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Research Article

Varietal Response to Different Hormonal Concentrations in Callus Induction in *In vitro* Culture of Sugarcane

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Abstract

An experiment containing callus induction has been conducted at the Laboratory of Breeding Division of Bangladesh Sugarcane Research Institute (BSRI) during the period of 2011 at BSRI farm, Ishurdi, Pabna. Leaf sheaths of small segments of young meristem of five varieties viz. Isd 2-54, LJ-C, Isd 17, Isd 37 and Isd 40 were used as explant. These segments were cultured in MS medium containing different concentrations of 2,4-D (viz. 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) for homogeneous callus induction. Varietal response on application of 2, 4-D, the variety Isd 17 showed the best results in callus induction. Among the five concentrations of 2, 4-D, the best response was exhibited in 3.0 mg/l of 2,4-D. Regarding interaction effect of varieties and doses of 2,4-D, the highest percentage (100%) of explants induced callus was recorded in variety Isd 17, Isd 40 and Isd 2-54 on 3.0 mg/l of 2,4-D respectively.

INTRODUCTION

Commercial sugarcane plants are inter-specific poly-aneuploid hybrids with chromosome numbers usually in excess of 100. Breeding of improved cultivars of sugarcane is difficult because of the complexity of the sugarcane genome. Consequently, most traits in sugarcane are multigenic and or multi-allelic and are quantitatively inherited. On the other hand, high ploidy, low fertility, large genome and complex environmental interactions make conventional breeding arduous for sugarcane improvement. Sugarcane is vegetatively propagated crop and all the varieties do not flower under natural condition. In Bangladesh condition, most of the sugarcane germplasm are non-flowering. Climatic conditions are not favorable for flowering. Every year around 250 varieties/clones are found to flower, among the flowering ones, some are flowering early and some are late. So, it is a serious problem to use all the germplasm as parent material in hybridization programme.

In vitro culture technique offers unique opportunity for creation of genetic variability and rapid isolation of clones with desired characteristics in sugarcane [1-5,12,16]. This technique provides a promising future in sugarcane breeding programme, where the production of the natural viable seeds is a major problem due to the non-sporadic flowering and scarcity of artificial condition for hybridization [6,17]. In sugarcane, the pioneer work on tissue culture was done by Nickl [7-11,23] and Heinz and Mee [12]. In sugarcane, tissue culture derived plants induces considerable phenotypic variability [4]. The success of *in vitro* culture depends mainly on the growth conditions of the source material [5,8,24] medium composition and culture conditions [13,25] and on the genotypes of donor plants. Among those factors, the genotype appears to be important factor influencing the efficiency of *in vitro* culture. Considering the scope and importance of *in vitro* culture in creation of variability in sugarcane, the present investigation was under taken to study the varietal response to different hormonal concentrations in callus induction under *in vitro* culture of sugarcane.

MATERIALS AND METHODS

The *in vitro* callus culture was conducted at the Laboratory of the Breeding Division, BSRI, Ishurdi, Pabna during the period of 2011. Five non-flowering commercial sugarcane varieties such as Isd 2-54, LJ-C, Isd 17, Isd 37 and Isd 40 were used as explant. The young leaf sheaths collected from 7-8 months old field grown sugarcane varieties were used as *in vitro* culture materials. Five hundred callus of five sugarcane varieties were cultured. All instruments, glassware and culture media were sterilized in different procedure. The test tubes, flasks containing the media were autoclaved with 1.16 kg/cm^2 of pressure at 121^{o}C for 20 minutes. Unexpanded young leaf sheaths were taken in a

beaker and treated with 1% savlon for 5-6 minutes. The explants were transferred in autoclaved plastic pot and treated with 0.5% sodium hypochlorite (NaOCl) for 20 minutes. Chen et al. [6], has described surface sterilization of leaf explants with 95% ethanol for 5 minutes. Chengalrayan and Gallo-Meagher [7], observed surface sterilization of leaf explants by 0.5% sodium hypochlorite for 20 minutes. The concentration of 0.5% NaOCl was used as sterilizing agent while savlon 3% (w/v) was used as antiseptic, detergent and surfactant. Murashige and Skoog [14-21,22], medium was used in callus induction.

