



Review Article

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Standardization of Macro Propagation in Banana cultivars - A Review

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ABSTRACT

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Banana in India is mostly a crop of marginal farmers with little affordability to tissue culture plants which are 4-8 times higher than the sucker cost. Hence, a simple and farmer friendly method has been developed to bridge the gap in supply of healthy planting material with an affordable cost through macro-propagation. This method generates plantlets from sword suckers and initial explants so farmers can adopt this especially to enhance the planting material production of traditional cultivars.

Introduction

Banana and plantains are propagated vegetatively through sword suckers and other types of planting materials like bits, butts and peepers. But the most common limiting factor for enhanced productivity is the non-availability of clean and disease free planting material. To address the problem of poor suckering nature of the crop, tissue culture technology is used for the mass production of the planting material. India's requirement is approximately 2500 million plantlets, but only 60-80 million tissue culture plantlets are produced per year, which accounts only 2.5 per cent of total requirement and suckers constitute 95-97% of the planting material (Uma *et al.*, 2010)., so an attempt has therefore been made to review a wide range

of information available to the researcher. Banana is cultivated in a wide range of agro-ecological zones (Wanja, 2010). The major banana growing areas of the world are geographically situated between 20° and 30° North and South of Equator. The climate of these regions is characterized by wide temperature fluctuation between day and night and between summer and winter, and poorly distributed low rainfall (Robinson, 1996). The suitable mean temperature and rainfall for banana cultivation are 26.67° C and 100mm per month respectively (Morton, 1987).

Banana and plantains are monocotyledonous plants in the genus *Musa*. They are the largest herbaceous flowering plants. The aerial shoot is called a pseudostem and grows to a height

of 2 to 8 m depending on the variety, climatic conditions, soils and management. Each pseudostem can produce a single bunch of bananas. After fruiting, the pseudostem dies, but offshoots may develop from the base of the plant (Robinson and Sauco, 2010). The centers of origin of the crop is in South-East Asia and Western Pacific regions where their inedible, seed bearing, diploid ancestors can still be found (Robinson, 1996). Areas of secondary diversity are found in Africa. The plants are distributed on the margins of tropical rain forests roughly between latitudes 30° N and 30° S of the Equator (Morton, 1987; Wong *et al.*, 2002). Banana fruits come in a variety of sizes and colors when ripe, including yellow, purple, and red (Robinson and Sauco, 2010).

Banana (*Musa paradisiaca*) belongs to the family *Musaceae*. The cultivated bananas differ from their wild relatives by being seedless (reproductive features of flower are dysfunctional) and parthenocarpic (Heslop-Harrison and Schwarzacher, 2007). *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome), represent the two main progenitors of cultivated banana varieties (Robinson, 2007). Many of the domesticated bananas have proven to be triploid, $2n=3x=33$, with genome constitution of AAA (mainly sweet desert bananas) representing only a fraction of world production.

Sources of banana planting materials

Banana is a crop with dual propagation abilities, sexual through seeds and asexual through suckers. Seed propagation is common in wild species and the extent of seed set, germinability and dormancy depends on the species. All cultivated commercial bananas are triploid and sterile except for a few parthenocarpic AA and AB which are diploids. Banana seedlings can be obtained through three methods namely; natural

regeneration, tissue culture and macropropagation (Singh *et al.*, 2011).

Natural regeneration

In natural regeneration several types of propagating materials such as maiden suckers, water suckers, sword suckers, butt, peeper and bits are used in establishment of banana plantations but they vary in their suitability (Robinson, 2007). Suckers are the main planting materials and normally remain true-to-type (Heslop-Harrison and Schwarzacher, 2007). Two types of suckers, sword and water suckers, are normally used. Sword suckers have a well-developed base, pointed tip and narrow leaf blades while water suckers are small, less vigorous, broad leaved and emerge in clumps (Singh *et al.*, 2011). Natural regeneration has been in existence for decades because of its simplicity. It is cheap and does not require sophisticated skills. However, it is not recommended (Robinson and De Villiers, 2007). This is because sucker excavation damages roots of the mat and consequently reduces fruit yield. The method also contributes to the spread of nematodes and soil-borne diseases (Robinson and Nel, 1990). In addition, this method cannot produce enough planting materials for medium and large-scale producers (Rasheed, 2002). Growth of suckers is also very slow due to hormone-mediated apical dominance of the mother plant. A plant produces only 5-20 suckers during its life time (Singh *et al.*, 2011).

