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Orange Juice, a Natural Source for Enhancing *in Vitro* Regeneration in Saccharum spp.

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Abstract Orange juice is a natural source of various vitamins especially Vitamin C, as well as sugar, potassium, thiamine, folate, flvonoids and an antioxidant hesperidins. Therefore the effect of orange juice on in vitro regeneration of sugarcane was investigated. For this study, genotype S-2003-us-359 was selected, in which we had already established in vitro regeneration system and a media combination had been selected with highest regeneration potential. In this study, this media combination was used as control and also modified the regeneration medium of this media combination by adding orange juice and dry milk. An increase in regeneration potential of this media combination was observed by adding orange juice in regeneration medium.

Keywords Orange juice; Sugarcane; *In vitro* regeneration

Introduction

Globally, sugarcane has got a prime candidate as a cash crop which fulfilled 80% world sugar demand and emerging biofuel crop in near future due to its dexterous biomass production (Gallo-Meagher et al., 2000). This tropical grass is the member of family Poaceae which contains high polyploidy level (2n=80~270) (Heinz and Mee, 1969). This grass is the most suitable promising crop which could be utilized mainly for sugar production and then for power generation, paper making, live stock feed, chipboard, cane wax, fertilizer, bioethanol, syrup and mulch. Recently, additional revenue being produced in the form of bioplastics by sugarcane stalk and bagasse from sugarcane. Sugarcane as a perennial crop is the world's most resourceful crop in converting solar energy into chemical energy. The preliminary but significant results led to the utilization of *in vitro* cell and tissue culture for a range of functions such as micropropagation (Hendre et al., 1983), breeding (Krishnamurthy and Tlaskal, 1974), germplasm conservation (Taylor and Dukic, 1993), the eradication of systemic pathogens (Parmessur et al., 2002) and genetic engineering (Arencibia et al., 1995) of sugarcane. (Taylor et al., 1992) tested sugarcane cultivars readily produce embryogenic callus from young leaf tissue and readily regenerate plants from callus. Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology especially gene introduction (Villalobos and Arias, 1987). Therefore, it is significant for every genotype to optimize media with different levels of plant growth regulators along with growing conditions so that optimum regeneration could be attained (Anjum et al., 2012). Lack of suitable multiplication procedure and contamination by systemic diseases are the serious problem to multiply an elite genotype of sugarcane in the open field.

Problems associated with the traditional breeding methods have been overcome by the use of plant tissue culture techniques for the propagation of sugarcane and these techniques ensure disease free multiplication of the plants by reducing the time period required for the multiplication (Khan et al., 2006). Large-scale production of disease-free quality planting material of sugarcane can also be achieved through micropropagation. Use of plant tissue culture techniques reduces the breeding cycle (Lloyd and Pillay, 1980).

1 Results and Discussion

Lack of proficient and reproducible regeneration system to produce transformed plants at an adequate

rate is still the key factor seriously restricting the improvement of crops through genetic transformation (Popelka and Alpeter, 2003). Tissue culture is a powerful tool for studying and solving, problems in plant biotechnology. A tissue culture system provides considerable quantities of target tissue.

To check the response of fresh orange juice and dry milk on in vitro regeneration, we used selected media combination that we were reported already (Ijaz et al., 2012). This media combination consisted of CIM3 (Callus induction medium 3), genotype; S−2003−us−359, Callus age; 21 days old calli and RM3 (Regeneration medium 3). CIM3 contained 3 mg/L 2, 4−D, 40 g/L sucrose and basal MS salt. RM3 contained 0.1 mg/L 2, 4−D, 0.25 mg/L BAP, 40 g/L sucrose and basal MS salt. We just modified the RM3 in this media combination by adding orange juice RM3 (a) and dry milk RM3 (b). Calli of genotype S−2003−us−359 were induced on CIM3 and 21 days old calli were transferred to RM3 (control), RM3 (a) (modified regeneration medium by adding orange juice) and RM3 (b) (modified regeneration medium by adding dry milk) (Figure 1).

Figure 1 RM3 (b) medium modified by adding dry milk

The analysis of variance (ANOVA) table depicts the significant effect of regeneration media on *in vitro* regeneration (Table 1). This comparative study revealed that the RM3 (a) (modified RM3 by adding orange juice) gave maximum number of plants (Figure 2) the mean value 166.33 shoots per explant. This response of RM3 (a) was statistically significantly different from control (RM3) and RM3 (b); modified RM3 by adding dry milk. But RM3 and RM3 (b) are statistically similar with mean value of 134.67 and 137.77 shoots per explant respectively (Table 2, Figure 3).