To maintain and ensure aseptic condition precautions were taken in every step of works. All inoculation and aseptic manipulation were carried out by using a laminar air flow cabinet. Hands were properly washed with soap before starting work in laminar airflow cabinet. During the operation hands were rubbed with 70% ethyl alcohol frequency with cotton and wiped cabinet base for maintaining clean condition.

The excised explants were inoculated into test tube containing MS media with various concentrations and combination of hormonal supplement for *in vitro* callus culture.

To investigate *in vitro* plant regeneration ability via callus culture following parameters were recorded on days to callus initiation and explants induced callus (%).The collected data were analyzed statistically following the analysis of variance (ANOVA) technique and the mean differences were adjudged by Duncan's Multiple Range Test (DMRT) using the statistical computer package program, MSTAT-C Gomez and Gomez [22,10].

RESULTS AND DISCUSSION

High frequency of callus of leaf sheath explants offers a feasible propagation method in sugarcane which can be utilized for the year round, rapid, pathogen free and quality plantlet production. It is also be useful for preservation of valuable germplasm and for the future crop improvement programme. The present investigation was taken in *in vitro* callus induction of sugarcane.

Varietal response to application of 2, 4-D

The effects of varieties on callus induction and days to callus initiation are shown in (Table 2). The days to callus initiation ranged from 13.27 to 16.47 which indicated significant variation among the varieties. The highest days to callus initiation (16.47) were found in variety Isd 37 followed by 14.60 in LJ-C, 14.20 in Isd 2-54 and 13.53 in Isd 40. The lowest days to callus initiation (13.27) was found in variety Isd 17.

The highest percentage of induced callus (74%) and the shortest days to callus induction (13.27) was found in variety Isd 17 followed by Isd 40 and Isd 2/54 respectively. The lowest percentage of callus was observed 53.00 in variety Isd 37. Significant difference was observed among varieties (Table 2). Based on the parameter, Isd 17 presented the best response compared to other genotypes. These results showed that callus induction capacity is genotype depended found in sugarcane [4].

Hormonal response to application of 2, 4-D

The surface sterilized leaf sheath explants of five non-flowering sugarcane varieties viz. Isd 2-54, LJ-C, Isd 17, Isd 37 and Isd 40 were cultured in MS media with five different concentrations of 2, 4-D (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) to induce callus. In each treatment 10-15 explants were used and the experiment was repeated thrice. In all the five hormonal treatments the cultured explants started to initiate callus within 2-3 weeks until the nutrient of the medium became exhausted. The effects of different varieties of sugarcane and various concentrations of 2, 4-D on days to callus initiation and percentage of callus formation are presented in (Table 3).

Significant variation among different concentrations of 2, 4-D for days to callus initiation and explants induced callus (Table 3). The shortest days (11.13) required for callus initiation was observed for the cultivation of 3.0 mg/l of 2, 4-D. On the contrary, the highest days to callus initiation was found in 1mg/l of 2, 4-D (19.67) followed by 17.13 in 2 mg/l, in 12.20 in 5 mg/l of 2, 4- D and 11.93 in 4mg/l of 2, 4-D. MS medium containing 3mg/l of 2, 4-D gave the similar result with the medium containing 4mg/l of 2, 4-D. This value was significantly superior over other treatments. The maximum amount of callus was observed at 3.0 mg/l and the minimum at 1 mg/l of 2, 4-D. Khattak et al. [18], and Khamit et al. [15], reported that the 3mg/l of 2, 4-D is ideal for maximum callus growth of sugarcane. According to Rahman et al. [24], all the varieties cultured with 3-4 mg/l of 2, 4-D showed the best performance in the callus induction on modified MS media. Callus was initiated after 10-15 days of incubation reported by Shamim et al. [26], Kumar et al. [20], suggested that 2, 4-D was suitable for induction and growth of callus. Similar observation was obtained from Jaisal and Narayan [14], Begum et al. [3,26], and Chen et al. [6], who found that MS medium supplemented with 2, 4-D was the best auxin for callus induction as in monocotyledonds and even in dicotyledons.

