PLANT SCIENCE TODAY
Vol x(x): xx-xx
https://doi.org/10.14719/pst.9027

eISSN 2348-1900

RESEARCH ARTICLE

In vitro regeneration of some promising genotypes of sugarcane (*Saccharum Officinarum* L.) in Bangladesh

Rebina Ferdous[1, 3], Md Jakir Hossain[2*], AKM Nazmul Huda[3] & Rahimul Alam[4]

[1]Basic & Applied Research on Jute Project, Bangladesh Jute Research Institute, Dhaka 120 7, Bangladesh
[2]Agarwood Research Laboratory, Bangladesh Forest Research Institute, Chattogram 421 1, Bangladesh
[3]Department of Biotechnology & Genetic Engineering, Islamic University, Kustia 700 3, Bangladesh
[4]Bangladesh Sugarcane Research Institute, Ishurdi, Pabna 662 0, Bangladesh

*Correspondence email - mjakirbotru@gmail.com

Received: 22 April 2025; Accepted: 28 August 2025; Available online: Version 1.0: 10 October 2025

Cite this article: Rebina F, Md Jakir H, Nazmul HAKM, Rahimul A. *In vitro* Regeneration of Some Promising Genotypes of Sugarcane (*Saccharum Officinarum* L.) in Bangladesh. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9027

Abstract

This study explores the *in-vitro* regeneration of sugarcane (*Saccharum officinarum* L.) genotypes in Bangladesh, aiming to optimize micropropagation protocols that can enhance sugarcane productivity and genetic diversity. The research evaluates the effects of various concentrations of auxins and cytokinins on callus induction, shoot regeneration and rooting in three sugarcane varieties: Isd 37, Isd 40 and ZH 110-65. The results demonstrated that 3.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) was the most effective for callus induction, yielding a high initiation rate of 97.33 % in Isd 40. For shoot regeneration, the optimal concentration of 1.0 mg/L Benzylaminopurine (BA) produced the highest number of shoots per culture, reaching 17.0 shoots per culture in Isd 40. Rooting was most successful with 5.0 mg/L Naphthalene acetic acid (NAA) which resulted in an average of 9.36 roots per shoot in Isd 40. Acclimatization trials showed that 65-80 % of the rooted plantlets survived under ex vitro conditions. This research provides a robust, reproducible tissue culture protocol for sugarcane that can be utilized to propagate disease-free, high-yielding and genetically diverse genotypes. The findings hold significant potential for improving sugarcane breeding and commercial production in Bangladesh, addressing the growing demand for high-quality sugarcane and supporting the sustainable growth of the local industry. The study lays a foundation for future crop improvement programs and germplasm conservation.

Keywords: callus induction; genotypic variation; germplasm conservation; *in-vitro* regeneration; micropropagation; phytohormones; sugarcane; tissue culture

Introduction

Sugarcane (*S. officinarum* L.) has been an essential crop for over 2,500 years, with origins in India and China before spreading to Western Europe in the eighteenth century (1, 2). Modern sugarcane cultivars are derived from complex crosses between *S. officinarum* (2n = 80) and *Saccharum spontaneum* (2n = 40:128), followed by backcrossing interspecific hybrids with the *S. officinarum* parent (3). Globally, sugarcane is the primary source of white sugar, contributing approximately 70 % of the world's sugar production. Additionally, it serves as a crucial raw material for biofuels, biofertilizers and other industrial products (4, 5).

In Bangladesh, sugarcane is a cornerstone of the agricultural economy, serving as the sole source of white sugar and ranking among the country's top three major field crops (6). However, production has faced challenges, with outputs declining from 38.6 lakh tons in 2016-2017 to 34.03 lakh tons in 2017-2018, contributing only 0.81 % to the national GDP (7). The rising global demand for sugar and biofuels has amplified the need for high-yielding, disease-resistant sugarcane varieties that are locally adapted.

Traditional propagation methods, such as stem cuttings, face several limitations, including labor-intensive processes, low propagation rates and vulnerability to pathogen transmission. Additionally, sugarcane's polyploidy and high heterozygosity complicate the development of pure lines and prolong breeding cycles to 10-15 years (8). In Bangladesh, these challenges are exacerbated by biotic and abiotic stresses, underscoring the urgent need for innovative solutions to improve sugarcane propagation.

Tissue culture offers a transformative approach to sugarcane micropropagation by enabling rapid multiplication of disease-free, genetically uniform plantlets. It allows for year-round production, higher yields and improved genetic traits such as disease resistance and increased sugar content (9). Tissue culture has been shown to accelerate the breeding of elite genotypes and support germplasm conservation efforts. Despite its potential, sugarcane tissue culture research in Bangladesh remains limited, with inefficient protocols hindering the rapid development and commercialization of high-quality planting material.

This study seeks to address these challenges by optimizing *in-vitro* regeneration protocols for three sugarcane varieties: Isd

40, Isd 37 and ZH 110-65. By evaluating the effects of auxins and cytokinins on callus induction, shoot regeneration and rooting, the research aims to develop efficient and reproducible micropropagation techniques. These findings will contribute to advancing sugarcane breeding programs, improving productivity and promoting the sustainable development of the sugarcane industry in Bangladesh.

