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Short Communication

Micropropagation of Banana

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The importance of plants in human life is not to be numbered. Banana is credit to have arisen in South Eastern Asia with India as one of the centers of origin apart from Indonesia, Phihppines, and Malaysia etc. Banana is one of the favoured fruits in India by reason of its low cost and free availability. Banana provides a steady diet than other fruits. Banana is composed of mostly carbohydrates water and which produced energy. It is substantial in calcium minerals and phosphorus. Banana is generally propagated vegetatively through suckers but traditionally method is laborious time consuming and not very efficient as far as production of homogenous plant concerned [1]. Tissue culture-based micro propagation systems are well developed for bananas and, consequently, can be exploited to multiply elite genotypes. As regards yields performance in banana, tissue culture plants have been reported produce 39% higher yield than sword suckers [2-3]. During 1994 there were 14 units engaged in micro-propagation of fruit crop, 12 units are only for banana. All sorts of varieties (dessert, cooking, brewing) are grown throughout Ethiopia. These varieties are not well studied [4].

The concentration of external cytokine in appears to be the main factor infecting multiplication. There are reports on the use of diphenyl urea derivatives in various cell-culture systems including both callus cultures and micro propagation of many woody-plant species [5-9]. The researchers state that cultivars responded significantly better to BAP in their shoot proliferation and that BAP was more economical than adenine-based cytokinins.

The Planting material of banana varieties viz. Robusta and Grand Nain were collected from the fields of local farmers. The sword suckers were collected after fruiting at the time before harvesting and were brought to the laboratory. The suckers were maintained and treated with anti-fungal agent (Bavistin/Mancozeb 4%) under the shaded nets at the site. Sword suckers were desirable for propagation compared to the water suckers (one developed out of injured or discarded corms, are of superficial origin and have broad leaves), as they are more vigorous, narrow tipped with a well-developed base and sword shaped leaves in early stage and these suckers come to bearing early.

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The suckers were washed thoroughly with Labolene (10-15%), and then were kept under running tap water for 1hr, so as to remove all the mud and tracts of contaminants on the surface. The suckers were then cut into the size of about 2-3 keeping a part of rhizome and pseudo stem and were brought to the laboratory. After removing from tap water the suckers were dipped in antifungal agent bavistin (4%) for 30 min. than the suckers were dipped in IPA for 30 sec and were air dried for 25 min. These air-dried suckers were kept in 0.2% mercuric chloride solution on a rotary shaker for 15-20 min depending on the size of sucker. After 15 min the solution was drained off and the suckers were washed thrice with sterile distilled water so as to remove traces of mercuric chloride as it is highly toxic. The suckers are kept in an antibiotic mixture containing oflaxacin 100 ppm, ceftazidime 100 ppm, ampicillin 100 ppm, gentamicin 100 ppm and rifampicin 50 ppm for 10 min after cutting them to 2-3 cm size for inoculation in induction media.

Murashing and Skoog's basal medium supplemented with different concentration of BAP (6-Benzyl Amino purine), Kinetin, NNA (Nepthal Acetic Acid) and IAA (Indole Acetic Acid) in different combinations was used tried for induction, multiplication and rooting. Culture were maintained until the first individual bud appeared. Subsequently each explant was cut in to two parts and a central meristem was given a vertical cut and culture ware inoculated on medium for 25 days. Transfer to fresh medium and sub culturing were carried out every 25 days up to three cycles.

In the multiplication stage, shoots explants were counted and the average shoot numbers from the sample was determined. The shoots were separated from the explants in the 3rd subculture. These shoots were inoculated in rooting media containing 0.5 mg/l IBA and 0.5 mg/l IAA. The culture was maintained at 3000 lux for the photoperiod of 16 hours at 25°C. After 20 days of incubation these shoots reached height of 5-7 cm with 3-4 leaves and 3-4 roots. They were transferred to Green House for primary Hardening.

During research it was observed that increasing the concentration of BAP during the initiation stage enhanced the percentage of buds formation. The importance of the application of high BAP concentration to being bud establish from explants were reported by [10-11] in Cavendish banana cultivar Brasilian (AAA). Previous researchers [12-14] indicated that 5 mg/l (22.2 µM) BAP was the optimum concentration for most banana cultivars.

Upon subculture for shoot proliferation, the highest number of normal and elongated shoots was derived from

media supplemented with 6 mg/l BAP and 0.5 mg/l NAA. It was observed that when BAP concentration was increased more (7 mg/l), the bud formation was seen but they turned albino. In low concentration of BAP (4 mg/l), bud proliferation (8 per explants) was seen but shoot elongation was slow. It was also observed that increased concentration of BAP (7 mg/l) along with high concentration of NAA (0.5mg/l) increased the growth of abnormal shoots [15] describe that phenomena of somaclonal variants in red banana (AAA) is due to the presence of high concentration of BAP in the culture medium. This study suggested that high concentration of BAP after bud initiation was not vital for shoot propagation due to the reduction in the number of shoots and prevalence rate of abnormality.

The results have shown that shoot multiplication and elongation was significantly better in the presence of NAA than BAP alone [16] reported that combinations of BAP with IAA or IBA were effective for *in vitro* multiplication of bananas and plantains [17] reported high shoot multiplication but a reduction in the length of shoots in media with a combination of BAP and IAA in triploid cultivar by using inflorescence explants [18] reported a reduction in the number as well as length of shoot that occurred with exposure to high levels of BAP alone (44.44 µM) in banana cv. Nanjanagudu Rasabale (AAB). Synergistic reaction of plant growth regulators have determine the cultural response in banana shoot proliferation and elongation.

Significantly highest number of roots was produced by 0.5 mg/l IAA + 0.5 mg/l IBA. The treatment, 0.5 mg/l IAA + 1.5 mg/l IBA produced 3.0 roots per explant. No roots were produced by control treatment. The present result is similar with the finding of [19] obtained 8.28 number of roots per plantlet on 0.5 mg/l IBA followed by 6.33 roots, 0.6 mg/l IBA. They also observed 3.89 and 3.97 number of roots in 0.2 mg/l IBA and 0.3 mg/l IBA, respectively [20] obtained similar results.

Fig 1. **[A]** Shoot multiplication on MS basal medium supplemented with BAP (6 mg/l) + NAA (0.5 mg/l), **[B]** Culture transferred in fresh medium after removal of Shoots, **[C]** Plantlets separated from culture and inoculated on MS basal medium + IAA (0.5 mg/l) + IBA (0.5 mg/l) + 2 mg/l Activated Charcoal, **[D]** Plants after 20 days with profuse rooting and ready to be transferred to Green House

SUMMARY

In the *in-vitro* multiplication of banana (*Musa spp*.) cv. Robusta and Grand Nain were studied. In the culturing of banana most widely used species is Grand Naine banana species. Before harvesting the sword suckers were collected and were treated with anti-fungal agent and cultured on Murashige and Skoog basal medium supplemented in multiplication or rooting media with 6mg BAP [Benzyl Aminopurine], 0.5mg NNA [Nepthal Acetic Acid] and 0.5 mg IAA [Indole Acetic Acid]. Observations were recorded for four sub culturing. Evaluations were done by counting the number of new shoots, leaves, roots produced at each subculture. Under *in vitro* conditions shoot tips coming from different rhizomes behaved differently. On the average 25 plants were produced from each shoot tip after four sub culturing. The important aspect of *in-vitro* propagation of banana is the potential use of this technique in developing new varieties of banana with desired traits. *In-vitro* propagation technique can be used as a tool in breeding for improvement of a triploid and sterile crop like banana, since more number of clonal propagated material can be obtained by this method.

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