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Development of low cost technology for *in vitro* mass multiplication of potato (*Solanum tuberosum* L.)

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Production of potato is constrained by lack of disease-free planting materials. This can be circumvented through tissue culture but the technology is costly limiting its adoption. As composition of culture medium used for shoot regeneration has a great influence on cost and there is a potential for use of locally available low cost resources as alternatives to the conventional costly laboratory resources. There is therefore, need to put in place interventions that will reduce the cost of production hence, making tissue culture products affordable. The present study describes a highly cost effective *in vitro* mass multiplication protocol for potato. The developed low cost medium can be used to boost the production of affordable disease-free potato seedlings, besides, this practice could be helpful in achieving more than 95% reduction in media cost.

Key words: Potato, micropropagation, low cost, medium, carbon source, tap water, plantlets.

INTRODUCTION

Potato is the third most important food crop in the world after rice and wheat which is consumed by more than a billion people worldwide. India is the second largest potato producer in the world after China; with the highest potato productivity among the top four potato producers in the world (CPRI vision, 2030). In potato cultivation, seed is the single most expensive input accounting for 40 to50% of production costs. On account of cumbersome methods involved in maintaining the nucleus stocks free from viruses and other diseases under conventional method of breeder's seed potato production, *in vitro* micropropagation techniques are gaining popularity. The recent advances in tissue culture techniques have facilitated the production, multiplication and maintenance of disease-free potato clones.

High production cost has been an impediment to tissue culture adoption which has further limited the technology to a few institutions and rich farmers while locking out the resource-challenged subsistence farmers. One factor contributing to the high cost of production is the cost of the culture nutrient medium which requires chemicals that are often very expensive (Savangikar, 2002). In order to increase application of tissue culture technology in potato farming, innovative approaches are needed to lower the cost of micro-propagule production. Various factors should be considered in developing plant tissue culture technique. One of the most important factors governing the *in vitro* shoots regeneration is largely determined by the composition of the culture medium (Rashid et al., 2000). Much number of researchers influenced to find alternatives materials to substitute alternatives to gelling agents, use of household sucrose, and some medium components objectively to reduce cost in media culture preparation. For example Raghu et al. (2007) have tried

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household sugar and tap water to substitution laboratory sucrose and double distilled water used in plant tissue culture. Besides successful in promoting the plantlet regeneration by using the substitution items provide cost reducing in media culture preparation. Many gelling agents are used for plant tissue culture media, such as agar technical Oxoid, agarose, phytagel and gelrite (Debergh, 1983).

Agar is the most commonly used as gelling agent for media preparation (Afrasiab and Jafar, 2011). From more than 100 years ago (Henderson and Kinnersely, 1988) until today, agar has been widely used as a gelling agent in plant tissue culture technique. This is because Henderson and Kinnersely (1988) reported its stability, high clarity and non toxic nature. As reported by Deb and Pongener (2010), agar substance often used in plant tissue culture as supporting agent, but because of the relatively high material costs caused them to do research for alternative materials with lower cost. Instead of high price of pure grade agar, there are some doubts about its nontoxic nature that influenced researcher to find alternatives material (Sharifi et al., 2010). Nagamori and Kobayashi (2001) used various types of starch and gums as cheaper alternatives for in commercial micro-propagation.

In addition to the gelling agents, the carbon sources such as grade sucrose that is often used in the micro-propagation of plants at laboratory contribute about 34% of the production cost (Demo et al., 2008). Sucrose has been reported as a source of both carbon and energy (Bridgen, 1994). Sugar was sucrose derived from sugar cane, in which the sucrose content in sugar is a combination of a glucose molecule with a molecule of fructose. Zapata (2001) cited in Kumara et al. (2010) reported success in reducing by 90% the cost of tissue culture banana trees are carried out due to the use of sucrose being replaced with table sugar. The quality and performance of plantlets grown on sucrose and on local commercial sugar resulted in all the sources of carbon fostered vigorous plantlet growth (Demo et al., 2008). Many research laboratories have used table sugar in the plant propagation medium (Kaur et al., 2005). Cost difference between household sugar and laboratory grade sucrose is quite big (97% difference). For the fact that laboratory grade sucrose is expensive, the use of 30 g/l household sugar can be proposed replacing the 30g/l sucrose as a way to reduce medium costs. Further studies should be conducted to identify the use of sugarcane juice directly to replace sugar. Buah et al. (2011) reported that costs of providing culture media using sugarcane juice are further cheaper than commercial sugar. In India, Ganapathi et al. (1995) reported that commercial grade sugar can replace analytical grade sucrose, with no significant change in the frequency of shoot formation in banana. The cost of commercial micropropagation has to be reduced drastically without compromising on the quality of micropropagules especially in the developing countries (Kuria et al., 2008). However, no such low cost protocol has been developed for potato till date. Therefore, this study was undertaken to observe the interaction effects of the different gelling agents, type of water and carbon sources during *in vitro* mass multiplication of potato in order to evolve a low cost mass multiplication technology by adopting low cost substituent in the culture medium to make this system more economical and affordable.

