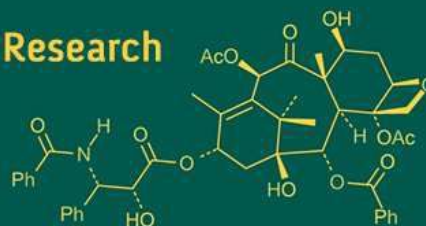


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Evaluation of low cost sucrose alternatives for *in vitro* potato plantlet production

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Abstract

In present experiment effectiveness of inexpensive alternative sucrose sources (Table sugar, Jaggery, Sugar cane juice and Rock sugar) used for *in vitro* potato plantlet production of two cultivars, Kufri Himalini and Kufri Jyoti was evaluated. These alternatives were compared against a control sucrose 30 g/L, which is known to be expensive. Among the different treatments, treatment B₄ (MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L), significantly improved plantlet height, number of leaves, root length, number of roots and number of nodes per plantlet in both Kufri Himalini and Kufri Jyoti. Which was on par with the treatment control (MS salts + Agar 6 g/L+ Sucrose 30 g/L + Calcium pantothenate 1 mg/L) and treatment B₃ (MS salts + Agar 6 g/L+ Table sugar 30 g/L+ Calcium pantothenate 1 mg/L). Conversely, treatment B₁₀ (MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L + Calcium pantothenate 1 mg/L), resulted in the poorest growth performance across all measured growth parameters in both cultivars, indicating that sugar cane juice as a sucrose source might be detrimental for plantlet development. These findings suggest that both jaggery and table sugar are effective substitutes for agar *in vitro* culture media.

Keywords: Potato, Kufri Himalini, Kufri Jyoti, tissue culture, low cost, sucrose, table sugar, jaggery

1. Introduction

The potato (*Solanum tuberosum* L.) is a widely grown tuber crop, originally from the Americas and now cultivated globally. As a member of the Solanaceae family, it is the fourth most produced food crop and the top non-cereal crop worldwide. Potatoes are highly nutritious, providing essential vitamins, proteins, carbohydrates and minerals, making them valuable in addressing malnutrition (Aksoy *et al.* 2021) ^[1]. Their high yield compared to other crops makes them an important food source for combating hunger and poverty. Potatoes are also versatile in cooking and play a key role in human nutrition by offering health benefits through their macronutrient and micronutrient content (Hellmann *et al.*, 2021) ^[12]. As a staple food, they contribute significantly to meeting daily dietary needs and promoting overall health.

Potatoes are vegetatively propagated using tubers, leading to rapid deterioration of seed stock, which ideally requires replacement every two years (Singh *et al.*, 2018) ^[24]. Quality seeds are often scarce or expensive, compounded by the limited availability of aphid-free regions for seed production in the country. Studies indicate that use of high-quality seeds can enhance productivity by 15 to 20 percent (Shaheb *et al.*, 2016) ^[23].

The shortage of adequate planting material is a critical issue affecting crop yield (Singh and Sharma, 2018) ^[25]. Seed potato production and distribution are particularly challenging due to the bulkiness, high seed rate and slow multiplication rate of potatoes (five to six times) (Shaheb *et al.*, 2016) ^[23]. There is a significant gap between the demand and supply of certified seed potatoes in India. Central Potato Research Institute (CPRI) of the Indian Council of Agricultural Research (ICAR) produces around 2,400 tons of breeder's seed annually, with 80 percent supplied to states and other agencies for multiplication (Anon., 2020) ^[4]. Following standard multiplication stages, this stock can produce approximately 0.5 million tons of certified seed, meeting only 10 percent of the total seed requirement, resulting in a deficit of about 4.9 million tons (Buckseth *et al.*, 2020) ^[6].

Micropropagation techniques offer a solution to many issues associated with traditional seed production systems (Singh *et al.*, 2019) [26]. These techniques support seed potato production by providing nuclear stock material, such as microplants or microtubers, for use in production channels like aeroponics, apical rooted technology and minituber production (Chindi *et al.*, 2014) [7]. However, the high unit production costs of *in vitro* techniques, particularly due to the expensive culture nutrient medium, have prevented many small and medium-scale laboratories from fully benefiting from plant tissue culture technology. This has led to the closure of several commercial micro-propagation units in India and stimulated research into low-cost production protocols (Saraswathi *et al.*, 2016) [21].

One of the most significant contributors to the high production costs in tissue culture is the expense of the culture nutrient medium, which requires costly chemicals. By adopting low-cost alternatives, the cost of tissue culture can be significantly reduced, making it accessible to small, medium and average farmers. Using these alternatives can reduce tissue culture plant production costs by 50 to 90 percent (Naik *et al.*, 2020) [16].

