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Initiation protocol optimization of (*Saccharum officinarum* L.) variety US- 718 through micropropagtion techniques

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Abstract

The sugarcane variety US-718 experimentally was carried out in changed concentration of BAP and kinetin under established condition of *invitro* along with replications in complete randomized design. The purpose of the study was to determine the optimum concentration in presence of combination of BAP and kinetin under *invitro* condition .In MS neutering media the variety US-718 shown maximum result of 90.32% shoot initiation in 3.5#0.5 of shoots per explant in concentrations of 1.5mg/l BAP without kinetin exhibited a better response than other concentration.

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Introduction

Sugarcane belong to Poaceae family is a strong and major crop of Pakistan. It consists high sugar content. (*Saccharum officinarum*) contributes 60-70% of annual sugar production in the world (Shakra Jamil 2017). Sugarcane is a perennial grass family crops that reproduction through sexual and asexual modes. It reproduces asexually by three or two buds stem cutting called sets, and advanced methodologies of in vitro propagation by taking parts of it such as shoot tip, apical merited, axillary bud and leaf. It also reproduces through seed propagation via flower (Fuzz), which is used for breeding purposes (Sime M-2013). The sugarcane is used in production of paper, antibiotics, dextran, waxes and fats, epoxy polymers and bio fertilizers as reported by DSD, 2013. FAO 2014 .The sugarcane is economically vital industrial crop and well propagated conservatively by sets, but enormous land requirement, potential transfers of pathogens through seed and conventional method from generation to generation are the main factors for low rate propagation The *invitro* propagation is a greatest substitute to overcome such factors and pave the way to produce disease free and enough amount of planting material. (Belet Getnet 2017).

Clonal propagation through tissue culture is the one of the extensively accepted methods used for the commercial production of genetically enhanced quality of crops (Tiwari *et al*., 2012). During tissue culture, pathogenic and non-pathogenic fungi and bacteria are exposed as contaminants and therefore can regularly be disposed of. Systemic viruses which are much more difficult to eliminate the sugarcane intensive persistent contamination has been frequently reported in many tissue culture system, despite, the use of strong surface sterilization treatments of explants (Anonymous. 2009). Micropropagtion is however actual subtle methods which needs sterile condition in each stage (0-4) specifically for establishment of surface sterilization of explants initiation and establishment of aseptic culture, multiplication, rooting and acclimatization. These steps manifested the sensitivity of tissue cultures techniques in determination of contamination and economic significance rather than conventional propagation. These techniques facilitates to grower to permit the sugar industry estates to produce adequate planting material with short time period and cost effective (Lakshman p. *et al.,* 2006).

Shoot tip initiation observed at different concentration by BAP and Kin in many plant culture systems (G. Zahra *et al.,* 2010). The success of the sugarcane tissue culture is also dependent of sugarcane varieties. (C. Ganonou *et al.,* 2005), the tissue culturing medium and use of growth enhancer and regulators (V. Saharan *et al* 2004). Therefor the present study on sugarcane variety US-718 was carried out by plant tissue culture laboratory Khoski Sugar mill Badin, to detect the influence of dissimilar media compositions on the initiation of sugarcane variety US-718. We have also carried out optimization of initiation protocol for *in vitro* propagation of sugarcane variety US-718.

Material and method

The present research work was carried on in the plant tissue culture laboratory Khoski sugar mill Badin during 2018.

Media preparation for initiation of explant.

The MS basal medium (Murashage and skoog1962) considered of 30g/L sucrose for initiation of the apical buds. The pH of the medium was adjusted to 5.7 by using of 0.1N NaOH and HCl before being gelled with 6.0g/L agar and autoclaved at 121C for 20 mints. Although liquefied, the medium (40mL) was distributed into glass culture jar for culturing and stored under an aseptic condition.

Explant surface sterilization

The outer layers were removed and the explant was washed with the solution of three drops of Tween-20, three drops of liquid soap under running tap water for 15 minutes. Then the explants surface sterilized for 15 minutes in the solution of 50% bleach, 0.1% HgCl and two drops of Tween -20 for 15 minutes. This step was twice and followed by three times washing each for 10 minutes with sterile distilled water.

The explants were incubated at 25±2 C, and 16- hour photoperiod regime marinated under fluorescent light having 2500umol m-2S-1 light intensity with 70-80% humidity.

Experimental design

Experiments were performed in a factorial completely randomized design with three replications, each with ten explant per replicate.

Result

Effective system for in vitro propagation of sugarcane has been improved and results obtained in the above experiments are summarized here. Good eminence growth for newly formed shoots obtained from the apical meristem was based on production of broad and dark green colored leaves, healthy stems and small germination buds at the bottom of the stem.

