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A novel *in vitro* shoot excision technique for enhancing proliferation in banana cv. Chini Champa

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Abstract

Micropropagation technology has been commercially exploited for mass multiplication of banana. Several parameters such as nutrient media, explants, culture conditions etc have been standardized. However, a novel *in vitro* shoot excision technique designed to enhance the proliferation rate of banana cv. Chini Champa has been examined for the first time. We meticulously examined the impact of excision angles (45º and 90º) and excision site (Tip, middle and base) during shoot proliferation stage. Our findings unequivocally demonstrate that employing a 45º angle excision and tip excision yield the highest multiplication rates and biomass accumulation, surpassing other excision angles and methods. The substantial enhancement in shoot numbers, growth and biomass underscores the potential of this technique for improving banana propagation protocols, offering a valuable tool for sustainable banana production.

Key words: Excision, micropropagation, multiplication, proliferation.

Introduction

Banana holds a prominent position as the fourth most valuable global fruit crop prized for its nutritional richness, affordability and consistent availability throughout the year. Leading the charge in banana production, India boasts a remarkable annual yield of 33.06 million tonnes cultivated across 0.92 million hectares with a commendable national average of 35.93 tonnes per hectare (NHB, 2020-2021). This bountiful output contributes significantly accounting for approximately 37% of India's total fruit production. The diverse Indian climate supports the cultivation of 20 distinct banana cultivars with the Cavendish types reigning supreme commanding 1.16 million metric tonnes and encompassing a substantial 49.1% share of the nation's banana production (Anonymous a, 2022). The primary banana growing regions of India include the North-east and South, although Maharashtra in West Central India leads the way (Anonymous b, 2022). The bananas grown in India are mostly used for dessert, culinary and dual purpose. The popular banana cultivars grown in India are Grand Naine, Rasthali, Poovan, Red banana, Robusta, Dwarf Cavendish, Nendran and Chini Champa. Chini Champa predominately cultivated in North East region of India (Borborah *et al*., 2016), West Bengal, Bihar, Odisha (Mallick *et al*., 2020). Micropropagation technology for some of indigenous banana cultivars such as Chini Champa is not available. Chini Champa can withstand water-logging, tolerant to *Fusarium wilt* and fairly resistant to bunchy top disease.

Nowadays banana propagation through tissue culture raised plantlets is in high demand and the farmers are growing banana by using tissue culture techniques due to quality and yield of the crop. Development of *in vitro* techniques has enabled rapid clonal propagation of genetically uniform superior clones, production of secondary metabolites, and ex situ conservation of valuable germplasm (Wilken *et al*., 2013). Banana tissue culture has significantly contributed to the propagation and cultivation of bananas, helping to meet the global demand for this popular fruit crop. Micropropagation of banana has acquired commercial preposition and *in vitro* technique is routinely being utilized for multiplication of banana (Bachchan, 2016). The advantages of *in vitro* propagation include high multiplication rate, physiological uniformity, availability of disease-free material (Agbadje *et al*., 2021). In response to the rising demand for tissue-cultured banana plants, commercial enterprises have become increasingly interested in cultivating *in vitro* banana plants. Due to rising consumer demand for healthy, disease-free plantlets, tissue culture technology is no longer restricted to research institutions. This technology is adopted by numerous commercial firms. (Resmi and Nair, 2007). However, there is a pressing need to devise cost-effective methods to enhance multiplication rates in the case of Chini Champa which is still being multiplied through conventional means. Despite earlier research delving into manipulating nutrient media, explant type, growing conditions, etc. for enhancing proliferation rate under *in vitro* condition (Teisson and Cote, 1997; Vuylsteke, 1998; Youmbi and Ngaha, 2004; Kumar *et al*., 2011) an unexplored avenue involves understanding how shoot cutting angles and positions influence multiplication.

This study aims to bridge this gap by developing a pragmatic approach to enhancing multiplication rates through deliberate shoot cutting angle and position manipulation. This technique holds the potential to benefit researchers and commercial enterprises alike by streamlining tissue culture production, thus optimizing costs.

Material and methods

Plant materials: The mother block of banana cv. Chini Champa was maintained at ICAR-Central Institute for Subtropical Horticulture, Lucknow during 2020-2022. The sword sucker

from healthy mother plants were removed and washed under running tap water. The basal portion of the corm is cut in to 12 x 12 x 15 mm in size. The trimmed portion is further kept in a solution containing Tween-20 and washed under running tap water for 30 minutes. Washed explants is then treated with pre-washed solution containing 0.1 % Carbendazim + 100 mg/L Cefotaxim + 100 mg/L Ascorbic Acid and kept under Incubator shaker for 60 minutes in agitation (100 RMP). The explant is then again washed with distilled water 3-4 times. The washed explants are brought inside laminar air flow cabinet and treated with 0.1 % mercuric chloride for 8 minutes. Then explants were washed with sterile distilled water for 6 times inside laminar air flow. The washed explants are then dried on absorbent paper. The explants are further trimmed down to 8 x 8 x 10 mm using sterilized surgical blade. The excised explants then inoculated on MS medium fortified with 4.5 mg/L BAP.

