sterilized double distilled water. MS media supplemented with 2, 4-D alone (2.00, 3.00, 4.00, 5.00, 6.00, mg l⁻¹) was used for induction of callus whereas MS media was supplemented with nine combinations of IBA and NAA i.e. 2.00 mg l⁻¹ IBA + 1.00 mg l⁻¹ NAA; 2.00 mg l⁻¹ IBA + 2.00 mg l⁻¹ NAA; 2.00 mg l⁻¹ IBA + 2.50 mg l⁻¹ NAA; 2.50 mg l⁻¹ IBA + 1.00 mg l⁻¹ NAA; 2.50 mg l⁻¹ IBA + 2.00 mg l⁻¹ NAA; 2.50 mg l⁻¹ IBA + 2.50 mg l⁻¹ NAA; 3.00 mg l⁻¹ IBA + 1.00 mg l⁻¹ NAA; 3.00 mg l⁻¹ IBA + 2.00 mg l⁻¹ NAA and 3.00 mg l⁻¹ IBA + 2.50 mg l⁻¹ NAA were used for regeneration of roots from induced callus. MS media was prepared by addition of requisite stock solution, growth regulators, sucrose (30 gm/litre) and agar (8 gm/liter) as per standard procedure. pH of the medium was adjusted to 5.8 and all culture containers filled with media autoclaved at 121.6°C at 15 psi for 20 minutes.

2.2 Sterilization of explant

Sword sucker were used as explants for initiation of callus culture. Selected explants were washed thoroughly under running tap water for half an hour to separate on mother plant. Explant was surface sterilized by using ethyl alcohol (70%) for a period of 5 minutes followed by thoroughly washed with double distilled water. This process was carried out to remove the adhering chemicals on the surface of the explants.

2.3 Preparation of explant and culturing on MS media

Explant was inoculated in test tubes under aseptic conditions of laminar air flow chamber. Further, inoculated culture vessels were kept under light intensity of 30.0 µm dm⁻¹s⁻¹ white light fluorescence tubes and temperature of 25±2 °C with humidity at 65 %, photoperiod of (2000- 3000 lux) of 16 hours light and 8 hours dark in culture room.

2.4 Data analysis

One-way ANOVA was used for analysis of callusing data whereas two-way ANOVA was used for analysis for rooting data.

3 Results and Discussion

3.1 Initiation of callus

Six different combination of 2,4-D was investigated for induction of callus. Significant enhancement (days) in average callus induction was observed from 24.41 to 39.42 days after inoculation. Minimum time (24.41 days) of callus induction was observed using 2,4-D @ 4.00 mg l⁻¹ concentration, it was followed by 2,4-D 3.0 mg l⁻¹ (26.76 days), 2,4-D 6.0 mg l⁻¹ (27.90 days) and 2,4-D 2.0 mg l⁻¹ (28.73 days) respectively, while maximum time (39.42 days) for callus induction was observed in untreated explants (Figure 1 & 2). The same pattern of observations was recorded by Darvari et al. (2010), Rashid (2012), Kumar et al. (2013), Sultan et al. (2011), Jafari et al. (2011) and Shukla et al., (2018).

3.2 *In vitro* establishment of root

3.2.1 Days taken for root initiation:

Effect of IBA and NAA on time taken for root initiation was observed for period of 25 days. Minimum time (11.733 days) was observed in MS medium supplemented with NAA (2.00 mg l⁻¹ and IBA 2.5 mg l⁻¹) where as maximum time duration (22.77 days) was observed in MS medium supplemented with only 3.0 mg l⁻¹ IBA (Figure 3). Further it has been observed that increase in concentration of IBA has also increased the time duration for root initiation. From these results it was concluded that IBA is able to initiate root only in presence of optimum concentration of NAA. The same pattern of performance was observed by Sultan et al. (2011), Kumar et al. (2013), Jafari et al. (2011), Karule et al. (2016), Ali & Mehmood (2017) and Nandhakumar et al. (2018).

3.2.2 Number of roots per plantlet

It was evident from the data that there was significant increase in number of roots per plantlet treated with suitable combinations of NAA and IBA in MS medium. The current study revealed that maximum number of roots (7.03) per plantlet was recorded in established culture treated with IBA, (2.50 mg l⁻¹) and NAA, (2.00 mg l⁻¹) followed by 6.16, 6.03 and 5.96 with the treatments of IBA, (2.00 mg l⁻¹) + NAA, (2.00 mg l⁻¹); IBA, (2.00 mg l⁻¹) + NAA, (1.00 mg l⁻¹); IBA, (3.00 mg l⁻¹) + NAA, (2.00 mg l⁻¹) and IBA, (2.00 mg l⁻¹) respectively; whereas minimum number of roots (1.30) was recorded in MS media supplemented with IBA, (3.00 mg l⁻¹) (Figure 4). These results also correlated with the previous experiment and validated that number of roots per plantlet also increased in MS medium supplemented with optimum concentration of IBA and NAA. Therefore IBA (2.50 mg l⁻¹) and NAA (2.00 mg l⁻¹) was selected for maximum proliferation of roots (Figure 4). The same pattern of root development was noted by Sultan et al. (2011), Kumar et al. (2013), Jafari et al. (2011), Lohidas & Sujin (2015) and Karule et al. (2016).

