National Journal of Advanced Research
ISSN: 2455-216X
Impact Factor: RJIF 5.12
www.allnationaljournal.com
Volume 4; Issue 1; January 2018; Page No. 63-65

Optimization of the protocol for initiation, multiplication and acclimatization of sugarcane variety US-633

[*1] Naseem Khatoon, [2] Beena Naqvi, [3] Ghulam Abass, [4] Faraz Ahmed
[1, 3] Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan
[2] PCSIR Complex Karachi, Pakistan
[3] Food Science technology Sindh Agriculture University Sindh, Jam, Pakistan

Abstract
Studies were carried out for rapid micropropagation of the sugarcane variety US- 633. The explants were surface sterilized with 40% sodium hypochlorite for 20 minutes. The culture were initiated on MS medium contains BAP (0.5 to 1.5 mg/L) with kin (0.5 to 1mg/L). The multiplication of the shoots was maximum observed in 3mg/L BAP and 2mg/L kin. Rooting of the plantlets was obtained in 3mg/L NAA with 3mg/L IBA.

Keywords: BAP, 6- benzyleaminopurine, NAA naphthalenaceticacid, kinetin

Introduction

Sugarcane (*Saccharum officinarum L.*) is one of the most resourceful converts of solar energy into sugars and other renewable forms energy. It is an in to important industrial cash crop of Pakistan and ranks fourth in acreage after wheat, cotton and rice [1]. There is terrible need to increase sugarcane productivity and quality enhancement as Pakistan occupies fifth position in cane producing countries and fifteenth one among sugar producing countries [2]. Sugarcane is a perennial herb and belongs to family *Graminae*. It is mainly propagated by vegetative means sets having three buds each, and a low multiplication rate (1:6 to 1:8) due to which seed material production of newly released varieties is invariably slow [3]. Due to global importance as an agricultural product, much research has been focused on sugarcane crop improvement through breeding and recently through biotechnological approaches like micropropagtion [4].

Plant tissue Culture techniques like micro propagation and somatic embryogenesis can be used for successful sugarcane propagation by controlling a lot of problems which are faced during convential method practices. The technique ensure diseases free multiplication of best varieties and minimizes time span required for mass production [5].

Standardization of protocol of production for in vitro multiplication of sugarcane through leaf sheath callus culture, axillary bud and shoot tip culture have been reported by authors [6, 7]. But the nutritional requirements for in vitro culture are differs according to genotype as well as explants used [8].

The present study was targeted to optimize a complete tissue culture protocol which allows multiplication of explants of plantlets from the tip of the sugarcane. Proportional nutritional values of in vitro grown, acclimatized sugarcane plantlets and field grown sugarcane plants was also discovered in current study. US-633 was selected for this study based on its early maturity, tremendous sugar content and resistance to pest in Sindh, Punjab area of Pakistan [9].

Materials and Methods

Source of Explants

Sugarcane variety US -633 was investigated in present study conducted from Feburary-2017 to January- 2018. Explants material was obtained from the Cane Department of Khoski Sugar mill Badin and grown in the locally in Dist. Badin. Green tops were collected from 6 -8 months old healthy plants of sugarcane var. US 633.For explants preparation, 6-7cm long segments, were dissected removing with 4-6 outer layers and washed thoroughly running tap water for 20-25 minutes.

Surface sterilization of sugarcane explants

After washing young apical were surface sterilized by dipping in 40% Sodium hypochlorite) for 20 minutes followed by 15 minutes immersion in 0.1% mercuric chloride solution congaing 2 drops of tween-20 per 100 ml. These explants were washed thrice with autoclaved distilled water. The immature apical were exposed for initiation on to solid MS medium [10] supplemented with 4% sucrose along with different combination of plant growth regulators mentioned were used) discarding of outer most layers. Ascorbic acid (1.0 ml/L) was added in the medium to control the browning of the explants due to the production of phenolic compounds. The pH of the MS media to the adjusted to 5.7 using of 0.1 N HCL/NaOH to the addition of agar 7.5 g/L bacteriological grade, Sigma) the media was homogenized by cooking it on a microwave oven for 10 mints dissolving of gelling agent. The prepared media was then dispensed in the culture jars and autoclaved for 20 min at 121 C at 15 Ibs psi pressure. All the cultures were incubated in a culture room provide with a 16 h photoperiod of cool white fluorescent light 3000 Lux) and the temperature was maintained at 25±1 C with 60 to 70 % humidity in the culture room.

Initiation Medium

For apical induction MS medium supplemented with various concentration of (Kin and BAPmg/L) were optimized. Shoots

were regenerated after 25 -30 days of incubation period under controlled condition.

