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Development of an Efficient *In vitro* Propagation and *Ex vitro* Establishment Protocol for BARI Mukhikachu-1 (*Colocasia esculenta* cv. antiquorum L.)

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Key words: Tissue culture, Shoot multiplication, Acclimatization, Aroid crop

Abstract

An efficient *in vitro* micropropagation and *ex vitro* establishment protocol was developed for *Colocasia esculenta cv.* antiquorum L (BARI Mukhikachu-1). This protocol produced suckers from *in vitro* plantlets after hardening and the plant establishment stage. Shoot tips from the sprout of cormel were used as explants. MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA produced the highest number of shoots, 4.88 shoots per explant, after 35 days of culture. The highest number of roots, 22 roots per plantlet, was found in ½MS + 0.5 mg/l IBA after 35 days of culture. Rooted plantlets were acclimatized for 14 days in potting media containing ash and cocopeat at a 1 : 1 ratio. Established plants were transferred to a plastic pot containing 50% soil + 50% compost for 60 days to promote further growth and development. Each plant produced an average of 5.83 suckers, which can be used separately as planting materials. This protocol can be used for the large-scale production of uniform planting materials.

Introduction

Mukhikachu (*Colocasia esculenta* cv. antiquorum L) belongs to the family Araceae and is an important aroid crop in Bangladesh. It is rich in valuable phytochemicals, fibers, minerals, and carbohydrates and is used as a vegetable (Samadder et al. 2025). A total of 132,880 tons of Mukhikachu corm and cormels were produced from 24,971 hectares of land, and gradually their area and production have increased from 22,779 hectares in 2019-2020 to 24,971 hectares in 2023-2024 (BBS 2025). The propagating materials of Mukhikachu are cormels, which are kept alive in the field by vegetative propagation (Adelegn 2018). Since the cormels are often not kept in cold storage, they are kept at room temperature (average 27°C) from May to August. High temperature and humidity

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during storage rapidly deteriorate cormel quality and induce premature dormancy breaking. Taro species are known to be susceptible to a variety of viral infections (Bunawan et al. 2019, Mignouna et al. 2019, Oladimeji et al. 2025). Lack of improved varieties and planting materials, rare natural flowering and seed setting, transmission of pathogens, particularly dasheen mosaic virus (DsMV) during vegetative propagation, which can cause yield loss up to 90% or may cause loss of genotypes, are major constraints of taro (Mikami and Tsutsui 2019, Ahmed et al. 2020, Oladimeji et al. 2022). Compared with conventional propagation, tissue culture enables rapid production of large quantities of uniform and healthy propagules within a limited space and time throughout the year (Wijerathna-Yapa and Hiti-Bandaralage 2023). A sophisticated vegetative propagation method called micropropagation can produce a large number of homogeneous, pathogen-free transplants in a short period (Hasnain et al. 2022, Sarchi et al. 2025). BARI Mukhikachu-1 is one of the popular varieties in Bangladesh, but seed availability is the main problem to increase production area. The present study was conducted to develop an efficient *in vitro* propagation and *ex vitro* establishment protocol for large-scale production of BARI Mukhikachu-1.

Materials and Methods

The variety BARI Mukhikachu-1 was used in this study. The experiment was carried out at the Tissue Culture Lab, Tuber Crops Research Centre (TCRC), Bangladesh Agricultural Research Institute (BARI) during 2024-2025. Corms were collected from TCRC and BARI and washed with running tap water to remove the surface dust. The clean corms were kept in a container having a solution of water and liquid soap for five minutes, followed by thorough washing with running tap water until the bubbles were cleaned. All the corms were dried by airflow and kept in a brown paper bag, and placed in a place at room temperature for sprouting. When the sprouts reached an optimal size of 1.0-1.5 cm, they were excised using a sterile surgical blade and thoroughly washed under running tap water. The outer leaves were removed until the inner clean tissue was exposed, and the shoots from the sprouts were used as explants. Explants were then carried out in a laminar airflow hood and immersed in 70% ethanol for 1 min. Then dipped into 10% Clorox, and 2-3 drops of Tween 20 were added and kept for 15 min, followed by washing three times with autoclaved distilled water. The margins of the explants were cut off to remove the damaged parts. Shoot tips (0.5-1.0 cm) were cultured in ready MS medium supplemented with 3% (w/v) sucrose (Merck, Darmstadt, Germany) and plant growth regulators. Medium was solidified with 0.7% (w/v) agar (Duchefa, Netherlands). Six different concentrations of BAP, viz. 0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/l, and 0.5 mg/l IBA was used for the induction of multiple shoots. The auxin IBA 0.5 mg/l, combined with BAP, enhances multiple shoots (Sofian et al. 2018). For rooting, ½MS medium supplemented with 3 different concentrations of IBA, viz. 0, 0.5, and 1.0 mg/l, was used. The pH of the medium was adjusted to 5.8 by using 0.1N NaOH or 0.1N HCl with a digital pH meter. Each glass jar contained 50 ml of media, and the media was autoclaved