Materials and Methods

Plant materials

Leaf sheaths of sugarcane (*S. officinarum L.*) were collected from 3-4-month-old plants of three varieties: *Isd 37*, *Isd 40* and *ZH 110-65*. These varieties were chosen for their unique traits: *Isd 37* for its performance in poor soil conditions, *Isd 40* for its tolerance to various stresses (drought, flood, salinity and waterlogging) and *ZH 110-65* for its adaptability to local environmental conditions in Bangladesh. The explants were obtained from the experimental fields of the Bangladesh Sugarcrop Research Institute (BSRI) and used for *in vitro* propagation.

Surface sterilization of plant material

Surface sterilization of explants was carried out following the protocol described by Hossain *et al.*, 2024, with minor modifications. Actively growing leaf sheaths were excised into sections measuring 7-8 cm and initially washed in a 3 % (w/v) Savlon solution for 5 min to remove surface contaminants. The sections were then rinsed thoroughly with sterilized distilled water. Subsequently, the explants were treated with 70 % ethanol for 1 minute, followed by additional rinsing with sterile distilled water. For further sterilization, the explants were immersed in a 0.1 % (w/v) mercuric chloride ($HgCl_2$) solution for 10 min with vigorous shaking to ensure uniform exposure. After this step, the explants were rinsed thoroughly 3-4 times with sterilized distilled water to remove residual mercuric chloride. The sterilized explants were then dried on sterile tissue paper under aseptic conditions before inoculation onto the culture medium. To monitor contamination rates and ensure the effectiveness of the sterilization protocol, a hormone-free medium was used as a control during sterilization trials. This step confirmed that aseptic conditions were consistently maintained throughout the procedure.

Preparation of culture media

Murashige and Skoog (MS) basal medium was prepared with macronutrients, micronutrients, vitamins and plant growth regulators (PGRs). Stock solutions for macronutrients (10x), micronutrients (100x), iron (100x) and vitamins (100x) were prepared and stored at 4 ± 1 °C. Growth regulators, including 2,4-D, BA, NAA, indole-3-butyric acid (IBA) and kinetin (Kn), were dissolved in appropriate solvents (e.g., ethanol, NaOH, or HCl) and diluted to working concentrations. The media was adjusted to pH 5.8, autoclaved at 121 °C and 1.16 kg/cm^2 for 20 min and poured into sterilized Petri dishes.

Culture techniques

All inoculations were performed under a laminar airflow cabinet sterilized with UV light and 70 % ethanol. Instruments were autoclaved and treated with a hot bed sterilizer before use. The work area and hands were routinely disinfected with 70 % ethanol to maintain sterility throughout the process. The surface-sterilized explants from the three sugarcane varieties (*Isd 37*, *Isd 40* and *ZH 110-65*) were inoculated into MS medium supplemented with various growth regulators to optimize *in vitro* propagation. For callus induction, the explants were cultured on MS medium containing different concentrations of auxins, including 2,4-D at 1.0, 2.0, 3.0 and 4.0 mg/L and NAA at 1.0, 2.0 and 3.0 mg/L. The cultures were incubated in the dark at a controlled temperature of 25 ± 2 °C. Observations were recorded for the number of days required for callus initiation and the percentage of explants forming callus.

Following successful callus induction, the calli were transferred to shoot regeneration media containing cytokinins such as BA or Kn at concentrations of 0.5, 1.0 and 2.0 mg/L. To explore synergistic effects, BA (1.0 mg/L) was combined with auxins, including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and NAA at concentrations of 0.1, 0.2 and 0.5 mg/L. The cultures were maintained under a 16 hrs photoperiod with fluorescent light at an intensity of 2000-3000 lux, a temperature of 25 ± 1 °C and approximately 70 % humidity. Parameters such as the number of days to shoot initiation, the number of shoots per culture and shoot length were monitored to evaluate shoot regeneration efficiency.

For rooting, juvenile shoots measuring at least 5 cm in height were transferred to rooting media supplemented with auxins, including NAA, IBA and IAA, at concentrations ranging from 3.0 to 6.0 mg/L. The cultures were incubated in a temperature-controlled growth room under similar photoperiod and light conditions. Observations were recorded for the number of days to root initiation, the number of roots per shoot and root length. Sub culturing was carried out every two weeks during callus induction and every 4-5 weeks during shoot regeneration and rooting to maintain the cultures and ensure optimal growth.

Acclimatization

Rooted plantlets were carefully removed from culture vessels, washed under running water to remove residual gel and transplanted into pots containing sun-dried soil mixed with sand (2:1 ratio). Potted plants were covered with perforated polythene bags and maintained in a growth chamber (80 % humidity) for 10 -12 days before gradual exposure to ambient conditions. The survival rate was recorded and factors influencing plantlet success were evaluated.

Statistical analysis

Data on callus induction, shoot regeneration and rooting were collected from five replicates per treatment. Statistical analysis was performed using ANOVA to assess significant differences at a 95 % confidence level, followed by Tukey's HSD test for post-hoc comparisons. Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 26 (22).

Results

Effects of auxins in callus induction

The surface-sterilized leaf sheath explants of sugarcane varieties Isd 37, Isd 40 and ZH 110-65 were inoculated onto MS medium containing 2.0 mg/L 2,4-D and NAA for callus induction. Callus initiation was observed 16 days post-culture, with vigorous callus growth until the medium's nutrients were depleted. The data on days required for callus initiation and the percentage of explants

showing callus induction are presented in Fig. 1. Results showed that 2,4-D significantly reduced the time to callus initiation compared to NAA. Isd 40 exhibited the shortest callus initiation time (16 days) and the highest percentage of callus induction (65 %) under 2,4-D treatment. Conversely, NAA required longer durations, with ZH 110-65 demonstrating the fastest response (22 days) and a maximum callus induction rate of 20 %. These findings underscore the superior efficacy of 2,4-D over NAA in promoting callus induction.