The highest percentage (84.00%) of callus initiation was observed in 3.0 mg/l of 2,4-D followed by 80.78% in 4.0 mg/l and 75.81% in 5.0 mg/l of 2, 4-D. Gill et al. [9], and Badaway et al. [2], have also reported similar results. The lowest percentage (50.80%) of callus initiation was recorded in 1.0 mg/l of 2, 4-D. Heinz and Mee [12], demonstrated the occurrence of large variations in both chromosome number and morphological characters in the plants regeneration from callus. The causes of variations were so far unknown, although they may be associated with variation in chromosome balance [19]. It appears that they are caused by a combination of physical and chemical phenomenon. A physical factor is the environment suitable for un-inhibited growth while the chemicals in the nutrients such as 2, 4-D may induce abnormal division. Sugarcane callus cultures show a considerable variation from cell to cell and among differentiated plantlets. Larkin and Scowcroft [21,27], have discussed in details, various factors responsible for somaclonal variation which include karyotype change, cryptic changes associated with chromosome rearrangement, transposable elements, somatic gene rearrangements, gene amplification and depletion, somatic crossing over and sister-chromatiod exchanges.

Interaction effect of variety and doses of 2, 4-D

The results of interaction effects of different varieties of sugarcane and various concentrations of 2, 4-D is presented in (Table 4).

The highest percentage (100%) of explants induced callus was recorded in variety Isd 2-54, Isd 17 and Isd 40 on 3.0 mg/l of 2,4-D. The lowest percentage (10%) of explants induced callus was recorded in variety Isd 2-54 on 1.0 mg/l of 2, 4-D. The highest percentage (100%) of callus was observed in the media supplemented with 3.0 mg/l of 2,4-D followed by 4.0 mg/l of 2,4-D (95%) in Isd 17 by 4.0 mg/l of 2, 4-D, (90%) in Isd 2-54 and by 4mg/l of 2, 4-D in Isd 40 (95%).

The days to callus induction ranged from 10.00 to 21.67 (Figure 1.2 and 1.3). The highest days to callus induction (21.67) was found in the media supplemented with 1.0 mg/l of 2, 4-D in variety Isd 37. The early days to callus induction was noticed in the media supplemented with 3.0 mg/l of 2, 4-D in variety Isd 40 (Figure 1.4).

The results of the present investigation were agreed with the results of Hossain et al. [13], Ali et al. [1] and Gopitha et al. [11], who found that the concentration of 3.0 mg/l of 2, 4-D induced callus in the highest percentage of explants. Begum et al. [13], found 3-4 mg/l of 2, 4-D produced highest percentage of callus in Bangladeshi sugarcane varieties (viz. L. Jaba, Isd 16, Isd 20 and clone 1/123).

Varietal response to application of NAA for callus induction

Five different concentration of NAA were used for callus induction in five sugarcane varieties. Varietal response with respect to days to callus initiation and result of explant callus on application of auxin (NAA) in *in vitro* culture of sugarcane are presented in (Table 1). Among sugarcane varieties, Isd 2-54 was found to induce callus which required 21 days. The explants of Isd 2-54 grown in MS medium supplemented with 1 and 2 mg/l NAA were found to induce callus. Other concentrations and varieties did not respond in callus induction but unwanted root were grown from 3 to 5 concentrations for Isd 2-54, but it was 1mg/l for Isd 37 and Isd 40. The variety LJ-C and Isd 17 did not show any response to their hormone.

The Table 5 clearly revealed that NAA was not suitable for callus induction from the explants of these varieties. NAA is commonly used for root formation in tissue culture derived shoots. Similar finding was observed by Gopitha et al. [11], at used different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) of NAA. Rooting hormone do not sufficient callus induction.