at 121°C with a pressure of 15 psi for 20 min. A single explant was cultured in each jar, and six replicated glass jars were used in each treatment and kept in a growth room at 24 ± 1°C with 75% relative humidity and 16/8 hrs photoperiod (16 hrs light and 8 hrs dark) and light intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent tubes. Data were recorded on days to shoot initiation, number of shoots/explant, days to root initiation, number of leaves/plantlet, number of roots/plantlet, length of shoot (cm)/ plantlet, length of root (cm)/plantlet, fresh weight of shoot (g)/plantlet, and dry weight of shoot (g)/plantlet after 35 days of culture (Fig. 1).

Fig 1. *In vitro* plantlets production from shoots of the corm sprout: (a) initiation of shoot, (b) multiple shoots obtained from MS + 8.0 mg/l BAP + 0.5 mg/l IBA after 35 days of culture, and (c) rooted plantlets obtained from ½MS + 0.5 mg/l IBA after 35 days of culture.

The plantlets were removed from the culture vessel and washed with distilled water. Shoot and root parts were separated using a surgical blade and placed on an analytical balance, and the fresh weight was recorded in grams. Shoots were kept in a drying oven at 65°C for 72 hrs. Then the dry weight of the shoots was recorded in grams on an analytical balance. The experiment was designed with a completely randomized design (CRD) including 3 replications. Data were analyzed using R (Version 4.6.0; R Core Team, 2026). The significance of differences among means was determined by using Duncan's Multiple Range Test (DMRT). Statistical significance was considered at $P \leq 0.05$.

Plantlets obtained from the *in vitro* rooting treatments were transferred from the growth room to normal room temperature (27 ± 1°C) and kept for 3 days for pre-hardening. After 3 days, plantlets were removed from the media carefully without disturbing the roots and washed thoroughly with tap water to remove the adhesive media with root. Then, plantlets were transferred to a small plastic pot (10.5 cm × 10.0 cm) filled 2/3-part with a ratio of 1 : 1 ash and coco dust. A single plantlet was transferred to the pots and placed in a shady place for 14 days. Watering was carried out twice daily, and survival % was recorded. After 14 days, established seedlings were shifted in large size plastic pot (21.5 cm × 18.0 cm) containing 1 : 1 (soil and compost) media. Different cultural management, such as watering and weeding, was carried out as necessary and kept for 60 days for growth and development. Data on plant survival (%), number of suckers/ plant, number of leaves/ plant, number of roots/ plant, length of shoot (cm), and length of root (cm)/ plant were recorded (Fig. 2).

Fig. 2. Acclimatization and multiple sucker development stage: (a) well-developed rooted plantlet, (b) established plants after 14 days of planting, and (b) mother plant with newly developed sucker after 60 days of planting.