Among the tested 2,4-D concentrations (1.0, 2.0, 3.0 and 4.0 mg/L), 3.0 mg/L was the most effective, consistently inducing the highest percentage of callus across all three varieties (Table 1). Isd 40 recorded the highest callus initiation rate (97.33 %), whereas Isd 37 showed the lowest (64 %). The performance of 4.0 mg/L and 2.0 mg/L ranked second and third, respectively, while 1.0 mg/L demonstrated the weakest effect. The callus characteristics varied, with Isd 37 producing yellowish-white semi-compact calli (Fig. 2E), Isd 40 forming cream-colored loose calli (Fig. 2A) and ZH 110-65 generating white compact calli (Fig. 2I). These observations emphasize the genotype-specific response to 2,4-D and highlight the optimal concentration of 3.0 mg/L for callus induction in sugarcane.

Effects of cytokinins on shoot regeneration

Among the cytokinins tested, BA consistently outperformed Kn in promoting shoot regeneration, as shown by shorter shoot initiation times, higher shoot numbers per culture and greater shoot lengths (Table 2). No shoot proliferation occurred at the lowest Kn concentration (0.5 mg/L). For both BA and Kn, 1.0 mg/L proved the most effective concentration. At this level, ZH 110-65 exhibited the earliest shoot initiation (15 days) and the highest number of shoots per culture (8.0 ± 2.31). Isd 37 recorded the longest shoots (2.78 ± 0.51 cm), followed by Isd 40 (2.61 ± 0.54 cm). These findings highlight the optimal efficacy of BA at 1.0 mg/L for *in-vitro* shoot regeneration in sugarcane (Fig. 2B, F&J).

Effects of cytokinins combined with auxins on shoot regeneration

The combination of BA (1.0 mg/L) with NAA (0.5 mg/L) significantly enhanced shoot regeneration across all parameters and varieties (Table 3). This treatment led to the shortest time to shoot initiation (9 days) in Isd 40 and ZH 110-65, the highest shoot proliferation rate (17.0 ± 0.3 shoots per culture) in Isd 40 and the longest shoots (5.43 ± 0.28 cm) in Isd 37 (Fig. 2C, G & K). The results illustrate the synergistic effect of BA with NAA at 0.5 mg/L in promoting shoot differentiation.

Rooting of juvenile shoots under *in-vitro* conditions

The rooting performance of juvenile sugarcane shoots was assessed with MS medium supplemented with NAA, IBA, or IAA at 3.0 mg/L (Table 4). NAA exhibited superior efficacy in all rooting parameters. Isd 40 showed the shortest root initiation time (9.33 days) and the highest number of roots per shoot (6.88 ± 2.17), while ZH 110-65 displayed the longest roots (3.63 ± 0.62 cm) under NAA treatment. IBA provided moderate results, whereas IAA demonstrated the weakest performance, with Isd 37 recording the longest root initiation time (20 days) and the lowest root production (1.0 ± 0.03 roots/shoot).

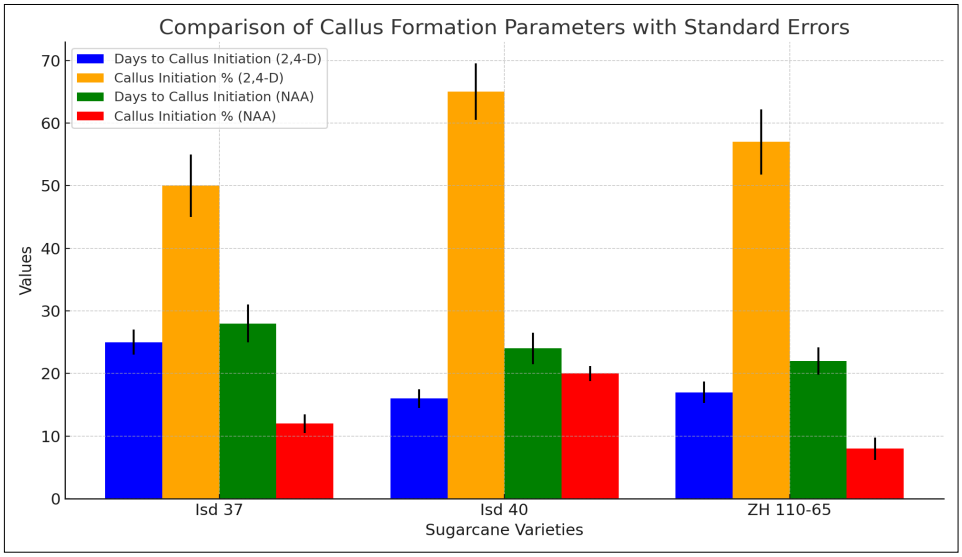

Fig. 1. Effects of auxins (2,4-D and NAA) for callus induction from the leaf sheath explants of three sugarcane varieties (Isd 37, Isd 40 and ZH 110 -65). Bars represent standard errors from five replications.