Shoot multiplication medium
The multiplication of were cultured on MS medium supplemented with different concentrations of BAP (0.5 to 3.0mg/L), with Kinetin (0.5 to 2mg/L) alone and in combination with GA3 (0.5 to 1mg/L) for shoot regeneration.

Rooting medium
Shoot lets measuring about 5to 6 cm in length were transferred aseptically from bunch of shoots growing in the culture jars and transferred to MS media with supplemented with different concentration of IBA (0.5 to 3.0mg/L), and NAA (0.5 to 5 mg/L individually or in combination.

Acclimatization
Plantlets with developed mature roots were removed from the culture jars to transfer them to plastic bags for hardening in green house. After 15 days plants were irrigated with ¼ MS basal salt solution and farmyard manure with distilled water every week. After the 2 months the plantlets were transferred into the soil in the filed condition.

Result and Discussion
Optimum shoot growth was recoded at 0.5 mg/L BAP in combination with 1mg/L Kin supplement in MS medium was observed to be most favorable among all concentration / Combinations optimized with respect to the numbers of shots produced as shown in table: 1. Muafia Shafique *et al*. 2015, [11]. Reported the BAP in combination with Kin is good for shoot enhancing of sugar cane as compared to Kin and GA3.

Table 1: Effect of Cytokinin (BAP, kin) on initiation of apical of sugarcane variety US-633.

Growth regulators supplements (mg/L)		shoots regenerated
BAP	**Kin**	
0.5	0.5	++
0.5	1	+++
1	1	+
1.5	1	+

Multiplication of shoots was observed at different concentration of BAP with kin. US 633 variety response towards the concentration used however BAP (3mg/l with 2 mg/L kin) were found best for multiple shoots formation. A maximum of 14 cm shoot length, 10 tillers were observed in 15 days (Table: 2). Present result are supported by Karim *et al*. 2002. [12]. who reported that BAP played efficient role in shoot multiplication of sugarcane with kinetin.

Table 2: Effect of growth hormone (BAP, Kin) on shoot multiplication of sugar cane variety US-633.

Hormonal supplements (mg/L)		Shoot length (cm)	No. of tillers
BAP	Kin		
1	0.5	4.00	5
1.5	1	6.0	5
2	1.5	9.23	7
2.5	2	9.65	8
3	2	13.00	10

Rooting and acclimatization
Different types of auxin were used at different concentrations and combination to induce roots in microprpagted shoots. Maximum rooting observed in MS medium supplemented with 3mg/L NAA and 3mg/L IBA (Table 3) and highest number of roots 15.00 which rook only 9 to 15 days with root length 2.3 cm. In present study various concentration of NAA alone or in Combination with IBA was found to give better response for root formation in the shoots of variety US-633.

Table 3: Effect of different concentration of auxin on root formation of sugarcane variety US-633

Hormonal supplements (mg/L)		No. roots per micro shoots (cm)	Day to emergence of root
NAA	IBA		
0.5		---	_
1		___	_
5.0		7.00	15-20
3	3	15.00	10-15
5	2	11.00	10-15
4	2	9.00	10-20

Acclimatization
The plants with healthy shoots and roots were successfully transplanted in soil after acclimation with 90% survival rate in field condition. (Shown in figures).

Conclusion
Present study described the protocol for sterilization, initiation, regeneration and rooting, acclimatization of varity US 633. It was observed that the best medium for initiation was MS medium supplemented with 0.5mg/L BAP, and 1mg/L kin. The multiplication of the shoots was maximum observed in 3mg/L BAP and 2mg/L kin. Rooting of the plantlets was obtained in 3mg/L NAA with 3mg/L IBA.

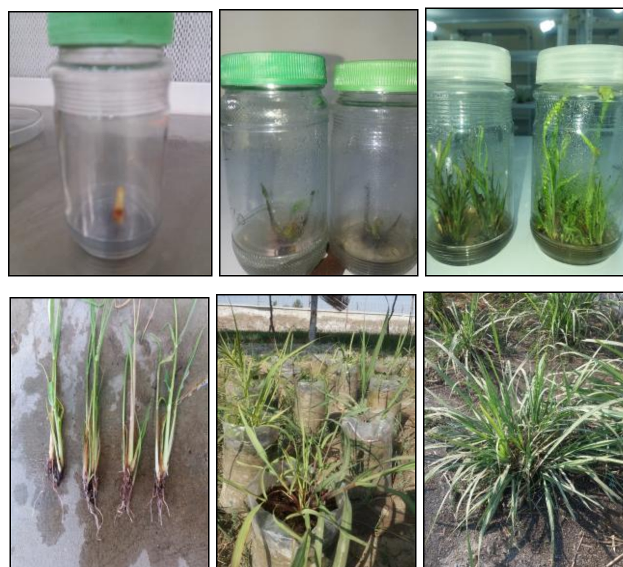

Fig 1

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