Tissue culture (Micropropagation)

Tissue culture refers to growing and multiplication of cells, tissues and organs on defined nutrient medium under aseptic and controlled environmental conditions (Ogero, 2012). Any part of the banana plant including pseudostems, suckers, peepers, lateral buds or even small eyes which contain a shoot

meristem can be used as explants in TC (Jarret *et al.*, 1985; Vuylsteke and De Langhe, 1985). However, though all of them behave similarly under *in vitro* conditions, peepers and sword suckers are preferred because they are easy to handle and only minimal damage is caused on the parent stool during their removal. Tissue culture requires special media which is often expensive.

The success of *in vitro* cultures depends largely on the choice of nutrient medium including its chemical composition and physical form (Murashige, 1974). Several media formulations have been reported for banana shoot tip culture but nearly half of them are modified Murashige and Skoog (MS) media. Other popular media include B5 (Gamborg *et al.*, 1968), SH (Schenk and Hildebrant, 1972), N6 (Chu *et al.*, 1975), and (LS) (Linsmaier and Skoog, 1975) media. The culture media have similar chemical composition with variations in concentrations.

In banana tissue culture (TC), a sucker is detached from the parent plant and brought to a laboratory where the outside tissue is pared away until only the growing point of approximately 10 mm³ remains (Robinson and De Villiers, 2007). This is sterilized and introduced into a nutrient medium under aseptic conditions. The cultures are then transferred into a growth chamber with controlled temperature and photoperiod. The growing points subdivide into several shoots which are then sub-cultured into fresh media. After reaching a height of approximately 4 cm, the plantlets are transferred onto a rooting medium. After rooting they are transferred to the greenhouse for acclimatization to natural conditions. A key feature of this technology is the ability to produce many disease free/healthy plants within a short time (Kahangi *et al.*, 2003; Dubois *et al.*, 2006). The sterile operational nature of TC procedures excludes fungi, bacteria and pests

from the production system. However, viruses such as the banana bunchy top virus and banana streak virus are not eliminated by this process unless virus indexing is done or other measures such as thermotherapy and use of meristem tips as explants are used (Macharia *et al.*, 2010). TC eliminates the necessity to harvest suckers from a commercial plantation normally associated with reduction in yields (Robinson and Nel, 1990). In addition, TC plants have inherently high level of juvenile vigor which renders them more photosynthetically active compared to plants derived from suckers (Robinson and De Villiers, 2007). Tissue culture plants have also been proven to have higher yields as compared to plants raised from conventional suckers (Robinson *et al.*, 1993). Furthermore, TC allows easy transfer of thousands of plants to farmers. Although the technology is highly efficient the initial cost of establishing tissue culture laboratories is very high and involves complex protocols (Vuylsteke and Talengera, 1998). This precludes its adoption amongst small scale banana seedlings entrepreneurs (Gitonga *et al.*, 2010). There is therefore a need for a feasible and easy to implement technique banana seedling production (Lopez, 1994). This research attempts to address this gap by proposing macropropagation technology which is affordable and easy to implement among small scale farmers.

Macropropagation technology

In macropropagation a whole sucker, a large piece of the parent corm or a sword sucker can be used to produce planting materials (Faturoti *et al.*, 2002). The technology can be implemented in two ways and can be done either in the field (*in situ*) or in the nursery (*ex situ*) (Singh *et al.*, 2011). Repression of apical dominance is usually done through complete/partial decapitation or by detached corm method to stimulate lateral bud development and increase suckering rate.

Effect of corm quality and cultivars on macropropagation

Kwa (2003) pointed that in vivo macropropagation is an alternative technique that involves disinfecting, desheathing banana corms to expose axillary buds and decorticating the apical meristem to suppress the apical dominance and enhances sucker productivity in plantain cultivars than Cavendish banana cultivars.

Joab, (2004) stated that the suckering ability of 'ItokeSege' is very low with an average of about 3 suckers per year per stool depending on agro-climatic conditions and managerial practices.