To reduce the unit cost of plant propagules low cost micropropagation was practiced (Savangikar *et al.*, 2002). Reducing the cost of media had a huge effect on horticulture, agriculture and the floriculture sector especially in developing countries. Sucrose is the preferred carbohydrate for most studies due to its ability to be easily translocated and its resistance to enzymatic degradation, owing to its non-reducing nature (Pontis, 1978) [17]. While sucrose is commonly used in the vast majority of *in vitro* shoot induction and development studies in woody species, it is not always the most effective carbon source. Additionally, the high cost of sucrose, which accounts for 21.7 to 30 percent of the media cost (Prakash *et al.*, 2004; Demo *et al.*, 2008; Swamy *et al.*, 2010; Dantas *et al.*, 2021) [18, 10, 27, 8]. Considering the importance of finding alternative sucrose source for the mass multiplication of potato plantlets, the experiment was designed to find an inexpensive sucrose source for *in vitro* potato plantlet production.

2. Materials and Methods

This experiment was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Bangalore, with an objective to examine the effectiveness of low cost alternative sucrose sources for *in vitro* potato plantlet production. The four carbon sources, used for experiment were table sugar, jaggery, sugarcane juice, rock sugar and sucrose. Sucrose, a widely recognized standard in tissue culture, served as the control and was sourced from HiMedia.

2.1 Alternative sucrose sources

2.1.1 Table sugar

It is a crude sucrose derived by crushing and extraction of sugarcane (*Saccharum officinarum*). Sugar is hard, white, dry crystals, lumps or powder, sweet taste, odourless soluble in water and very slightly soluble in alcohol (Arthur and

Rose, 1996) [5]. Raw cane sugar contains 96 to 97 percent sucrose, 0.75 to 1.0 percent reducing sugar, 0.75 percent moisture, 0.5 percent ash and remainder, organic non-sugars (Anon., 1972) [3]. In the present study, the medium was supplemented with common grade sugar (DMart Yelahanka, Bengaluru, India) at 20, 30 and 40 g/L of the medium for plantlet production.

2.1.2 Jaggery

Jaggery is the product obtained on concentrating sugarcane juice with or without prior purification, into a solid or semi solid state. It is also called gur, contains all the constituents of cane juice, some of them having undergone slight changes during boiling. Percent composition of gur is: sucrose, 65 to 85; invert sugar, 10-15; ash, 2.5; and moisture, 3 to 6. It also contains carotene, 280 I.U./100 g.; nicotinic acid, 1.0 mg./100 g.; vitamin B1, 20 ug/100 g.; and traces of iron and copper (Anon., 1957) [2]. In the present study, the medium was supplied with jaggery (DMart Yelahanka, Bengaluru, India) at 20, 30 and 40 g/L of the medium for plantlet production.

2.1.3 Rock sugar

Rock sugar, a confectionery mineral with large sugar crystals, forms from a supersaturated sugar-water solution crystallizing onto a surface. Heating the solution enhances sugar dissolution, yielding larger crystals after several days. Originating in India and Iran, rock candy is used to sweeten tea and as a mouth freshener, often with aniseed. It also relieves cough. In this study, the medium was supplemented with rock sugar at concentrations of 20, 30 and 40 g/L for potato plantlet production (DMart Yelahanka, Bengaluru, India).

2.1.4 Sugarcane juice

Sugarcane juice is an opaque liquid, grey to dark green in color, containing in solution all the soluble constituents of cane. Sugar cane juice (specific gravity; 1.07; pH, 5.8 to 6.4) contains; water, 80 to 85 percent; sucrose, 13 to 18 percent; glucose, 0.3 to 1.0 percent and non-reducing sugar, 1.0 percent. The analysis of the non-sugars is as follows: nitrogen, 0.019; protein, nil; ash, 0.37; calcium, 0.021; and phosphorus, 0.032 g/100 cc. juice (Anon., 1957) [2]. In the present study, medium was added with sugarcane juice (Cane-O-La, Jayanagar, Bengaluru, India) at 100, 150 and 200 ml/L of the medium.

2.2 Ex-plant and culture conditions

Explants used in this experiment were collected from CIP (International Potato Centre) Bengaluru. Single nodal cuttings were used as explant for this experiment. Cultivars selected were Kufri Himalini and Kufri Jyoti. Observations were recorded three weeks after the culturing on growth parameters like plantlet height, number of leaves, number of nodes, root length and number of roots per plantlet for statistical analysis. During the growth stage temperature was maintained at 22±2 °C with 16 hours of light period and 8 hours of dark period.