Nutrient formulation: MS medium fortified with 4.5 mg/L BAP, 1.0 mg/L IAA, 60 mg/L Mg SO_4, 100 mg/L M-Inositol, 100 mg/L Glutamin, 30 g/L Sucrose and 7 g/L Agar was sterilized in autoclave at 121°C temperature and 15 psi pressure. The medium was allowed to cool and kept for 72 hours prior to utilization.

Explant preparation: The *in vitro* developed shoots of banana were brought to laminar air flow. Each clump consists of 7-8 healthy shoots. In first experiment, the shoots were excised either from tip, mid portion or base of the clump and inoculated on MS medium fortified with 4.5 mg/L BAP, 1.0 mg/L IAA, 60 mg/L Mg SO_4, 100 mg/L M-Inositol, 100 mg/L Glutamin, 30 g/L Sucrose and 7 g/L Agar and in second experiment, the shoots were excised from mid portion in 45 degree and 90 degree and inoculated on same medium and kept under dark at 25+2°C with 50-55% RH in growth rooms along with control and data was recorded periodically for different parameters.

Plant biomass and regeneration frequency: Shoots were taken out from culture bottles and washed to eliminate attached media and fresh weight was taken. Then these shoots were kept in hot air oven at 38 degrees Celsius. Dried shoots are taken out and weighed. Regeneration frequency was calculated by dividing total number of shoots regenerated from initial number of inoculated shoots over a fix time interval.

Statistical analysis: All statistical analyses were carried out using Minitab software (version 21.2). The results were analyzed using one-way ANOVA followed by Duncan's test at the 5% level. The data on regeneration frequency percentage was transformed to arcsine transformation before analysis.

Results and discussion

The data obtained during course of investigation have been depicted in table and graph and discussed in the light of available references**.**

Impact of angle of incision on *in vitro* shoot proliferation: In this present study, we used two different angles for excision 45° and 90° (Table 1 and Fig. 1), respectively and describe the impact of the angle of excision on the proliferability of *in vitro* shoots of banana cv. Chini Champa. Data revealed that almost all the parameters *viz.*, the number of shoots regenerated (38.200±2.860), regeneration frequency (2.385±0.179), fresh (9.305±1.745 g) and dry weight (1.725±0.285 g) of shoot, average width of shoot (0.347±0.041 cm) were maximum when the shoots were excised at 45° angle, with the exception of the average length of the shoot (3.306±0.183 cm) which was higher at 90° excision. It was evident that, both 45° and 90° excision had significant effects on number of shoots regenerated, fresh weight of shoot and average length of shoot.

Shoots excised at a 45° angle exhibit a higher multiplication rate and shoot biomass suggesting that this angle supports the development of more substantial shoot mass. However, when considering the dimensions of the shoots, the 90° angle leads to the generation of longer shoots, while the 45° angle results in shoots that are thicker on average. Overall, the 45° excision angle appears to be more favorable for enhancing shoot regeneration and mass, whereas the 90° angle produces fewer but longer shoots.

Table 1. Effect of angle of excision on *in vitro* proliferation of shoot in banana cv. 'Chini Champa'

Variables	Angle of excision (45°)	Angle of excision (90°)
Number of shoots regenerated	38.20±2.86^a	32.80±1.48^b
Regeneration frequency	2.39±0.18^a	2.05±0.09^b
Fresh weight of shoot (g)	9.31±1.75^a	7.26±0.45^b
Dry weight of shoot (g)	1.73±0.29^a	1.19±0.13^b
Average length of shoot (cm)	3.31±0.18^b	4.42±0.24^a
Average width of shoot (cm)	0.35±0.04^a	0.28±0.01^b

Note**:** Values indicated by different letters are significantly different at the 5% level by Duncan's multiple range test (n=30) SD standard deviation

The angle of excision significantly influences the proliferation of banana shoots. Shoots excised at a 45° angle consistently outperformed those excised at a 90° angle in terms of various parameters, including the number of shoots regenerated, regeneration frequency percent, fresh and dry weight of shoots, and average width of shoots. These findings are particularly noteworthy as they suggest that the angle of excision can have a substantial impact on shoot proliferation. The enhanced shoot proliferation observed at a 45° angle of excision may be attributed to the removal of a larger portion of the shoot, potentially disrupting apical dominance and leading to the development of multiple shoots. While this concept has not been extensively explored in existing literature, our findings provide valuable empirical evidence to support this hypothesis.