From the (Table 1), it is noted that in US-718 best result for shoot formation were obtained in medium (MS medium containing 1.5 BAPmg/without kinetin), in this medium all explant showed shoot proliferation within 15 days with maximum number of 3.5±0.22 shoots per explants (Fig. 1).

Table 1. Effect of BAP and KIN on Initiation of US- 718 .

BAP mg/l	Kinetin mg/l	No of explant cultured	Days for shoot formation	Number of shoot per explant	Shoot length
0	0	10	Not survive	0±0.00	0±0.00
0.5	0	10	Not survive	0±0.00	0±0.00
0	0.5	10	10.7±0.9432	0.5±0.21	2.03±0.31
0.5	0.5	10	10.5±0.5441	0.5±0.10	2.74±0.22
0.5	1.0	10	11.3±0.3898	1±0.06	3.08±0.20
1.0	0.5	10	12.1±0.5736	0.5±0.26	3.91±0.21
1.0	0	10	12.4±0.5179	1±0.06	2.65±0.06
0	1.0	10	12.3±0.6395	1±0.01	2.37±0.01
1.0	1.0	10	13.9±0.451	1.5±0.32	3.65±0.04
1.5	0	10	13.2±0.6731	3.5±0.22	4.00±0.24
0	1.5	10	13.1±0.4533	3±0.12	3.71±0.42
1.5	1.5	10	13.4±0.6423	2.5±0.04	3.02±0.07
2.0	0	10	12.5±0.6651	2±0.056	2.76±0.41
0	2.0	10	13.5±0.5571	1±0.04	2.28±0.03
2.0	2.0	10	13.7±0.7211	1.5±0.01	2.31±0.05
2.5	0	10	13.5±0.6610	3±0.27	3.70±0.39
0	2.5	10	13.2±0.4456	2.5±0.03	3.10±0.13
2.5	2.5	10	13.8±0.7721	2.5±0.23	2.92±0.21

10.68 1.315 2.537

Fig. 1. Showed Microprogation of sugarcane variety US-718 using of various levels of BAP and Kinetin.

By increasing concentration of BAP alone or along with Kinetin shoot proliferation was decreased and time taken for shoot formation was also delayed. The best shoot length response in US-718 was obtained in MS medium containing 1.5mg/l BAP with 0 kin (table 1) in this medium 4.00±0.24 shoot length was noted.it is evident that the shoot length per explant increased only adding of BAP without kinetin. In the present investigation shoot apical meristem of approximately one inch sizes was used. As shown in Fig. 1, time for shoot formation was increased by decreasing the size of meristem. This size exhibited (90.32%) survival with 90% regeneration potential within 10 days of inoculation. For shoot formation solid media were used. Best results were obtained on media solidified with Phytagel at 3.0mg / l.

Discussion

The process of producing a large number of identical clones through in vitro culture is widely used in different types of plants. It is also reported that there is no culture on the initiation medium without growth regulator (Belay Tolera *et al.,* 2014).

(George EF, *et al.,* 2008). It also reported that the size of the meristem shoot formation and proliferation. In the present investigation, the best results of shoot formation and proliferation were obtained when a one-inch-sized meristem was used. The present studies also show the effect of phytohormones for the shoot formation and multiplication and shoot length of the explants. Amongst the various phytohormones, mainly two cytokines, namely BAP and kinetin, were used alone or together in the MS medium. The differences in the concentration of receptor molecules (protein) to plant growth regulators of target tissue of the varites also control the response potential (Singh R 2003). The concentrations of BAP are required for establishment shoot initiation and are dependent on the crop species (Biradar *et al.,* 2009). BAP at the concentration of 1.5mg/L showed maximum frequency of tip and shoot initiation establishment than other concentration but according to (Biradar *et al.,* 2009), BAP promoted higher apical initiation at 2.0mg/L. The control recorded the lowest culture growth (6.21%) and (0.00±0.00 shoots per explants), though, this highlights the need to supplement MS medium with the essential growth regulator, which confirm the similar reports by (Mekonen *et al.,* 2014, A. T. Asare *et al.,* 2017). Alongside, Danso *et al.,* (2011) established 5mg/BAP, 2mg/GA3 and 3g/l activated charcoal as well as antibiotics as nutrient supplemented to the MS medium to get single or multiple apical tip tissue culture.

Conclusion

During research study it is observed that among the various concentrations of BAP alone and as well as in combination with kin. a maximum numbers of bud initiation and shoots were observed after treatment with combination of containing full-strength MS medium supplemented with 1.5mg/L of BAP for sugarcane variety US-718 resulted in best response for in vitro aseptic culture establishment

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