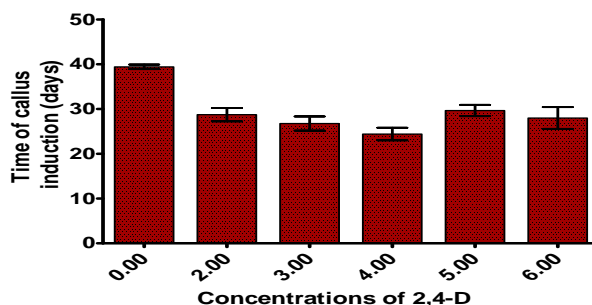


Figure 1 Effect of different concentrations of 2, 4-D with MS media on time of callus induction inoculation (days) in banana cv. Udhayam

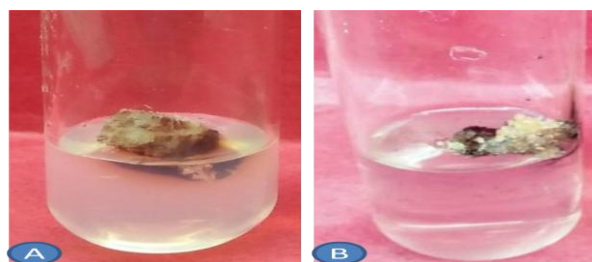


Figure 2 A. Initiation of callus.

B. Proliferation of callus.

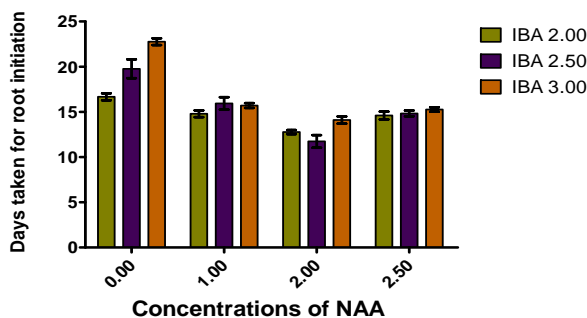


Figure 3 Effect of IBA and NAA on days taken for root initiation of banana cv. Udhayam under MS medium

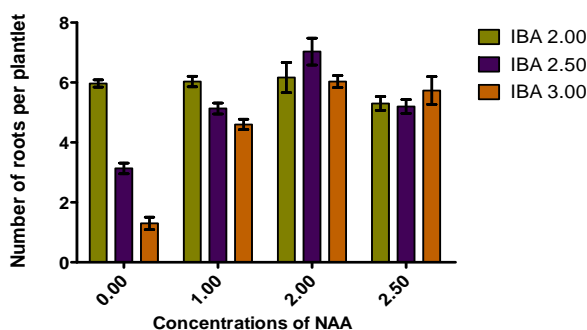


Figure 4 Effect of different concentrations of IBA and NAA on rooting of shoots (%) in plantlet regenerated *in-vitro* after 40 days (5 weeks) under MS medium

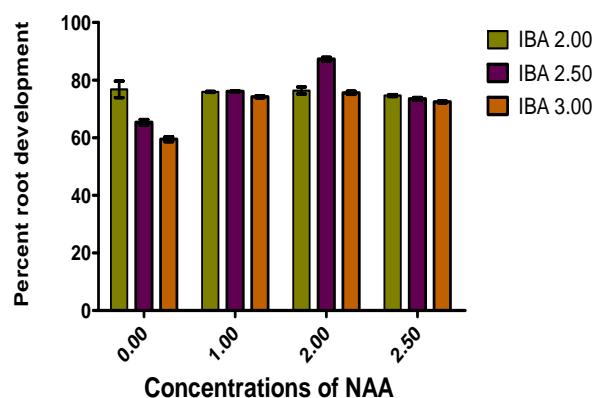


Figure 5 Effect of IBA and NAA on number of roots per plantlets of established culture of banana cv. Udhayam under MS medium



Figure 6 Root proliferation in MS medium supplemented with 2.50 mg l⁻¹ IBA and 2.00 mg l⁻¹ NAA

3.2.3 Percent root formation per shoot after 40 days

The data pertaining to percentage of roots per shoot development for primordial emergence are presented with suitable combination of IBA and NAA in MS medium. The current study revealed that maximum rooting percent was observed (87.30%) in plantlet of established culture treated with IBA, (2.50 mg l⁻¹) + NAA, (2.00 mg l⁻¹) followed by 76.78, 76.36 and 76.10 with the treatments of IBA, (2.00 mg l⁻¹) alone, IBA (2.00 mg l⁻¹) + NAA (2.00 mg l⁻¹) and IBA (2.50 mg l⁻¹) + NAA (1.00 mg l⁻¹) respectively, where as minimum percentage of roots per shoot (59.46%) was recorded in IBA (3.00 mg l⁻¹) treated explants (Figure 5 & 6). The result is similar to the findings of Govindaraju et al. (2012), Kalimuthu et al. (2007), Liu xuenhong et al. (2006), Karule et al. (2016) and Nandhakumar et al. (2018).

Minimum time duration (24.41 days) for induction of callus was obtained in MS medium supplemented with 2,4-D (4.00 mg l⁻¹). Minimum days taken for root initiation (11.73 days) were found using IBA, (2.50 mg l⁻¹) and NAA, (2.00 mg l⁻¹) combination. Maximum number of roots (7.03) and maximum rooting percent (87.30%) per plantlet was recorded under the treatment of IBA, (2.5 mg l⁻¹) and NAA, (2.00 mg l⁻¹). Therefore in this study it has been concluded that 2,4-D with concentration of 4.00 mg l⁻¹ has shown good response for callus induction whereas the combination of IBA (2.50 mg l⁻¹) and NAA (2.00 mg l⁻¹) was found better for regeneration of roots.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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