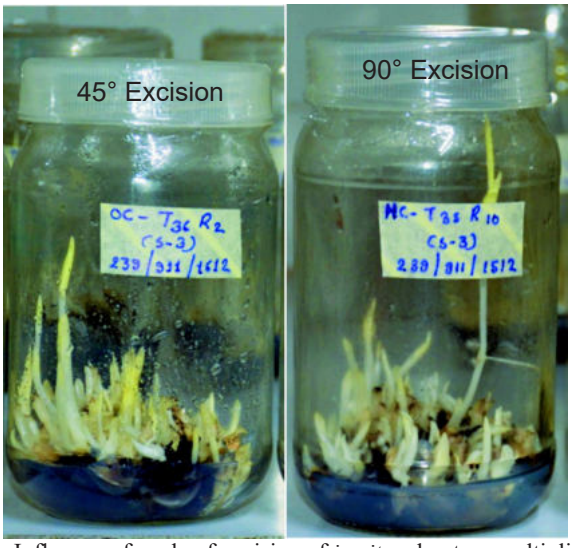

Fig 1. Influence of angle of excision of *in vitro* shoot on multiplication rate

There is dearth of information on impact of angle of excision on shoot proliferation under *in vitro* condition in any crop. However, Feri and Czako 1981 investigated differential response of tip and mid portion of pepper shoots under *in vitro* condition and found that apical portion produced shoot buds while mid portion produced roots when inoculated on MS medium fortified with 1 mg/L BAP and 1 mg/L IBA. The reason behind higher regeneration frequency with excision of shoots at 45° angle could be attributed to removal of more tissue leading to breaking of apical dominance and resulting in more number of shoots as compared to 90° angle excision.

These results have practical implications for tissue culture Labouratories and banana cultivators aiming to maximize the efficiency of shoot multiplication. Adopting a 45° angle for excision could lead to higher multiplication rate, reduced production costs, and increased productivity in banana propagation.

Impact of excision site on *in vitro* proliferation: Three distinct locations, including the tip, middle, and base of shoot clumps, were chosen for excision and their effects on the proliferation of shoot was evaluated (Table 2 and Fig. 2). It was evident that, tip cutting had significantly greater effect on all parameters, including number of shoots regenerated (41.800±1.095), regeneration frequency (1.425±0.095 %), fresh weight of shoot (11.202±1.535 g), dry weight of shoot (2.262±0.199 g) and average width of shoot (0.335±0.040 cm), with the exception of average length of shoot, which was greater in base excision (4.072±0.124 cm). Vegetative parameters such as number of shoots regenerated (41.800±1.095, 37.000±1.225, 35.600±1.817), fresh weight of shoot (11.202±1.535, 9.665±1.089, 9.123±0.798) and average length of shoot (3.244±0.162, 3.870±0.376, 4.072±0.124 cm) have significantly greater effect on all three place of excision (tip, medium and base).

Table 2. Effect of excision site on *in vitro* shoot proliferation of banana cv. Chini Champa'

Variables	Tip excision	Middle excision	Base excision
No. of shoots regenerated	41.80±1.10[a]	37.00±1.23[b]	35.60±1.82[b]
Regeneration frequency	1.43±0.10[a]	1.19±0.04[b]	1.15±0.06[b]
Fresh weight of shoot (g)	11.20±1.54[a]	9.67±1.09[ab]	9.13±0.80[b]
Dry weight of shoot (g)	2.26±0.20[a]	1.69±0.16[b]	1.55±0.22[b]
Average length of shoot (cm)	3.24±0.16[a]	3.87±0.38[a]	4.07±0.12[b]
Average width of shoot (cm)	0.34±0.0[4]a	0.17±0.01[b]	0.20±0.01[b]

Note: Values indicated by different letters are significantly different at the 5% level by Duncan's multiple range test (n=30) SD standard deviation