Baruahand Kotoky (2015) reported that mass multiplication of banana through macropropagation is a farmer's friendly method for disease free planting material generation at field level. The complete process of macropropagation takes 5-7 months (including a hardening period of 45 days) for production of suckers ready for planting. The length of this period varies with the prevailing temperature at the time of planting of the corms for propagation and it is also necessary to produce the suckers as per the proper time of planting in the particular region. Planting the decapitated and decorticated corms, weighing 1-1.5 kg, in the month of October, taking sawdust containing *Bacillus subtilis* initiation media and treating the corms with 40 ppm BAP resulted in production of 25-27 numbers of uniform tertiary suckers that are ready for field planting by the month of March-April, which is the recommended planting time for the state of Assam.

Effect of substrate on macropropagation

Beardsell and Nichols (1982) reported that the physical composition of the nursery potting medium can have a profound effect on the

supply of water and air to the growing plant as well as affect anchorage and nutrient and water holding capacity of the medium.

Baiyeri and aba (2005) conducted an experiment in Nsukka, Nigeria to study genetic and initiation media effects on number, quality and survival of plantlets at prenursery and early nursery stages. Ricehull and sawdust were evaluated as *Musa* sucker plantlet initiation media using five genotypes as test plants. Sword-sucker-corms whose apical dominance was physically destroyed were planted and evaluated for plantlet production during a period of about five months. The number, quality and pattern of plantlets produced and their survival were studied. Results showed that initiation media had statistically similar effects on most parameters measured. However, number of days to the emergence of the second and third plantlets was significantly ($P < 0.05$) earlier in ricehull. Variable genotypic responses to measured traits were in most cases significant.

Days to emergence of the first and fifth plantlet were shortest in 'FHIA 17' (a dessert banana hybrid) and longest in 'PITA 25' (a plantain hybrid). Emergence of the first three plantlets in landrace plantain ('Agbagba') was earlier than in dessert banana landrace ('Nsukka Local'). A higher proportion of plantlets excised from landrace genotypes had roots than those from the hybrids. Similarly, higher percentage of plantlets initiated in sawdust had roots (irrespective of genotypes). Survival of plantlets varied with genotypes, initiation media and rooting status of plantlets at the time of excision. In most cases plantlets excised with roots had higher percentage of survival. However, all plantlets of 'Nsukka Local' initiated in sawdust but were rootless survived. Slightly higher proportion of plantlets initiated in sawdust (irrespective of rooting status) survived than those initiated in rice hull.

Effect of growth regulators

Swennen and De Langhe, (1985) concluded that the higher shoot growth in corms treated with BAP at 1.5 mg L^{-1} corresponds well with the enhanced shoot emergence. An injection of BAP in plantain corms under field conditions enhanced bud formation as well as the speed of shoot development.

Talengera *et al.*, (1994) and Maerere *et al.*, (2003) reported that application of BAP at 3.0 and 6.0 mg/l has been recommended for enhancing *in vitro* shoot proliferation in plantains and bananas, respectively.

Osei (2005) and Kalimutha *et al.*, (2007) concluded that Cytokinin and auxin work antagonistically and thus an application of cytokinins decreases the apical dominance while an application of auxin increases the apical dominance. Benzylaminopurine is an adeninebased cytokinin popularly used for *in vitro* induction of axillary and adventitious shoots in banana.

Kalimutha *et al.*, (2007) stated that *in vitro* multiplication of 7 - 8 shoots per explant of plantain cultivars has been reported when MS basal medium was supplemented with BAP at 2.0 mg L^{-1} . Singh *et al.*, (2011) found that *in vivo* macropropagation combined with an application of BAP at concentration of 0.16 mg/l induces sprouting of axillary buds in Cavendish banana.