Table 1. Effects of 2,4-D on callus induction (%) at different concentrations were evaluated using leaf sheath explants from three sugarcane varieties, with each experiment involving 10 explants

Concentrations (mg/l)	Average performance of callus of three sugarcane varieties								
	Isd 37			Isd 40			ZH 110-65		
	Colour	Texture	Callus initiation (%)	Color	Texture	Callus	Colour	Texture	Callus initiation (%)
1.0	Yellowish white	Semi Compact	39.33±0.31	Cream	Loose	47.33±0.51	white	Compact	35.33±0.81
2.0	Yellowish white	Semi Compact	50.67±0.11	Cream	Loose	66.00±0.61	white	Compact	51.67±0.21
3.0	Yellowish white	Semi Compact	64.00±0.21	Cream	Loose	97.33±0.53	white	Compact	75.00±0.51
4.0	Yellowish white	Semi Compact	57.67±0.61	Cream	Loose	81.33±0.93	white	Compact	60.00±0.33

Table 2. The effects of cytokinins on *in vitro* shooting performance were evaluated from the calluses of three sugarcane varieties. Each treatment consisted of 10 cultures and the data (mean ± S.E.) were recorded after 35 days of culture (-, Indicates no initiation of shoot)

Cytokinins (mg/L)	Average shooting performance of three sugarcane varieties								
	Isd 37			Isd 40			ZH 110-65		
	Days to shoot initiation	No. of shoots/ culture	Ave. shoot length (cm)	Days to shoot initiation	No. of shoots/ culture	Ave. shoot length (cm)	Days to shoot initiation	No. of shoots/ culture	Ave. shoot length (cm)
BA 0.5	22	2.0±0.55	1.41±0.61	32	3.0±0.83	2.05±0.19	17	4.0±1.07	2.31±1.00
1.0	19	4.0±0.37	2.78±0.51	25	7.0±1.20	2.61±0.54	15	8.0±2.31	2.30±0.28
2.0	23	4.0±0.83	1.26±0.33	28	4.0±0.71	2.11±0.18	18	6.0±0.75	2.13±0.95
Kn 0.5	-	-	-	-	-	-	-	-	-
1.0	28	2.0±0.42	2.12±0.57	30	5.0±0.91	2.39±0.63	24	4.0±1.05	2.07±0.57
2.0	33	1.0±0.07	0.96±0.15	31	2.0±0.25	1.02±0.38	26	3.0±0.73	1.34±0.43

Table 3. Effects of the Cytokinin (BA) Combined with Auxins (IAA, IBA and NAA) at Different Concentrations on Shooting Performance from Calluses of Three Sugarcane Varieties in MS Medium. Each treatment consisted of 10 cultures and the data (mean ± S.E.) were recorded after 35 days of culture

Hormone (mg/l)	Average shooting performance of three sugarcane varieties								
	Isd 37			Isd 40			ZH 110-65		
	Days to shoot initiation	No. of shoots/ culture	Ave. shoot length (cm)	Days to shoot initiation	No. of shoots/ culture	Ave. shoot length (cm)	Days to shoot initiation	No. of shoots/ culture	Ave. shoot length (cm)
BA1.0+IAA0.1	30.00	2.45±1.03	3.58±0.51	20.00	3.0±1.04	2.66±0.71	21.33	1.87±0.68	2.35±0.48
BA1.0+IAA0.2	28.00	5.50±1.01	3.92±0.49	19.33	8.0±1.57	2.98±0.60	18.33	4.50±0.32	2.61±0.70
BA1.0+IAA0.5	26.67	7.15±0.85	4.12±0.66	17.67	10.0±0.2	3.51±0..62	15.67	6.34±0.39	3.3.12±0.25
BA1.0+IBA0.1	27.00	5.41±0.61	3.85±0.62	15.67	4.5±1.37	2.83±0.47	17.00	3.50±1.25	2.71±0.88
BA1.0+IBA0.2	25.67	8.35±1.21	4.00±0.38	15.00	10.2±2.0	3.01±0.75	13.67	7.25±0.53	3.00±0.50
BA1.0+IBA0.5	23.67	9.87±0.44	4.77±0. 82	11.67	13.0±1.5	4.19±0.53	10.33	10.00±1.7	3.82±0.65
BA1.0+NAA0.1	21.00	6.31±0.73	4.27±0.77	12.67	7.8±1.44	3.75±0.41	15.67	5.67±0.50	3.11±1.02
BA1.0+NAA0.2	13.67	7.92±0.59	4.43±0.96	11.33	9.4±0.37	4.60±1.05	12.67	8.67±1.29	3.50±0.85
BA1.0+NAA0.5	12.00	11.41±0.37	5.43±0.28	09.00	17.0±0.3	5.13±0.50	9.00	15.67±1.5	4.08±0.47

Table 4. Effects of different Auxins on the rooting performance of *in-vitro* grown microshoots from three sugarcane varieties cultured on MS medium. Each treatment consisted of 10 cultures and data (mean ± S.E.) were recorded after 35 days of culture