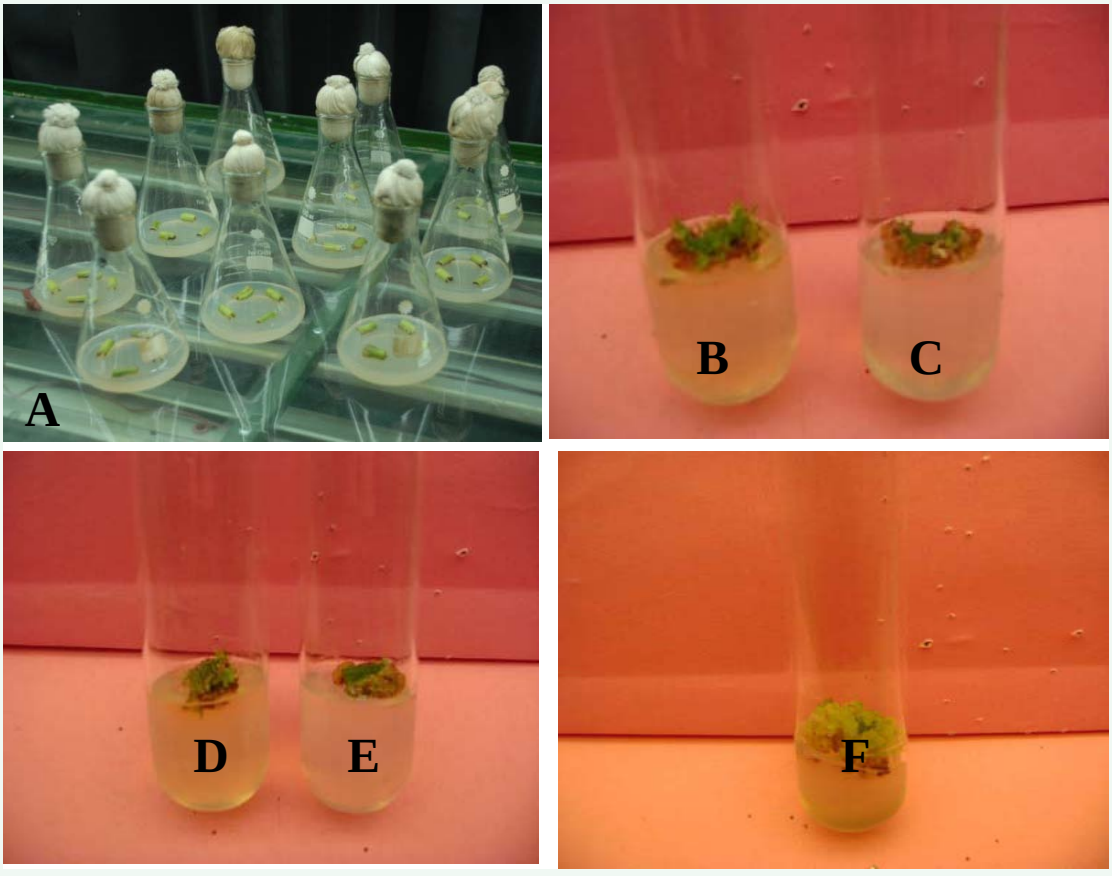

Figure 1
1.1: Leaf sheath explant cultured on MS medium supplemented with 3.0mg/l of 2, 4-D.
1.2: Callus induction from leaf sheath explant cultured on MS medium supplemented with 3.0mg/l of 2, 4-D. B) Isd 2-54 and C) LJ-C
1.3: Callus induction from leaf sheath explant cultured on MS medium supplemented with 3.0mg/l of 2, 4-D. D) Isd 17 and E) Isd 37
1.4: Callus induction from leaf sheath explant cultured on MS medium supplemented with 3.0mg/l of 2, 4-D. F) Isd 40

Table 1: MS media supplemented with different concentrations and combinations of Auxin.

MS + Auxin (2, 4-D mg/l)	MS + Auxin (NAA mg/l)	MS + Auxin (IAA mg/l)
MS + 1.0	MS + 1.0	MS + 1.0
MS + 2.0	MS + 2.0	MS + 2.0
MS + 3.0	MS + 3.0	MS + 3.0
MS + 4.0	MS + 4.0	MS + 4.0
MS + 5.0	MS + 5.0	MS + 5.0

Table 2: Varietal response to callus induction through application of 2, 4-D concentrations in *in vitro* culture of sugarcane.