Ali *et al.*, (2011) studied about the initiation, proliferation and development of micropropagation system for mass scale production of banana through meristem culture. The shoot apical meristem of different sizes was cultured on Murashige and Skoog's (MS) medium supplemented with different concentrations and combinations of 6-benzylamino-purine (BAP), kinetin (Kin) and α -naphthaleneacetic acid (NAA) either alone

or in combination with each other under different temperature conditions ranging from 23 to 27°C. Shoot formation response from shoot apical meristem showed that MS medium containing 1.0 mg/l BAP showed best response for shoot formation. For shoot multiplication, MS medium containing 1.0 mg/l BAP + 0.25 mg/l kin provided the best multiplication response which was 8 shoot per culture vial within 21.6 days after inoculation into shoot multiplication medium. Shoot formation and multiplication response was also affected by temperature variations. The best results were obtained at $27^\circ\text{C} \pm 1^\circ\text{C}$. By increase or decrease in temperature, the rate of *in vitro* response was also decreased. For rooting of well-developed *in vitro* shoots MS medium supplemented with 1.0 mg/l Indole-3-butyric acid (IBA)+ 0.5 mg/l NAA showed 3.6 roots per plant after 6.8 days of inoculation into rooting medium with an average root length of 2.4 cm. 100% hardening response was obtained in Peat moss after 21 days of transplantation in glass house.

Rai *et al.*, (2014) noted that from the study made on the effect of various combinations of auxins and cytokinins on micropropagation of "Grand Naine" cultivar of Banana (*Musa*) that the rhizomes bearing the meristematic shoot tips were taken as explants from greenhouse maintained plants. These were surface sterilized with different concentrations of bavistin and HgCl_2 for different intervals of time and cultured on MS media supplemented with different concentrations of BAP (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) and NAA (0.25 and 0.5 mg/l). BAP at 2.0 mg/l along with NAA at 0.5 mg/l proved to be the best combination and showed optimum shoot growth. Multiplied shoots were inoculated on rooting media incorporated with either IBA or NAA (0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and Charcoal (2 gm/l) for root induction. IBA (2mg/l) and Charcoal (2 gm/l) produced maximum number of roots with a

lot of root hairs. Shoots obtained in rooting media were hardened in pottrays containing different potting mixtures, of which the mixture of Cocopeat and sand (2:1) showed maximum (96%) survival of plantlets.

Dayarani *et al.*, (2013) reported decapitation of rhizome and treatment with 0.04% BAP has shown good results with high number of buds and high per cent of regeneration. Banana shows strong apical dominance, which when overcome by decapitation helped in producing adventitious buds. Time taken for initiation of first bud was significantly less in treatment (Sucker + Decapitation of rhizome) compared other two treatments as it has taken only 30 days for the first bud initiation, whereas other treatments have taken more than 40 days. Number of buds regenerated into plantlets was also high in Sucker + Decapitation of rhizome. The plantlets regenerated through all the three treatments showed good response at acclimatization stage with good survival rate. The plantlets without proper root system were treated with IBA (0.25%) before hardening and have showed a vigorous growth at acclimatization stage. Highest per cent of rooting was seen in Sucker + Decapitation of rhizome, with 92.4% survival.

Saraswathi *et al.*, (2014) conducted an experiment to devise an efficient method of micropropagation for a high yielding but recalcitrant banana cv. Udhayam (PisangAwak, ABB) using shoot tip explants. Virus-indexed shoot tips were established in medium comprising full-strength Murashige and Skoog (MS) basal salts and vitamins, supplemented with 50 mg l⁻¹ ascorbic acid, 100 mg l⁻¹ myo-inositol and 4.0 mg l⁻¹ benzylamino purine (BAP). Among the various media tested for shoot proliferation, MS medium with BAP (3.0 mg l⁻¹) and 5% coconut water (CW) was found optimum as it produced the maximum number of 6.3

multiple shoots in a minimum period of 7.5 days. Rooting was achieved in the MS medium fortified with indole butyric acid (IBA) 0.5 mg l⁻¹ + naphthalene acetic acid (NAA) 1.0 mg l⁻¹ + activated charcoal (AC) 250 mg l⁻¹. Fully hardened planting material ensured high survival upon field transplantation.

According to Kindimba and Msogoya (2014) the appropriate concentration of BAP for enhancing in vivo macropropagation of French plantain cv. 'ItokeSege'. In vivo multiplication response was evaluated based on number of days to first shoot emergence, number of shoots per corm, number of roots per shoot and shoot size. In vivo macropropagation combined with BAP at 1.5 mg L⁻¹ is a suitable technique for improving multiplication and sucker growth of French plantain cv. 'ItokeSege'. The findings of this study provided an opportunity for the use of in vivo macropropagation coupled with BAP at 1.5 mg L⁻¹ as an alternative simple and cheap technology for rapid and mass production of planting materials for recalcitrant plantain varieties.