Auxin (mg/l)	Average rooting performance of three sugarcane varieties								
	Isd 37			Isd 40			ZH 110-65		
	Days to root initiation	No. of roots/ shoot	Ave. root length (cm)	Days to root initiation	No. of roots/ shoot	Ave. root length (cm)	Days to root initiation	No. of roots/ shoot	Ave. root length (cm)
IAA 3.0	20	1.86±0.34	1.97±0.60	17	2.07±0.48	2.11±1.00	18	1.0±0.03	2.43±0.44
IBA 3.0	14	2.60±0.76	2.13±0.47	12	4.25±0.50	2.78±0.71	14	1.17±0.48	2.96±0.81
NAA 3.0	11	5.99±1.22	2.73±0.50	9.33	6.88±2.17	3.13±1.04	11.67	1.93±0.20	3.63±0.62

Higher concentrations of NAA (3.0-6.0 mg/L) were further tested to optimize rooting (Table 5). NAA at 5.0 mg/L yielded the shortest root initiation time (7.67 days) and the highest number of roots per shoot (9.36 ± 0.77) in Isd 40. The longest roots (4.37 ± 0.55 cm) were observed in ZH 110-65 under the same concentration. These findings confirm the optimal concentration of NAA at 5.0 mg/L for rooting, with genotype-specific responses (Fig. 2D, H & L).

Acclimatization of Rooted Plantlets

In-vitro regenerated shoots were successfully acclimatized by transferring rooted plantlets to soil after thorough root washing. Plantlets maintained in growth chambers under 80 % humidity showed a survival rate of 65-80 % (Fig. 3). The survival rate was positively influenced by plantlet health and root condition, with higher rates observed in plantlets having robust roots.

Discussion

Micropropagation is a highly effective tissue culture method for rapidly multiplying plants from a single shoot apex while ensuring disease-free, true-to-type material with uniform traits. It facilitates faster development of new varieties, rejuvenation of older ones, germplasm conservation and enhancement of plant vigour, ultimately leading to higher yields and sucrose content (10). Proper management of this approach can maintain seed quality for extended periods, providing a reliable alternative to traditional propagation methods. In this study, a reproducible *in-vitro* regeneration protocol for *S. officinarum* was developed, encompassing callus induction, shoot regeneration, multiplication and rooting using leaf sheath explants from three sugarcane varieties: Isd 37, Isd 40 and ZH 110-65. The protocol yielded high-frequency plant regeneration, demonstrating its potential for year-round, rapid, pathogen-free and high-quality plantlet production. These findings also underline the importance of this approach in preserving germplasm and supporting future crop improvement programs.

Callus induction experiments revealed that 2,4-D at 2.0 mg/L outperformed NAA in all three sugarcane varieties, requiring fewer days for callus initiation and achieving higher callus formation rates. The effectiveness of 2,4-D aligns with prior studies that demonstrated its superiority in callus induction in both monocots and dicots (11, 12, 19-21). Varietal differences were observed in the response to 2,4-D and NAA, supporting earlier findings on genotype-specific responses in tissue culture (12). The highest callus induction rate (97.33 %) was achieved at 3.0 mg/L 2,4-D, with a progressive decrease in performance observed at lower and higher concentrations, consistent with findings from previous reports (12, 13). This optimal concentration highlights the genotype-specific interaction of 2,4-

Table 5. Effects of different concentrations of NAA in MS medium on *in vitro* rooting performance from the cut end of microshoots of three sugarcane varieties. Each treatment consisted of 7-10 cultures and data (± S.E.) were recorded after 35 days of culture

Concentrations (mg/L)	Average rooting performance of three sugarcane varieties								
	Isd 37			Isd 40			ZH 110-65		
	Days to root initiation	No. of roots/ shoot	Ave. root length (cm)	Days to root initiation	No. of roots/ shoot	Ave. root length (cm)	Days to root initiation	No. of roots/ shoot	Ave. root length (cm)
NAA 3.0	11.00	5.99±1.22	2.73±0.50	9.33	6.88±2.17	3.13±1.04	11.67	1.93±0.20	3.63±0.62
NAA 4.0	10.00	6.05±01.43	2.83±0.85	9.00	6.91±0.84	3.43±0.42	10.67	2.25±0.34	3.87±0.71
NAA 5.0	9.33	8.11±1.10	3.06±0.47	7.67	9.36±0.77	3.67±0.25	9.67	3.12±0.74	4.37±0.55
NAA 6.0	9.67	7.0±1.55	2.77±1.00	8.67	8.47±1.36	3.47±0.39	11.67	2.85±0.41	4.00±0.83