2.3 Treatment details

Table 1: Alternative sucrose sources for *in vitro* potato plantlet production

Treatments	Treatment details
Control	MS salts + Agar 6 g/L+ Sucrose 30 g/L+ Calcium pantothenate 1 mg/L
B ₁	MS salts + Agar 6 g/L+ Table sugar 20 g/L+ Calcium pantothenate 1 mg/L
B ₂	MS salts + Agar 6 g/L+ Table sugar 25 g/L+ Calcium pantothenate 1 mg/L
B ₃	MS salts + Agar 6 g/L+ Table sugar 30g/L+ Calcium pantothenate 1 mg/L
B ₄	MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L
B ₅	MS salts + Agar 6 g/L+ Jaggery 30 g/L+ Calcium pantothenate 1 mg/L
B ₆	MS salts + Agar 6 g/L+ Jaggery 40 g/L+ Calcium pantothenate 1 mg/L
B ₇	MS salts + Agar 6 g/L+ Rock sugar 20 g/L+ Calcium pantothenate 1 mg/L
B ₈	MS salts + Agar 6 g/L+ Rock sugar 30 g/L+ Calcium pantothenate 1 mg/L
B ₉	MS salts + Agar 6 g/L+ Rock sugar 40 g/L+ Calcium pantothenate 1 mg/L
B ₁₀	MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L+ Calcium pantothenate 1 mg/L
B ₁₁	MS salts + Agar 6 g/L+ Sugar cane juice 150 ml/L+ Calcium pantothenate 1 mg/L
B ₁₂	MS salts + Agar 6 g/L+ Sugar cane juice 200 ml/L + Calcium pantothenate 1 mg/L

2.4 Statistical analysis

The complete data was analysed using CRD. The critical difference of the experimental data was tested by using F test at 1 percent level of confidence. The analysis was done using OPSTAT online analysis tool.

2.5 Cost analysis

The reduction in media cost for production of potato plantlets through standard MS media and alternative low cost media were computed. The percent cost reduction per litre of media was calculated by following formula.

$$\text{Cost reduction (\%)} = \frac{\text{Cost of alternative media}}{\text{Cost of standard MS media}} \times 100$$

3. Results and Discussion

3.1 Influence of alternative sucrose sources on plantlet height:

Among the different treatments, significantly highest plantlet height was recorded in treatment B₄ (MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L) with averaging 10.32 cm height for Kufri Himalini and 8.68 cm height for Kufri Jyoti. This was statistically at par with control (MS salts + Agar 6 g/L+ sucrose 30 g/L + Calcium pantothenate 1 mg/L) for both the cultivars Kufri Himalini (10.18 cm) and Kufri Jyoti (8.47 cm) and treatment B₃ (MS salts + Agar 6 g/L+ Table sugar 30 g/L+ Calcium pantothenate 1 mg/L) also performed comparable performance with averaging 10.12 cm height for Kufri Himalini and 8.41 cm plantlet height for Kufri Jyoti. The shorter plantlets were recorded in treatment B₁₀ (MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L+ Calcium pantothenate 1 mg/L) with averaging 6.06 and 4.61 cm plantlet height for Kufri Himalini and Kufri Jyoti, respectively.

Jaggery as a sucrose source increased plantlet height compared to HiMedia sucrose this might be because of its complex composition, which includes important minerals, vitamins and other organic substances in addition to sucrose. Plantlet growth is supported by a richer and more balanced environment, the presence of micronutrients potassium, calcium and iron, which can strengthen a plant's ability to absorb nutrients, increase its metabolic activity and speed up its growth. Results were in conflict with findings of Prakash *et al.* (2004) ^[18], Joshi *et al.* (2009) ^[14] and Rajavel and Stephan (2014) ^[19] in ginger, turmeric and *Wrightia tomentosa*, respectively.

3.2 Influence of alternative sucrose sources on number of leaves per plantlet:

Significantly greater number of leaves per plantlet were recorded in Treatment B₄ (MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L) for both the cultivars Kufri Himalini (9.30) and Kufri Jyoti (8.10). This was statistically on par with control (MS salts + Agar 6 g/L+ Sucrose 30 g/L+ Calcium pantothenate 1 mg/L) with averaging 8.80 leaves per plantlet Kufri Himalini and 7.80 leaves for Kufri Jyoti, and treatment B₃ (MS salts + Agar 6 g/L+ Table sugar 30 g/L+ Calcium pantothenate 1 mg/L) also performed comparable results with averaging 8.60 and 7.60 leaves per plantlet for Kufri Himalini and Kufri Jyoti, respectively. Conversely, least number of leaves per plantlet were recorded in treatment B₁₀ (MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L+ Calcium pantothenate 1 mg/L) with averaging 5.40 leaves per plantlet for Kufri Himalini and 5.10 leaves for Kufri Jyoti.