Results and Discussion

Significant variation ($P \leq 0.05$) was observed in various treatments for days to shoot initiation. The longest time required for shoot initiation (11.5 days) was recorded in the control treatment (MS medium without BAP and IBA). MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l IBA, which was statistically similar to MS medium + 10.0 mg/l BAP + 0.5 mg/l IBA, for shoot initiation, 7.5 days was recorded. The shortest time to shoot initiation (5.5 days) was observed in MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA (Fig. 3a). Significant differences among treatments were observed for the number of shoots per explant. The maximum number of shoots (5)/ explant as obtained in MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA, followed by MS medium supplemented with 6.0 mg/l BAP + 0.5 mg/l IBA, and MS medium supplemented with 10.0 mg/l BAP + 0.5 mg/l IBA, which showed 2.5 shoots/explant. The lowest number of shoots per explant (1.3) was recorded on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l IBA, 4.0 mg/l BAP + 0.5 mg/l IBA, and the control treatment (MS medium without BAP and IBA) (Fig. 3b). Significant variation was observed in the number of leaves/shoots. The maximum number of leaves per explant (4.5) was obtained in MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA; the lowest number of leaves, 2.5, was recorded from MS medium supplemented with 10.0 mg/l BAP + 0.5 mg/l IBA. No significant difference was observed for the number of leaves/plantlets for the other treatments (Fig. 3c). A significant difference was observed among the treatments for the length of the shoot. MS medium supplemented with 4.0 mg/l BAP + 0.5 mg/l IBA produced the longest shoot, 6.70 cm, BAP and IBA-free media (control) showed the lowest, 4.63 cm length of shoot. Moreover, the second highest length of shoot 6.15 cm was obtained from the treatment MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA (Fig. 3d).

There was a significant difference among the treatments regarding the *in vitro* root production. The highest number of roots/plant (1.6) was found in MS medium supplemented with 4.0 mg/l BAP + 0.5 mg/l IBA, 6.0 mg/l BAP + 0.5 mg/l IBA, and 8.0 mg/l BAP + 0.5 mg/l IBA. The lowest number of roots, 0.5, was obtained in MS medium with 10.0 mg/l BAP + 0.5 mg/l IBA (Fig. 4a). During *in vitro* culture, contamination and survival percentage were recorded, where less than 1% contamination and almost 99% plantlet survival were observed.

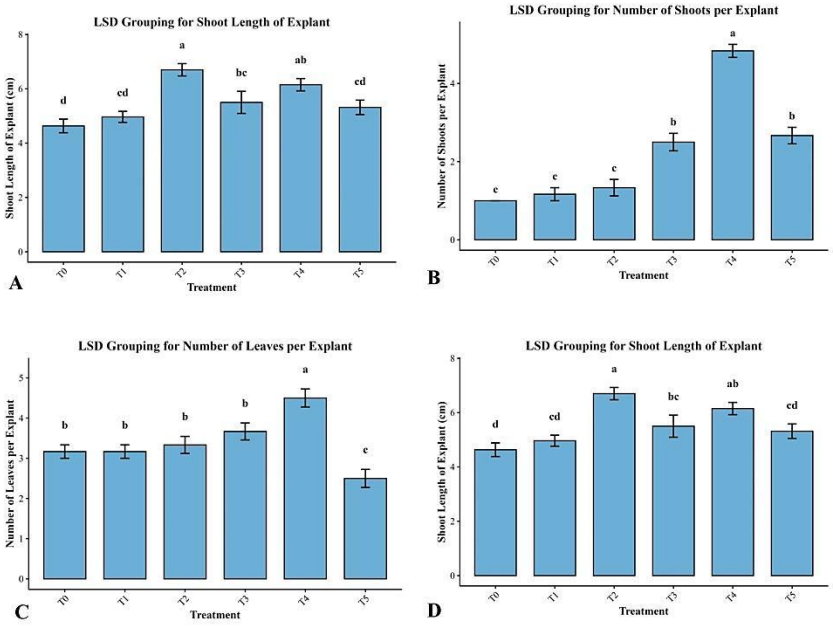

Fig 3. Effects of different concentrations of BAP in combination with 0.5 mg/l IBA on *in vitro* shoot induction and growth of BARI Mukhikachu-1: (a) days to shoot initiation, (b) number of shoots per explant, (c) number of leaves per explant, and (d) shoot length per explant (cm). Vertical bars represent the standard error of the means. Means followed by different letters are significantly different at P ≤0.05 according to DMRT.