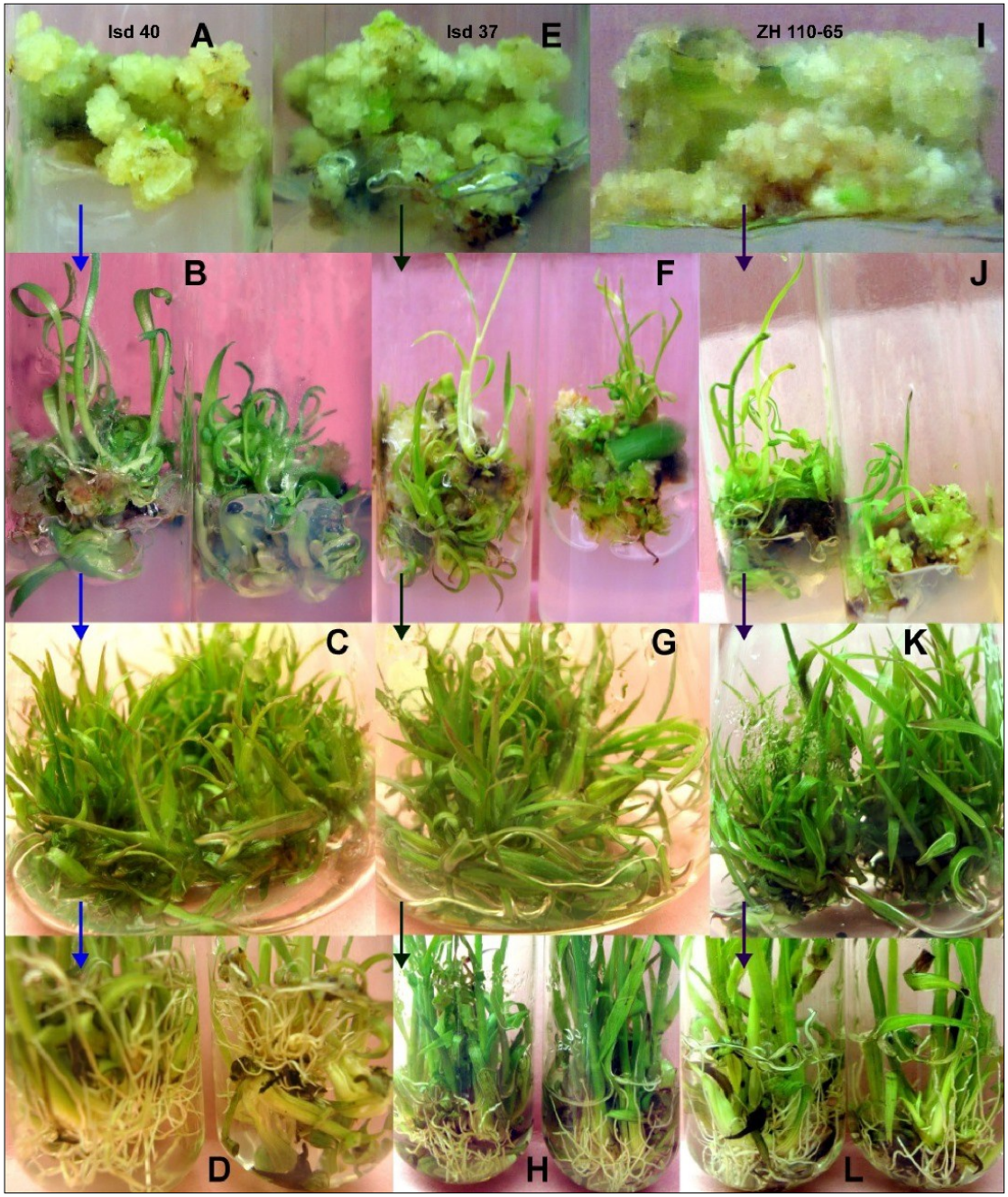

Fig. 2. Successive steps of *in vitro* regeneration of three sugarcane verities. **A.** Cream-colored loose calli of variety Isd 40 resulting from different concentration of 2,4-D and NAA, **B.** Emergence of microshoots from the calli of genotype Isd 40 from the effects of Cytokinin and Auxins, **C.** Multiplication and proliferation of shoots of genotype Isd 40 due to the synergistic effect of BA with NAA, **D.** Massive *in vitro* rooting of shoots of genotype Isd 40 under the superior efficacy of NAA at 5.0 mg/L, **E.** Mature yellowish-white and semi-compact calli of variety Isd 37 under the influence of varying concentrations of 2,4-D and NAA, **F.** Emergence of microshoots from the calli of genotype Isd 37 from the effects of Cytokinin and Auxins, **G.** Multiplication and proliferation of shoots of genotype Isd 40 due to the synergistic effect of BA with NAA, **H.** Massive *in vitro* rooting of shoots of genotype Isd 37 under the superior efficacy of NAA at 5.0 mg/L, **I.** Mature white and compact calli of variety ZH110-65 under the influence of different concentrations of 2,4-D and NAA, **J.** Emergence of microshoots from the calli of genotype ZH 110-65 from the effects of Cytokinin and Auxins, **K.** Multiplication and proliferation of shoots of genotype ZH110-65 due to the synergistic effect of BA with NAA, **L.** Massive *in vitro* rooting of shoots of genotype ZH110- under the superior efficacy of NAA at 5.0 mg/L.

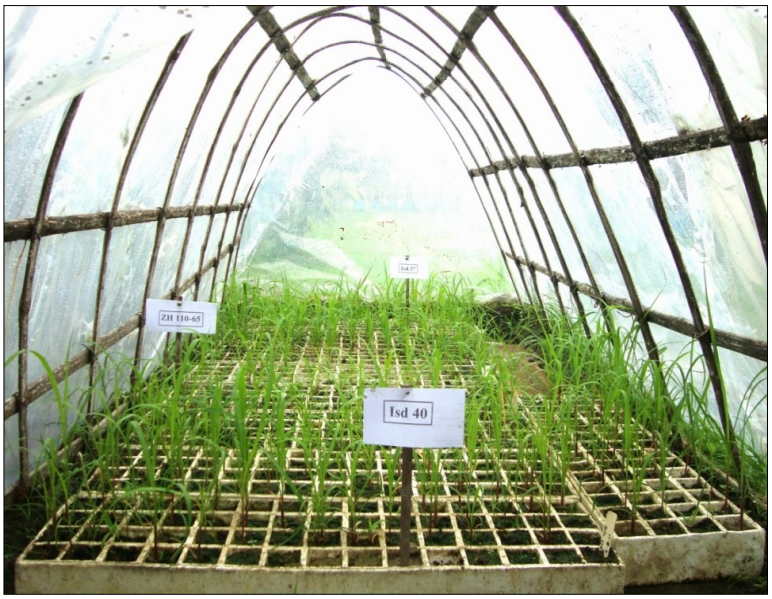

Fig. 3. Acclimatized plants from three genotypes under greenhouse condition.