Effect of biofertilizers on macropropagation

Sajith *et al.*, (2014) conducted an experiment to enhance the efficacy of decortication in elite cv. Bangladesh Malbhog using additives like bio-fertilizers and plant growth hormones. The trial was carried out with suckers weighing 1.0-1.5 kg and sawdust as substrate. All treatments tested, showed good response in terms of plantlet production and enhanced bud proliferation, growth and better root profiles compared to control. Treatment (*Bacillus subtilis* + BAP) produced the maximum number of primary buds (3.77) followed by Treatment (*Trichoderma viride*) and Treatment (AMF + *T. viride*) with 3.50 and 3.47 buds respectively as compared to

control (2.03 buds). Secondary bud production was also observed higher in Treatment (*Bacillus subtilis*+ BAP) with 5.70 buds per sucker followed by treatments (*Trichoderma viride*) and (AMF + *T. viride*) with 4.70 and 4.57, respectively. As far as tertiary bud production was compared, (*Bacillus subtilis*+ BAP) gave the highest of 7.33 buds followed by treatments (*Trichoderma viride*) (7.20) and (AMF + *T. viride*) (6.70) with a least of 3.33 buds in control. Addition of IBA and *Azospirillum* were observed to have good response in terms of root formation and enhanced bud regeneration (5.77 tertiary buds). Total number of buds produced was also observed highest in *B. subtilis*+ BAP (16.80) followed by (*Trichoderma viride*) (15.40) and (AMF + *T. viride*) (14.73) suggesting that treatment combinations, *B. subtilis*+ BAP and AMF + *T. viride*, were effective for macropropagation of cv. Bangladesh Malbhog.

Tripathi *et al.*, (2014) recorded that the height and girth of pseudostem (146.16 and 65.33 cm, respectively), total number of leaves (34.33), total number of functional leaves per plant at the time of emergence of inflorescence (17.33) and length of inflorescence (112.83 cm) were maximum in the plants treated with 50 g *Azotobacter*+ 50 g *Azospirillum*+ 50 g PSB + 50 g *Trichoderma harzianum* per plant as compared to non-treated ones.

Treatments with 50 g *Azotobacter*+ 50 g *Azospirillum*+ 50 g PSB + 50 g *Trichoderma harzianum* per plant also resulted maximum bunch weight (22.25 kg), number of fingers per hand and per bunch (16.66 and 143.00, respectively), number of hands per bunch (8.33), finger weight (135.83 g), length (19.16 cm), diameter (15.33 cm), TSS (19.00 OBrix), total sugars (18.68%), pulp (80.86%) and pulp:peel ratio (4.22) with reduced peel (19.14%) and titratable acidity (0.47%).

Effect of disease and pest on macro propagation

Batista Filho *et al.*, (1991), found that larval populations of the weevil are positively related to temperatures and rainfall. Some farms were severely affected and had high mats therefore corms could not be selected. Gettman *et al.*, (1992) concluded that the corms that were not heavily infested (10% - 25% damage) can be treated with boiling water to kill any larvae and eggs present. According to Abera (2000) many eggs are oviposited in bananas with high mat due to the exposed corms which increase susceptibility to weevil attack. Gold *et al.*, (2001) stated that Desert bananas such as Cavendish (AAA), Kampala and Sweet banana which are relatively resistant to the banana weevil. Gold *et al.*, (2003) reported that weevil damage is more where temperatures are higher and is inversely related to altitude. Masanza *et al.*, (2005) stated that farm sanitation use of pheromone traps, pseudostem traps and use of entomopathogens can be employed to control the weevil. Control can also be done through host plant resistance (Kiggundu *et al.*, 2003) and botanical pesticides such as neem (Musabyimana *et al.*, 2001).