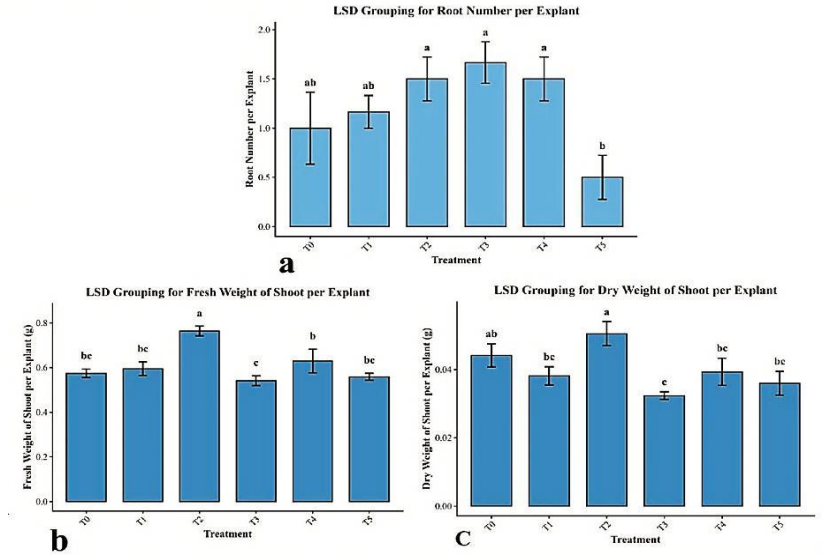

Fig. 4. Effects of different concentrations of BAP in combination with 0.5 mg/l IBA on *in vitro* growth and biomass accumulation of BARI Mukhikachu-1: (a) root number per explant, (b) fresh weight of shoot per explant, and (c) and dry weight of shoot per explant (g). Vertical bars represent the standard error of means. Means followed by different letters are significantly different at P ≤0.05 according to DMRT. ***

A significant difference was found in the fresh weight and dry weight of the shoot. Maximum fresh weight of shoot per plantlet was obtained from the treatment MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA (Fig. 4b), and the highest dry weight of shoot was recorded from MS medium supplemented with 4.0 mg/l BAP + 0.5 mg/l IBA (Fig. 4c).

A significant ($p \leq 0.05$) difference was observed among the different treatments (Table 1). ½MS medium supplemented with 1.0 mg/l IBA required a maximum of 7.5 days to initiate root, followed by ½MS medium supplemented with 0.5 mg/l IBA (4.7 days). The highest (22) number of roots/plant was recorded in ½MS medium supplemented with 0.5 mg/l IBA, followed by ½MS + 1.0 mg/l IBA (11). Moreover, the longest root, 7.9 cm, was found from the treatment ½MS medium supplemented with 0.5 mg/l IBA, followed by 3.9 cm from ½MS medium supplemented with 1.0 mg/l IBA. No root formation was observed in the control treatment (½MS medium without IBA).

Table 1. Analysis of variance for *in vitro* rooting of BARI Mukhikachu-1.

Source of variances	df	Days to root initiation (days)	Number of roots/plant	Length of root (cm)	Length of shoot (cm)	Number of leaf/plant
IBA	2	86.05***	726.22***	94.41***	24.42***	2.06**
Error	15	0.322	2.76	0.63	0.71	0.23

Notes: **, ***: significant at the 0.05 and 0.01 probability level, respectively, and df: degree of freedom.

There was a significant difference among the treatments regarding the growth and development of *in vitro* plants. ½MS media supplemented with 0.5 mg/l IBA showed the highest length of shoot, 7.9 cm, followed by ½MS + 1.0 mg/l IBA, which showed 5.5 cm shoot length. ½MS medium without IBA (control) produced the lowest 3.9 cm length of shoot. There were no significant differences observed regarding the number of leaves/ plant from the treatments ½MS + 0.5 mg/l IBA and ½MS + 1.0 mg/l IBA. Control treatment (only ½MS media) produced the lowest number of leaves, 2.6 per plantlet (Table 2).

Table 2. **Effects of different concentrations of IBA on *in vitro* rooting of BARI Mukhikachu-1.**

Treatment	Days to root initiation (days)	Number of roots/plants	Length of root (cm)	Length of shoot (cm)	Number of leaves/plants
½MS	NR	NR	NR	3.9c	2.6b
½MS + 0.5 mg/l IBA	4.7b	22.0a	7.9a	7.9a	3.8a
½MS +1.0 mg/l IBA	7.5a	11.33b	3.9b	5.5b	3.3a
CV	13.99	14.93	14.94	19.99	14.48

Notes: Different letters (a, b, c, etc.) in the same column are significantly different ($p \leq 0.05$) based on Duncan's test. Traits sharing the same letter are not significantly different from each other. NR=No response