D with sugarcane tissue, showcasing its significant potential for improving callus induction efficiency. Previous researchers reported higher (89 %) callus induction rate (23). These findings align with previous studies (24-26), which further emphasize the pivotal role of 2, 4-D in promoting callus formation in sugarcane.

Shoot regeneration experiments indicated that BA was more effective than Kn in all varieties, with 1.0 mg/L BA yielding the shortest time to shoot initiation, the highest number of shoots per culture and the longest shoots. These results are consistent with former studies (14, 15), which highlighted BA's superior role in shoot regeneration. Additionally, combining BA (1.0 mg/L) with NAA (0.5 mg/L) produced the best outcomes across all shoot regeneration parameters, including a maximum of 17.0 ± 0.3 shoots per culture in Isd 40 and reduced the time to shoot initiation to 9 days. These findings align with previous work (16, 13), reported similar synergistic effects of BA and NAA in sugarcane tissue culture. Variations in shoot regeneration performance among genotypes further emphasize the importance of tailoring hormonal treatments to optimize responses for specific varieties.

Rooting studies demonstrated that NAA at 3.0 mg/L was the most effective auxin, promoting the shortest time to root initiation, the highest number of roots per shoot and the longest root length across all varieties. These results agree with findings previous studies (17) which identified NAA as a superior auxin for sugarcane rooting. Further optimization revealed that increasing the concentration of NAA to 5.0 mg/L enhanced rooting performance, with Isd 40 exhibiting the shortest root initiation time (7.67 days) and the highest number of roots (9.36 ± 0.77 per shoot), while ZH 110-65 achieved the longest root length (4.37 ± 0.55 cm). These outcomes partially align with earlier studies (13, 18), reported the effectiveness of NAA at higher concentrations for sugarcane rooting. Notable varietal differences in rooting performance further underscore the influence of genetic variability on auxin sensitivity.

Overall, the optimized protocol developed in this study provides a reliable, efficient and genotype-specific framework for sugarcane micropropagation, addressing key challenges in propagation, including biotic and abiotic stresses. This approach supports sugarcane breeding programs, enhances germplasm conservation efforts and contributes to sustainable agricultural practices in Bangladesh. Further validation and refinement of this protocol can ensure its broader applicability and effectiveness in meeting the growing demands of modern agriculture.

Conclusion

This study successfully optimized *in vitro* regeneration protocols for sugarcane genotypes in Bangladesh, demonstrating high callus induction, shoot regeneration and rooting efficiency, particularly in the Isd 40 variety. The findings highlight the critical role of genotype-specific responses to auxins and cytokinins in enhancing micropropagation efficiency. The developed protocol offers a reliable method for producing disease-free, high-yielding sugarcane plantlets, supporting sustainable sugarcane cultivation and germplasm conservation efforts. The data supports the hypothesis that genotype-specific responses to plant growth regulators are critical for optimizing micropropagation. Furthermore, the acclimatization success rates of 65-80 % of rooted plantlets under *ex vitro* conditions are indicative of the practical applicability of this method in commercial sugarcane cultivation. These findings provide a robust foundation for improving sugarcane breeding programs, addressing the need for more resilient and productive varieties and promoting germplasm conservation. However, the findings are based on controlled laboratory conditions and further validation under field conditions is necessary to confirm the scalability and long-term viability of the protocol. Despite this limitation, the results present a significant step forward in advancing sugarcane breeding and improving productivity.

Acknowledgements

The authors express their sincere gratitude to the Bangladesh Sugarcrop Research Institute (BSRI) for providing the sugarcane plant materials used in this study. We are also thankful to BSRI for their logistical support and laboratory facilities. Finally, we extend our appreciation to all laboratory colleagues for their valuable feedback and suggestions that helped improve the manuscript.

Authors' contributions

RF conceptualized the study, designed the experiments, performed data collection and analysis and contributed to manuscript drafting. MJH prepared the first draft manuscript, revised for intellectual content, contributed to statistical analysis and supported manuscript editing and formatting. AKMNH supervised the research, assisted in experimental execution, contributed to statistical analysis and supported manuscript editing and formatting. RA supervised the research as supervisor and assisted in experimental execution. All authors read and approved of the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest