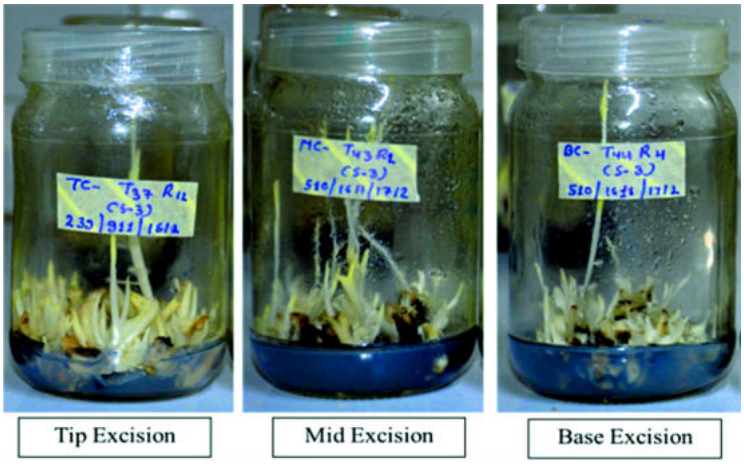

Fig. 2. Influence of excision sites of *in vitro* shoot on multiplication rate

Shoots excised from the tip show the highest regeneration, with a greater number of shoots and higher regeneration frequency compared to the middle and base excision sites. Additionally, shoots from the tip have the greatest fresh and dry weight, indicating more substantial growth. In contrast, the base excision site produces the longest shoots, although they are fewer in number and thinner compared to those from the tip. Shoots from the middle and base also have lower weights and regeneration frequencies. Overall, tip excision proves to be the most effective for promoting shoot proliferation, leading to more robust and thicker shoots, while base excision favors the growth of longer, but fewer and thinner shoots.

Remarkably, tip excision had a significantly greater effect on all parameters compared to middle or base excisions. This finding challenges the conventional practice of selecting the middle portion of shoots for sub culturing in banana propagation. While scientific literature has lacked evidence regarding excision site selection, our study provides empirical support for the superiority of tip excision. Usually, mid portion of shoots has been reported for subculturing of banana. However, no scientific evidence is available as to why mid portion is chosen for excision. Our study reveal that shoot tip excision promotes proliferation as compared to mid portion or base portion. However, no report is available on this aspect in literature.

Effect of angle and site of excision on dry weight biomass percent: The dry weight biomass percent was studied at both angle of excision and site of excision (Fig. 3 and 4). Greater dry weight biomass obtained at 45° angle of excision (17.98 %) as compared to 90° angle of excision (16.44 %). When compared the dry weight biomass at site of excision, we obtained higher dry weight biomass percentage at tip excision (19.64 %) followed by middle excision (16.67 %)

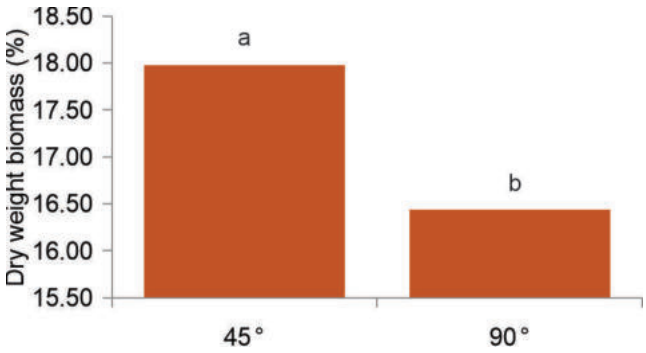

Fig 3. Dry weight biomass at different shoot excision angles

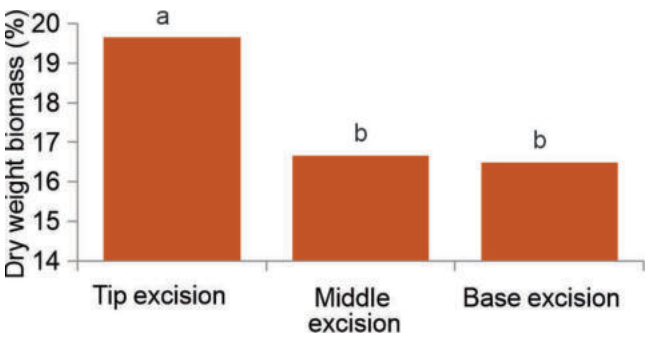

Fig 4. Dry weight biomass at different excision sites

and base excision (16.48 %). The reason could be attributed to higher multiplication rate obtained on excision of shoot tip at 45° angle. Higher multiplication rate coupled with thicker shoots led to higher dry mass accumulation.

The examination of dry weight biomass percentage further supports the advantages of tip excision at a 45° angle. Shoots excised at this angle displayed a higher dry weight biomass percentage compared to those excised at a 90° angle. The reason behind this could be the higher multiplication rate observed with shoot tip excision, coupled with thicker shoots, resulting in increased dry mass accumulation.

This study offers valuable insights for the optimization of *in vitro* propagation techniques for banana cv. Chini Champa. The choice of excision angle and location within the shoot clump significantly affects shoot proliferation. By challenging conventional practices and providing empirical evidence, our findings pave the way for improved methods in banana cultivation and propagation, which can ultimately benefit the agricultural sector. Our research demonstrates the effectiveness of a 45° angle excision and tip excision technique in enhancing the proliferation of Banana cv. Chini Champa under *in vitro* conditions. These techniques offer a promising avenue for improving banana propagation protocols, benefitting both researchers and growers by increasing plantlet production and improving yield. Further research is needed to delve into the underlying mechanisms and assess the long-term performance of plantlets produced using these techniques in field conditions.

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