Table 1 Analysis of variance (ANOVA) table for Regeneration

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
RM	2	1944.22	972.111	5.38*
Error	6	1084.00	180.667	
Total	8	3028.22		

Note: * = Significant (P<0.05)

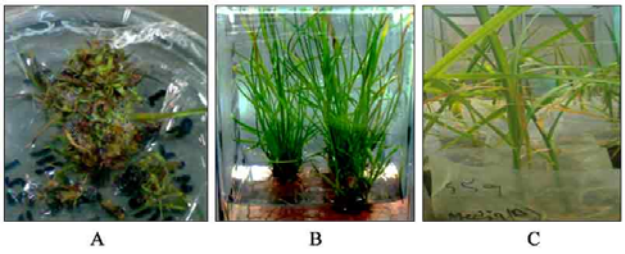

Figure 2 Comparative study of RM3(a)
Note: A: Regeneration of shoots on RM3 (a); B: Plant lets of RM3 (a) on root induction media; C: Plants of RM3 (a) in green house condition

Table 2 Means of regeneration media ± SE

Treatment	Mean ± SE
RM3	134.67±7.86 B
RM3(a)	166.33±8.76 A
RM3(b)	137.77±6.09 B

Note: Means sharing similar letters are statistically non-significant (P>0.05)

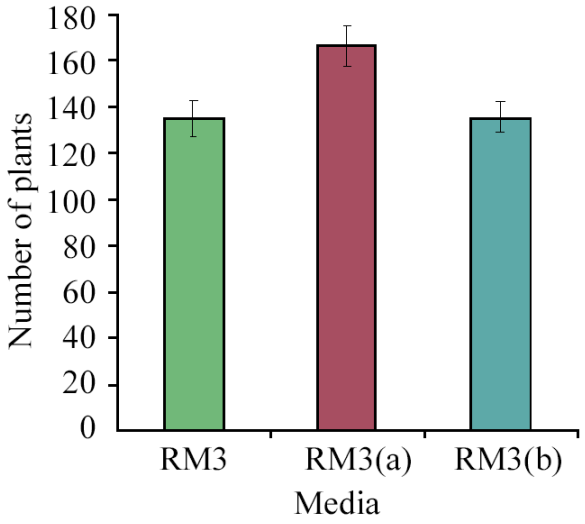

Figure 3 Regeneration response of genotype S−2003−us−359 on RM3 (control)
Note: RM3 (a): RM3 medium modified by adding orange juice; RM3 (b): RM3 medium modified by adding dry milk

Plant juices and liquid endosperms have been shown to possess stimulatory properties. The inclusion of liquid endosperm from immature corn (Netien et al,

1951), tomato juice (Nitsch, 1951; Straus and La Rue, 1954), immature fruits and seeds (Steward and Caplin,1952; Steward and Shantz, 1959), malt extract, yeast extract, leaf extracts from a number of plants and tumor extracts (Butenko, 1968), have been reported. These results are agreed with our results because by adding orange juice in our selected regeneration RM3), the regeneration response is enhanced.

2 Materials and Methods

The immature young leaves of sugarcane genotype S−2003−us−359 were cut into slices by using blades and scalpels and were cultured on callus induction medium containing 3 mg/L 2, 4−D, 40 g/L sucrose plus MS Salt in common. They were kept on dark conditions for callus induction.

Twenty one days old calli of this genotype were shifted on regeneration media. In this experiment regeneration medium reported by us (Ijaz et al., 2012) was modified by adding orange juice and dry milk (Table 3). Here reported regeneration medium was used as control. Data were collected on the basis of number of plants. Experiment was repeated three times.

Table 3 Regeneration media (Reported and Modified)

	RM $_{Reported}$	RM (a) $_{Modified}$	RM (b) $_{Modified}$
MS Salt	4.33 g/L	4.33 g/L	4.33 g/L
Sucrose	40 g	40 g	40 g
2, 4−D	0.10 mg/L	0.10 mg/L	0.10 mg/L
BAP	0.25 mg/L	0.25 mg/L	0.25 mg/L
Myoinositol	0.10 g/L	0.10 g/L	0.10 g/L
Nicotinic acid	1 g/L	1 g/L	1 g/L
Pyridoxine HCl	1 g/L	1 g/L	1 g/L
Thymine HCl	2 g/L	2 g/L	2 g/L
Glycine	4 g/L	4 g/L	4 g/L
Orange juice	0	50 mL	0
Dry milk	0	0	0.50%
Phyta gel.	2.66 g/L	2.66 g/L	2.66 g/L