Variety	Explants induced callus (%)	Days to callus induction
Isd 2-54	62.00	14.20
LJ-C	55.60	14.60
Isd 17	74.00	13.27
Isd 37	53.80	16.47
Isd 40	71.00	13.53
LSD value	6.024	1.002

Table 3: Effect of different concentrations of auxin (2, 4-D) on callus induction in *in vitro* culture of sugarcane.

Media + 2,4-D (mg/l)	Days to callus initiation	Explants induced callus (%)
MS + 1.0	19.67	50.80
MS + 2.0	17.13	70.19
MS + 3.0	11.13	84.00
MS + 4.0	11.93	80.78
MS + 5.0	12.20	75.81
LSD value	1.002	7.891

Table 4: Interaction effect of varieties and 2,4-D concentrations on callus induction in sugarcane.

Variety and Doses of 2,4-D (mg/l)	Explants induced callus (%)	Days to callus induction
Isd 2-54 × 2,4-D 1.0	10	20.00
× 2,4-D 2.0	30	17.00
× 2,4-D 3.0	100	10.33
× 2,4-D 4.0	90	11.33
× 2,4-D 5.0	80	12.33
LJ-C × 2,4-D 1.0	15	20.67
× 2,4-D 2.0	28	17.33
× 2,4-D 3.0	85	11.33
× 2,4-D 4.0	80	12.33
× 2,4-D 5.0	70	11.33
Isd 17 × 2,4-D 1.0	30	17.67
× 2,4-D 2.0	50	15.67
× 2,4-D 3.0	100	10.33
× 2,4-D 4.0	95	11.33
× 2,4-D 5.0	90	11.33
Isd 37 × 2,4-D 1.0	17	21.67
× 2,4-D 2.0	40	19.67
× 2,4-D 3.0	75	13.67
× 2,4-D 4.0	70	13.67
× 2,4-D 5.0	67	13.67
Isd 40 × 2,4-D 1.0	25	18.33
× 2,4-D 2.0	45	16.00
× 2,4-D 3.0	100	10.00
× 2,4-D 4.0	95	11.00
× 2,4-D 5.0	90	12.33
LSD (5%)	7.132	2.241

Table 5: Varietal response to application of auxin (NAA) for induction of callus in *in vitro* culture of sugarcane.

Varieties	Dose of NAA (mg/l)	Days to callus induction	Explants induced callus (%)
Isd 2-54	1	25	5
	2	21	4
	3	Rooted but no callus	-
	4	Rooted but no callus	-
	5	Rooted but no callus	-
LJ-C	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	-	-
Isd 17	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	-	-
Isd 37	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-
Isd 40	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-

Varietal response to application of IAA for callus induction

Varietal responses to media supplemented with different doses of IAA for callus induction in *in vitro* culture of sugarcane are presented in (Table 6).

All the varieties showed unwanted root induction at 5mg/l of IAA concentration. Other concentrations (1 to 4mg/l) and varieties did not respond in callus induction. The Table 6 clearly revealed that IAA is not suitable for callus induction from the

Table 6: Varietal response to media supplemented with different doses of IAA for callus induction in *in vitro* culture of sugarcane.

Varieties	Dose of IAA (mg/l)	Days to callus induction	Explants induced callus (%)
Isd 2-54	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-
LJ-C	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-
Isd 17	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-
Isd 37	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-
Isd 40	1	No change	-
	2	-	-
	3	-	-
	4	-	-
	5	Rooted	-

explants of these varieties. Gopitha et al. [11], reported that the different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) of IAA were used, but these concentrations were not responded in callus induction.

CONCLUSION

3.0 mg/l of 2, 4-D showed the best response among the concentrations. Regarding interaction effect of varieties and doses of 2,4-D, the highest percentage (100%) of explants induced callus was recorded in variety Isd 17, Isd 40 and Isd 2-54 on 3.0 mg/l of 2,4-D.

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