More number of leaves per plantlet was recorded in media prepared using jaggery as a sucrose source reason might be due to the presence of additional micronutrients and vitamins enhanced plantlet growth and increased the number of leaves per plantlet. These findings are inconsistent with the results of Prakash *et al.* (2004) ^[18], Joshi *et al.* (2009) ^[14], Rajavel and Stephan (2014) ^[19] in ginger, turmeric and *Wrightia tomentosa*, respectively. Who were of the argued that jaggery inhibited plant growth. They suggested that this inhibition could be due to nutrient imbalances osmotic stress and hormonal imbalance caused when jaggery was added to the media.

3.3 Influence of alternative sucrose sources on root length:

Significant difference in root length was recorded among various treatments. The longest root length (8.16 and 6.34 cm) was recorded in treatment B₄ (MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L) for both cultivar Kufri Himalini and Kufri Jyoti, respectively. This was statistically on par with treatment control (MS salts + Agar 6 g/L+ Sucrose 30 g/L+ Calcium pantothenate 1 mg/L) with averaging 7.91 cm root length for Kufri Himalini and 6.26 cm for Kufri Jyoti and treatment B₃ (MS salts + Agar 6 g/L+ Table sugar 30 g/L+ Calcium pantothenate 1 mg/L) also performed comparable results with 7.85 cm root length for Kufri Himalini and 6.11 cm for Kufri Jyoti. Contrast to this the lowest root length (4.78 and 4.64 cm) was recorded in treatment B₁₀ (MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L+ Calcium pantothenate 1 mg/L) for Kufri Himalini and Kufri Jyoti, respectively.

Root length was significantly higher in case of jaggery (as sucrose source + MS Salts + Agar 6 g/L + Calcium pantothenate 1 mg/L) performed better results concerning root length reason might be due iron content presence in jaggery enhanced the root growth. These results were inconsistent with findings of Tyagi *et al.* (2007) [30], Swamy *et al.* (2010) [27] and Tripathi *et al.* (2021) [29] reported that jaggery inhibited the plantlet growth in turmeric, patchouli and *Ocimum citriodorum*, respectively. They reported that, this inhibition could be due to nutrient imbalances and osmotic stress caused by changes in pH balance after addition of jaggery to the media.

3.4 Influence of alternative sucrose sources on number of roots per plantlet: Among the different treatments highest number of roots per plantlet were recorded in treatment B₄ (MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L) for both cultivars Kufri Himalini (7.10) and Kufri Jyoti (6.50). This was statistically on par with control (MS salts + Agar 6 g/L+ Sucrose 30 g/L+ Calcium pantothenate 1 mg/L) with averaging 6.80 roots per plantlet for Kufri Himalini and 6.40 roots for Kufri Jyoti, and treatment B₃ (MS salts + Agar 6 g/L+ Table sugar 30 g/L+ Calcium pantothenate 1 mg/L) with 6.70 roots per plantlet for Kufri Himalini and 6.10 roots for Kufri Jyoti. The lowest number of roots per plantlet (3.60 and 3.30) were recorded in treatment B₁₀ (MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L+ Calcium pantothenate 1 mg/L) for Kufri Himalini and Kufri Jyoti, respectively. Sugar cane juice and rock sugar were used as alternative sources for sucrose for both the cultivars Kufri Himalini and Kufri Jyoti examined during *in vitro* study. Sugar cane juice

was found to be unsatisfactory because to medium turned to a dark brown color that adversely affected the culture growth and concerned rock sugar inhibitors already present or formed during the autoclaving as reported by Maheswari *et al.* (1980) [15] and Hsiao and Bormann, (1989) [13]. These findings are also in conformity with the reports of Prakash *et al.* (2004) [18] and Joshi *et al.* (2009) [14] in ginger, turmeric and *Wrightia tomentosa*, respectively.