Significant differences among treatments were observed for plant survival, number of suckers, root number, shoot length, root length, number of leaves per sucker, number of roots per sucker, and shoot length per sucker ($P \leq 0.05$), whereas the number of leaves per mother plant was not significantly affected by IBA concentration. Well-developed plantlets from the treatments ½MS medium supplemented with 0.5 mg/l IBA and ½MS medium supplemented with 1.0 mg/l IBA were evaluated under pot conditions to determine the influence on plant survival, sucker formation, and plant development. The plantlets were transferred to potting media and maintained under ex vitro conditions for 60 days. The highest plant survival, 99%, was found in plants from ½MS + 0.5 mg/l IBA, and plantlets from ½MS +1.0 mg/l IBA showed 86% survival. The number of suckers per mother plant was higher in ½MS + 0.5 mg/l IBA compared to ½MS + 1.0 mg/l IBA, showing 5.83 and 2.28 suckers per mother plant, respectively. Mother plants from ½MS medium supplemented with 0.5 mg/l IBA produced a higher number of leaves (4.56) and roots (36.94) than those under ½MS + 1.0 mg/l IBA (3.22 and 24.61, respectively). Similarly, the average shoot length of 34.99 cm and root length of 30.11 cm of mother plants were recorded in ½MS medium + 0.5 mg/l IBA, 20.91 cm and 17.93 cm, respectively, were recorded from ½MS + 1.0 mg/l IBA. Number of leaves and number of roots/suckers were recorded as 2.59 and 9.79, respectively, from ½MS supplemented with 0.5 mg/l IBA, whereas 1.79 and 4.08 roots/sucker was found from ½MS medium supplemented with 1.0 mg/l IBA. The length of the shoot, 15.29 cm, was obtained in ½MS + 0.5 mg/l IBA and 7.65 cm in ½MS + 1.0 mg/l IBA (Table 3). This study concluded that for *in vitro* rooting, ½MS medium supplemented with 0.5 mg/l IBA is more effective for the ex vitro establishment and growth of Mukhikachu compared to higher concentrations of IBA. This treatment enhanced plant survival, sucker formation, and overall vegetative growth, suggesting its suitability for large-scale production of propagation materials of *Colocasia esculenta* cv. BARI Mukhikachu-1.

Table 3. **Effects of different concentrations of IBA on the growth and development stage of the mother plant in potting media at *ex vitro* conditions after 60 days of planting.**

Treatments	Plant survival (%)	Number of sucker/mother plant	Number of leaves/mother plant	Number of roots/mother plant	Length of shoot/mother plant(cm)	Length of root/mother plant(cm)	Number of leaves/ Sucker	Number of roots/ sucker	Length of shoot/ sucker (cm)
½MS + 0.5 mg/l IBA	99.22 a	5.83a	4.56 a	36.94 a	34.99 a	30.11 a	2.59 a	9.79a	15.29a
½MS + 1.0 mg/l IBA	85.61 b	2.28 b	3.22 a	24.61 b	20.91 b	17.93 b	1.79b	4.08b	7.65b
CV	3.31	17.52	34.40	10.36	1.84	7.01	4.27	6.82	11.69

Notes: Different letters (a, b, c, etc.) in the same column are significantly different ($p \leq 0.05$) based on Duncan's test. Traits sharing the same letter are not significantly different from each other.

Mukhikachu is a popular crop in Bangladesh due to its high nutritional value, market demand for off-season summer vegetables, and high productivity. In many parts of underdeveloped nations, taro makes a substantial contribution to people's diets in terms of providing carbohydrates (Alam and Abdul Kadir 2022). Most of the harvested