Njau *et al.*, (2011) studied some selected farms (in Central and Eastern regions of Kenya) for certification as sources of healthy banana corms for Macropropagation. In Eastern region, some plantations were heavily infested with weevils leading to a rejection rate of over 20% where the temperatures are warm, (25°C - 30°C) and favour thriving of the weevil. Although weevils are not transmitted from the corm to the suckers generated through macropropagation, the results show that chemical and cultural control measures should be taken to reduce weevil attacks and thereby increase availability of higher quality corms for

propagation. The corms that are lightly infested should be well pared to remove all larvae and cured fully before placing in the propagation chamber. In another survey to identify the key pests and pathogens of banana in Central and eastern regions of Kenya it was concluded that *Fusarium oxysporum* f. sp. *cubense* was isolated from less than 1% corms of sweet banana and Kampala varieties. *Radopholussimilis* was isolated from all the varieties but its incidence was highest (46%) in the Cavendish variety. Endophytes and non-pathogenic microorganisms were isolated from more than 90% of the corms. Over 98% of the propagated corms produced healthy seedlings and only less than 1% of the corms propagated rotted in the propagation media due to non-pathogenic causes. In areas with high weevil infestation it was difficult to obtain corms with the standard required for macro propagation. The information obtained showed that macro propagation technique effectively produces healthy banana seedlings.

Macropropagation in other crops

Banerjee *et al.*, (2011) conducted an accelerated protocol for large-scale propagation of *Dendrocalamus asper*, an edible bamboo, in which seven axillary shoots were induced in vitro from each excised tender node (15–20 mm in length) containing single axillary bud when nodal segments were inoculated in semisolid Murashige and Skoog (MS) medium fortified with 5 mg/l 6-benzylaminopurine (BAP). Maximum multiple shoot formation (14) was observed when in vitro generated axillary shoots were transferred to liquid MS medium containing 5 mg/l BAP and 40 mg/l adenine sulphate. A maximum of 93.33 % shoots were effectively rooted when transferred to liquid MS medium supplemented with 1 mg/l indole-3-butyric acid (IBA). A simple acclimatization

procedure of 55 days, primarily in cocopeat for 20 days and finally in a blend of sand, soil and farm yard manure (1:1:1 v/v), ensured a very high survival rate within next 35 days. After acclimatization, rooted plantlets were further multiplied by splitting of rhizomes, formed *in vivo* within 90 days of growth. After 90 and 180 days of acclimatization, plants were successfully transferred to the field and maintained in an unirrigated condition with the initial supplementation of farm yard manure @ 10 kg/pit; where around 85 % survivability with 25 culms per bush attaining an average height of 4.5 m was recorded up to four years.

Ahouran *et al.*, (2012) conducted an experiment which involved in vitro propagation of *Crocus cancellatus* with ornamental and horticultural value. Two different types of corm explants (apical and basal halves of corms) were cultivated onto Murashige and Skoog's (MS) medium supplemented with different levels of α -naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP). One to five cormlets emerged from every responding explant through direct organogenesis. Apical halves of corms were more highly responsive than basal halves and produced a maximum multiplication rate with 3.45 ± 0.06 cormlets per explant in $95.33 \pm 2.33\%$ of the explants in MS medium supplemented with 3% sucrose and 2 mg L⁻¹ NAA and 1 mg L⁻¹ BAP. The effect of cold storage temperature on in vitro cormlets sprouting was studied. Cormlets stored at 4°C for 8 weeks had more statistically significant positive effects on cormlets sprouting from the controls.

Baskaran *et al.*, (2014) conducted an experiment to study the effect of various bio-fertilizers and commercial formulations on growth and development of gladiolus. The results showed that early sprouting of corm (17.10 days) was obtained by *Azotobacter*,

maximum number of leaves (11.33) was produced by *Azospirillum*, maximum plant height (161.8 cm) was obtained by phosphorus solubilizing bacteria (PSB), early flowering (81 days) was recorded by Annapurna®, maximum diameter of floret (9.43 cm) was observed by PSB, maximum number of florets per spike (12.33) was recorded by Annapurna®. Significant increase in spike length (80.33 cm) was obtained by Sumangala®, maximum rachis length (54.67 cm) was obtained by both Annapurana® and *Azotobacter*, maximum number of corms per plant (2.90) was observed by *Azospirillum*, maximum number of cormels (7.30), maximum weight of corm (44.93 g), maximum weight of cormels per plant (10.40 g), maximum volume of corm (51.17 cm³), maximum corm diameter (5.67 cm) and maximum value of propagation coefficient (231%) was obtained by the application of Flower Booster®. Application of bio-fertilizers and commercial spray formulation products not only improved the qualitative and quantitative parameters but also improved the soil fertility and productivity.

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