Ethical issues: None

References

1. Kaur M. Irradiation induced mutagenesis in sugarcane. Ludhiana: Punjab Agricultural University; 2014.
2. Leal MRLV. The potential of sugarcane as an energy source. Proc Int Soc Sugar Cane Technol. 2007;26:23-34.
3. Ming R, Moore PH, Wu KK, D'hont A, Glaszmann JC, Tew TL. Sugarcane improvement through breeding and biotechnology. 2006. https://doi.org/10.1002/9780470650349.ch2
4. Arif S, Batool A, Nazir W, Khan RS, Khalid N. Physiochemical characteristics, nutritional properties and health benefits of sugarcane juice. In: Non-alcoholic beverages. Cambridge: Woodhead Publishing; 2019:227-57. https://doi.org/10.1016/B978-0-12-815270-6.00008-6
5. Aguilar-Rivera N. A framework for the analysis of socioeconomic and geographic sugarcane agro industry sustainability. Socioecon Plann Sci. 2019;66:149-60. https://doi.org/10.1016/j.seps.2018.07.006
6. Rahman MS, Khatun S, Rahman MK. Sugarcane and sugar industry in Bangladesh: An overview. Sugar Tech. 2016;18(6):627-35. https://doi.org/10.1007/s12355-016-0489-z
7. Bangladesh Sugar Crop Research Institute. Annual report. Pabna: Bangladesh Sugar Crop Research Institute; 2019.
8. Getnet B. Review on *in-vitro* propagation of sugarcane to advance the value of tissue culture. Agri Res Tech Open Access J. 2017;5(4):555670. https://doi.org/10.19080/ARTOAJ.2017.05.555670
9. Lakshmanan P, Geijskes RJ, Wang L, Elliott A, Grof CPL, Berding N, et al. Development and hormonal regulation of direct shoot organogenesis and somatic embryogenesis in sugarcane (*Saccharum* spp. interspecific hybrids) leaf culture. Plant Cell Rep. 2006;25:1007-15. https://doi.org/10.1007/s00299-006-0154-1
10. Redae MH, Ambaye TG. *In-vitro* propagation of sugarcane (*Saccharum officinarum* L.) variety C86-165 through apical meristem. Biocatal Agric Biotechnol. 2018;14:228-34. https://doi.org/10.1016/j.bcab.2018.03.005
11. Chen WH, Davey MR, Power JB, Cocking EC. Control and maintenance of plant regeneration in sugarcane callus culture. J Exp Bot. 1988;39:251-61. https://doi.org/10.1093/jxb/39.2.251
12. Begum S, Hakim L, Azam MA. Efficient regeneration plants from leaf base callus in sugarcane. J Plant Tissue Cult. 1995;5(1):1-5.
13. Tarique HM. Effect of genotype, media and explants on micropropagation of sugarcane [MSc thesis]. Dhaka: Sher-e-Bangla Agricultural University; 2008.
14. Yutaka TY, Tomohiro M, Toshikazu, Takeshi O. Plant regeneration via shoot organogenesis from cotyledons in two wild *Cucumis* species, *C. figarei* and *C. metuliferous*. JARQ. 1998;32:281-6.
15. Karim MZ, Alam R, Bhaksha R, Paul SK, Hossain MA, Rahman ABMM. In-vitro clonal propagation of sugarcane (*Saccharum* spp.) variety Isd 31. Pak J Biol Sci. 2002;5(6):659-61. https://doi.org/10.3923/pjbs.2002.659.661
16. Karim MZ, Amin MN, Hossain MA, Islam S, Hussain F, Alam R. Micropropagation of two sugarcane (*Saccharum officinarum*) varieties from callus culture. Online J Biol Sci. 2003;2(10):682-5. https://doi.org/10.3923/jbs.2002.682.685
17. Lal N. Assessment of IAA, IBA and NAA for *in-vitro* shooting and rooting plantlet growth in sugarcane. Indian Sugar J. 1992;42:205-8.
18. Ali K, Afghan S. Effect of auxin and cytokinins for *in-vitro* shoot and root development of different sugarcane. Pak Sugar J. 2003;17(6):36-9.
19. Nadir HM, Soepraptop S, Heinz DJ, Ladd SL. Fine structure of sugarcane (*Saccharum* spp.) callus and role of auxin in embryogenesis. Crop Sci. 1978;18:210-6. https://doi.org/10.2135/cropsci1978.0011183X001800020004x
20. Liu MC, Yew HS, Chen WH. Tissue and cell culture as aids to sugarcane breeding. A high sucrose and vigorously growing calliclone 71-4829. Taiwan Sugar J. 1984;31(3):77-83. https://doi.org/10.1007/BF00039143
21. Jaisal N. Regeneration of plantlets from the stem segments of adult plants of Ficus religiosa L. Plant Cell Rep. 1985;4:256-8. https://doi.org/10.1007/BF00269371
22. IBM Corp. IBM SPSS Statistics for Windows, version 26. Armonk, NY: IBM Corp.; 2019. https://www.ibm.com/products/spss-statistics
23. Awan MF, Iqbal MS, Sharif MN, Tabassum B, Tariq M, Murtaza S, et al. Evaluation of genotypic and hormone mediated callus induction and regeneration in sugarcane (*Saccharum officinarum* L.). Evaluation. 2019;4(6):70-6.
24. Sengar K, Sengar R. Effect of 2,4-D on *in-vitro* regeneration of callus in sugarcane. *In Vitro* Cell Dev Biol Plant. 2012;48:431.
25. Rao GG. Callus induction and organogenesis in sugarcane (*Saccharum officinarum* L.) var 93v297. Callus Induction and Organogenesis in Sugarcane (*Saccharum officinarum* L.) var 93v297. 2013;14-22.
26. Distabanjong K, Distabanjong C, Jang SW. Developing regeneration system for cryopreservation in sugarcane (*Saccharum officinarum* L.).2015;427-33. https://doi.org/10.17660/ActaHortic.2015.1083.55

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.