cormels are consumed as vegetables, while only a small portion is reserved for planting materials. Though cormels are used as planting materials and seed cormel supply is a limiting factor, *in vitro* plantlets can speed up the seed production program and overcome the unavailability of quality planting materials. Micropropagation is the most effective method for achieving a rapid increase in propagules on a year-round basis (Gunasena et al. 2025, Ravindra et al. 2025). Shoot tip culture is a useful and expanding method to rapidly obtain a large number of propagules (Tajaldeen 2021, Wada and Feyissa 2021). This technique is very effective since it can quickly and efficiently create large amounts of disease-free, genetically homogeneous plants. Plant growth regulators that support shoot propagation belong to the cytokinin group. In this study, MS medium was used for micropropagation of Mukhikachu. A study (Alam and Kadir 2022) reported that MS medium is the most suitable medium for *in vitro* production of *C. esculenta* cv. Bolosso I. compared to B5 media and Chu (N6) media. Cytokinins, such as BAP and kinetin, are known to promote cell division and shoot formation, making them essential for successful shoot induction (Elasi, M. F et al., 2024, Alizadeh et al. 2024). One study conducted by (Pham et al. 2024) used BAP for developing a successful regeneration strategy for the rapid propagation of *H. gigantea* species. Multiple shoots were induced using different concentrations and types of cytokinin alone and in combination with auxin hormones. Another research finding revealed that the percentage of shoot proliferation under different concentrations of BAP was found in the range of 83 to 100% (Abdulhafiz et al. 2020). In this study, MS medium supplemented with different concentrations of BAP + 0.5 mg/l IBA was used for multiple shoot production. Using BA alone (3-5 mg/l) for multiple shoot induction in 9 species of *Dieffenbachia* (El-Hassani et al. 2024). In this study, the maximum number of shoots per explant (4.88) was obtained in MS medium supplemented with 8.0 mg/l BAP with 0.5 mg/l IBA. This finding is consistent with the results reported by a previous study (Pourhassan et al. 2023). They suggested shoot multiplication, both BAP treatments and the hormone-free control were significantly more effective than either Kn or TDZ. (Chen and Yeh 2007) reported on the micropropagation of *Aglaonema sp*., the shoot number increased linearly with increasing concentration of BA. (Adelegn 2018) also reported that the maximum average number of shoots (8.53/corm and 5.8/sprout explants) was obtained in MS medium supplemented with 8.0 mg/l BAP + 3.0 mg/l IAA. Exogenously applied IBA induces rooting more efficiently than IAA, hence widely used as a rooting agent in agricultural applications (Alizadeh and Dumanoğlu 2022).

Besides, IBA is involved in other auxin-mediated developmental processes such as leaf epinasty, cell division, stem bending, and root hair elongation (Kepue 2024). Research has been conducted using ½MS and auxin for sufficient rooting (Donmez et al. 2022). In this research, *in vitro* shoots were cultured on ½ strength MS medium with the addition of different concentrations of IBA. Many studies have been conducted using ½ strength MS medium with IBA for obtaining the highest number of roots (Kang and Sivanesan 2025).

The highest number of roots per plantlet (22.0) was obtained on ½MS medium supplemented with 0.5 mg/l IBA after 35 days of culture. These results agreed with the previous study (Anbazhakan et al. 2025), where the highest number of roots, 9.63 per shoot, was recorded on half-strength MS medium augmented with 0.5 mg/l IBA. Successful micro-propagation depends on the ability to transfer *in vitro* raised plantlets to a potting mixture (Martinez et al. 2023), and acclimatization is a stage that has a specific objective to adapt the plantlets obtained from *in vitro* culture before being planted in the open field. This study conducted a two-step acclimatization process for producing new suckers from mother plants, which would be used as planting materials for corm and cormel production. After successful hardening, plants were transferred in large size plastic pots containing ½ soil + ½ compost for 60 days, and obtained an average of 5.83 new suckers.

This study described a complete *in vitro* protocol and *ex vitro* establishment of BARI Mukhikachu-1 plantlets. Shoot tip from the sprouted corm was used as an explant, and a successful response was obtained after surface sterilization. In case of shooting, MS medium supplemented with 8.0 mg/l BAP + 0.5 mg/l IBA showed superior performance regarding all the traits. The highest number of shoots, 4.88 per explant, was recorded after 35 days of culture. Moreover, ½MS supplemented with 0.5 mg/l IBA showed the maximum number of roots, 22 per shoot, including other rooting parameters. Furthermore, pot media containing 1 : 1 (ash and cocopeat), performed 99% plant establishment during acclimatization. In addition, after acclimatization, plants were grown for 60 days in large-sized plastic pots containing soil and compost (1 : 1) media. In this stage, a maximum of 5.83 new suckers per mother plant was obtained, and these suckers, along with the mother plant, will be used for seedlings as planting materials. This protocol can be utilized for year-round large-scale production of planting materials, thereby overcoming the shortage of quality seed cormels. The findings of this research will inform recommendations for optimizing the production of BARI Mukhikachu-1 using *in vitro*-derived seedlings. The developed protocol will support farmers interested in the commercial cultivation of this crop.

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