Reference

Anjum N., Ijaz S., Rana I.A., Khan T.M., Khan I.A., Khan M.N., Mustafa G., Joyia F.A., and Iqbal A., 2012, Establishment of an *in vitro* regeneration system as a milestone for genetic transformation of sugarcane (*Saccharum officinarum L.*) against *Ustilago scitaminea*, Bioscience Methods, 3(2): 7-20, http://10.5376/bm.2012.03.0002

Arencibia A., P.R. Molin G. de la Riva, and G. Selman-Housein, 1995, Production of transgenic sugarcane (*Saccharum officinarum* L.) plants by intact cell electroporation, Plant cell Rep., 14(5): 305-309, http://10. 1007 / BF00232033

Butenko R.G., 1968, In: plant tissue culture and plant morphogenesis, Isr. Prog. For Scient. Transla. Jerusalem, pp. 40-45

Gallo-Meagher M., English R.G., and Abouzid A., 2000, Thidiazuron stimulates shoot regeneration of sugarcane embryogenic callus, *In vitro* cell and dev. Boil. Plant, 36(1): 37-40, http://10.1007/s11627-000-0009-3

Hendre N.A., Iyor R.S., Kotwalm M., Kluspe S.S., and Mascarenhas A.F., 1983, Rapid multiplication of sugarcane by tissue culture, Sugarcane, pp.5-8

Ijaz S., Rana I.A., Khan I.A., and Saleem M., 2012, Establishment of an *in vitro* regeneration system for genetic transformation of selected sugarcane genotypes, Genet. Mol. Res., 11(1): 512-530, http://dx.doi.org/10.4238/2012.March.6.4

Khan M.S., Usman M., and Lilla M.I., 2006, Facile plant regeneration from tomato leaves induced with spectinomycin, Pak. J. Bot., 38(4): 947-952

Krishnamurthy M., and Tlaskal J., 1974, Fiji disease resistant Saccharum officinarum var. Pindar sub-clones from tissue cultures, Proc. Int. Soc. Sugar Cane Technol., 15: 130-137

Lloyd H.L., and Pillay M., 1980, The development of an improved method for evaluating sugarcane for resistance to smut, Proceedings of the south african sugar technologists' association ,168-172

Netien G., Beauchesne G., and Mentzer C., 1951, Influence du “lait de mais” sur la croissance des tissues de carotte *in vitro*, Comp. Rendus de L’Acad. Des Sci., 233: 92-98

Nitsch J.P., 1951, Growth and development *in vitro* of excised ovaries, Am. J. Bot., 38(7): 566-571, http://dx.doi.org/10.2307/2438018

Parmessur Y., Aljanabi S., Saumtally S., and Dookun-Saumtally A., 2002, Sugarcane yellow leaf virus and sugarcane yellows phytoplasma: elimination by tissue culture, Plant Pathol., 51(5): 561-566, http://dx.doi.org/10.1046/j.1365-3059.2002.00747.x

Popelka J. C., and Altpeter F., 2003, Evaluation of rye (*Secale cereale* L) inbred lines and their crosses for tissue culture response and stable genetic transformation of homozygous rye inbred line L22 by biolistic gene transfer, Theor. Appl. Genet., 107(4): 583-590, http://10.1007/s00122-003-1314-0

Steward F.C., and Caplin S.M., 1952, Investigation on growth and metabolism of plant cells: Evidence on the role of coconut milk factor in development, Ann. Bot., 16(4):491-504

Steward F.C., and Shantz E.M., 1959, The chemical regulation of growth (some substances and extracts which include growth and morphogenesis), Ann. Rev. Plant. Physiol., 10: 379-404, http://10.1146/annurev.pp.10.060159.002115

Straus J., and La Rue C.D., 1954, Maize endosperm tissue growth in vitro I. Cultural requirements, Am. Bot. S., 41(8): 687-694

Taylor P.W.J, and Dukic S., 1993, Development of an *in vitro* culture technique for conservation of Saccharum spp. hybrid germplasm, Plant Cell Tiss. Organ Cult., 34: 217–222, http://10.1007/BF00036105

Taylor P.W.J., KO L.H., Adkins S.W., Rathus C., and Birch R.G., 1992, Establishment of embryogenic callus and high protoplast yielding suspension cultures of sugarcane (Saccharum spp. Hybrid), Plant. Cell Tiss. Org. Cult., 28(1):69-78, http://10.1007/BF00039917

Villalobos I., and Arias O., 1987, Induccion Y multipucacion de callos *in vitro* en tres cultiv ares comerciales de caña de azúcar (*Saccharnm* spp.), Agron. Costarricense, 11(1): 39-44