3.5 Influence of alternative sucrose sources on number of nodes per plantlet: Among the different treatments highest number of nodes per plantlet 9.80 and 8.40 were recorded in treatment B₄ (MS salts + Agar 6 g/L+ Jaggery 20 g/L+ Calcium pantothenate 1 mg/L) for Kufri Himalini and Kufri Jyoti, respectively. This was followed by control (MS salts + Agar 6 g/L+ Sucrose 30 g/L+ Calcium pantothenate 1 mg/L) with averaging 9.60 nodes for Kufri Himalini and 8.10 nodes per plantlet for Kufri Jyoti, and treatment B₃ (MS salts + Agar 6 g/L+ Table sugar 30 g/L+ Calcium pantothenate 1 mg/L) with averaging 9.50 nodes per plantlet for Kufri Himalini and 7.90 nodes per plantlet for Kufri Jyoti. The lowest number of nodes per plantlet 6.30 and 5.40 were recorded in treatment B₁₀ (MS salts + Agar 6 g/L+ Sugar cane juice 100 ml/L+ Calcium pantothenate 1 mg/L) for Kufri Himalini and Kufri Jyoti, respectively. Table sugar as a sucrose source performed comparable results to HiMedia sucrose reason might be due to efficient translocation and assimilation of table sugar by explants leads to eventual growth which is similar to sucrose. The results of the present study was in accordance with previous studies reported by (Saeed, 2006; Das and Gupta, 2009) [20, 9].

Table 2: Influence of alternative source sources, combination of alternative sucrose on growth parameters of *in vitro* potato plantlet production cv. Kufri Himalini

Treatments	Plantlet height (cm)	Number of leaves	Root length (cm)	Number of roots	Number of nodes
Control	10.18	8.80	7.91	6.80	9.60
B ₁	7.67	6.50	5.71	4.30	7.40
B ₂	8.52	7.80	6.64	5.40	8.20
B ₃	10.12	8.60	7.85	6.70	9.50
B ₄	10.32	9.30	8.16	7.10	9.80
B ₅	8.75	7.60	6.67	5.90	8.60
B ₆	6.76	5.80	5.84	4.10	6.90
B ₇	7.84	6.40	6.07	4.40	6.70
B ₈	8.05	6.60	6.28	4.70	6.90
B ₉	8.27	7.10	6.46	4.90	7.30
B ₁₀	6.06	5.40	4.78	3.60	6.30
B ₁₁	6.39	5.70	4.96	3.70	6.60
B ₁₂	6.55	6.10	5.22	4.10	6.80
F-test 1%	**	**	**	**	**
S. Em ±	0.08	0.22	0.11	0.20	0.19
CD at 1%	0.31	0.82	0.42	0.75	0.71

** -Significant at 1% level; S. Em- Standard error mean; CD-Critical difference

Table 3: Influence of alternative source sources, combination of alternative sucrose on growth parameters of *in vitro* potato plantlet production cv. Kufri Jyoti

Treatments	Plantlet height (cm)	Number of leaves	Root length (cm)	Number of roots	Number of nodes
Control	8.47	7.80	6.26	6.40	8.10
B ₁	6.62	6.30	4.96	4.50	6.60
B ₂	7.81	6.80	5.27	5.30	7.20
B ₃	8.41	7.60	6.11	6.10	7.90
B ₄	8.68	8.10	6.34	6.50	8.40
B ₅	7.47	7.20	5.48	5.40	7.50
B ₆	5.83	6.40	4.87	4.90	6.70
B ₇	6.02	5.60	4.97	4.80	5.90
B ₈	6.49	5.90	5.18	5.10	6.20
B ₉	6.68	6.30	5.39	5.30	6.60
B ₁₀	4.61	5.10	4.64	3.30	5.40
B ₁₁	4.78	5.30	4.93	3.60	5.70
B ₁₂	4.85	5.70	5.24	3.80	5.90
F-test 1%	**	**	**	**	**
S. Em ±	0.12	0.16	0.08	0.17	0.15
CD at 1%	0.45	0.58	0.31	0.63	0.54

* -Significant at 1% level; S. Em- Standard error mean; CD-Critical difference

Table 4: Comparative cost analysis of sucrose and alternative sucrose sources used for *in vitro* potato plantlet production

Sucrose source	Concentration (g/L)	Cost (Rs.)	Reduction in cost (%)
Sucrose	30.0	34.8 (Rs.11400)	-
Jaggery	20.0	2.4 (Rs. 120/Kg)	93.10
Table sugar	20.0	1.44 (Rs. 48/Kg)	95.86

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5. Conclusion

The comparative cost analysis of different sucrose sources for *in vitro* potato plantlet production clearly indicates that using alternative sucrose sources such as jaggery and table sugar offers significant cost savings compared to standard sucrose. Jaggery and table sugar, reduce the media cost by 93.10 and 95.86 percent respectively, compared to sucrose. These alternatives provide a highly economical solution, without compromising the growth and development of the potato plantlets. This cost-effectiveness makes them viable options for scaling up tissue culture practices, especially for small and medium-scale labs and farmers in developing countries, thus promoting more accessible and sustainable potato plantlet production.

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