MATERIALS AND METHODS

Three different experiments with different tetraploid (2n=4x=48) potato (*Solanum tuberosum* L. ssp. *tuberosum*) cultivars *viz.*, Kufri Chandramukhi, Kufri Girdhari, Kufri Himalini, Kufri Bahar and Kufri Sindhuri belonging to different maturity groups were carried out at Central Potato Research Institute, Shimla during 2011 to 2012. Three double node cuttings dissected essentially from middle portion of the micro-plants were cultured per test tube (25 × 150 mm) containing 13 cm^3 MS medium (Murashige and Skoog, 1962). In the first experiment MS medium was prepared with nine types of water viz., rain, natural, tap, aquaguard, single distilled, double distilled, Type-I (Reverse osmosis), Type-II (Electronically de-ionized) and ultra-pure water, supplemented with sucrose at 30 gL^{-1} and solidified with agar (AR) at 7 $gL^{-1.}$ In the second experiment MS medium was prepared with double distilled water, supplemented with seven types of carbon sources viz., commercial sugar, commercial sugar (sulphur less), sucrose, fructose, dextrose, sugar cubes and galactose at 30 gL^{-1} and solidified with agar (AR) at 7 $gL^{-1.}$ In the third experiment MS medium was prepared with double distilled water, supplemented with sucrose at 30 gL^{-1} and solidified with four types of solidifying agent's viz., agar (PT), agar (bacteriological), agar (purified) and gelrite. The quantity of solidifying agent used was at 7 gL^{-1}except gelrite (2 gL^{-1}). All the three experiments were uniformly supplemented with 4.19 µM D-calcium pantothenate, NAA (0.05 µM) and GA_3 (0.29 µM). The experiment was carried out in a factorial completely randomized design (CRD) with four genotypes over a period of 28 days. The culture tubes were incubated under a 16 h photoperiod (irradiance of 60 µmol m^{-2} s^{-1}) at temperature of 22±1°C for 28 days in non-hermetic culture room.

After twenty-eight days of culturing, observations were recorded on morphological parameters such as micro-plant height (cm); number of green leaves, nodes and roots; inter-nodal and root length (cm); fresh as well as dry mass (mg) of microplants. As there were three micro-plants per culture tube, data was recorded for each micro-plant and averaged. In case of number of roots, only primary roots were counted, as there was secondary branching too. Root length was recorded for the longest root. Fresh and dry weight was taken for all the three plantlets. For dry weight, micro-plants were dried at 80°C for 48 h in the hot air oven and dry weight was recorded after bringing to room temperature. The experiment was repeated once again; data were pooled over individual experiments and analyzed statistically using the software AGRES for obtaining analysis of variance and means were separated according to the least significant differences at 0.05 level of probability.

RESULTS AND DISCUSSION

Carbon source

The analysis of variance showed that type of carbon

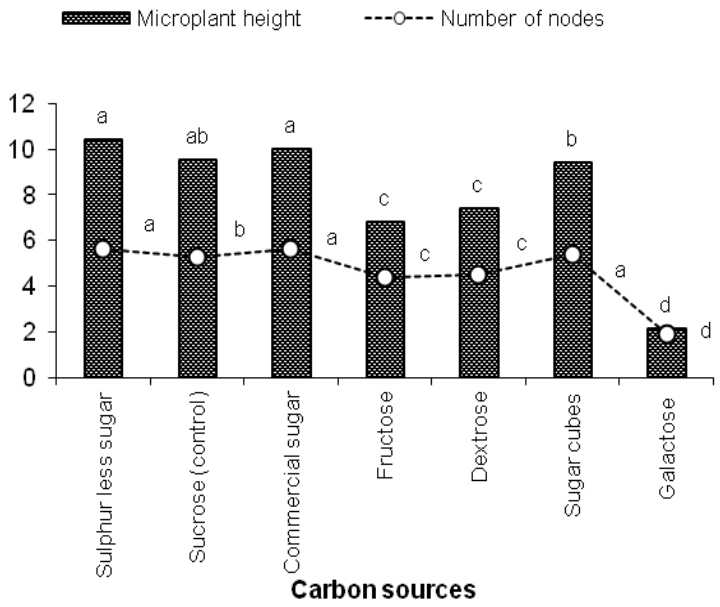

Figure 1. Effect of carbon sources on micro-plantlet height and number of nodes.

source had a major effect on all the morphological characters studied. Carbon source significantly ($P \leq 0.05$) influenced the microplant height, number of leaves, and number of nodes, inter nodal length, number of roots, root length, fresh as well as dry weight. Among the carbon sources sulphur less sugar (10.4 cm) significantly enhanced the microplant height which was also found at par with commercial sugar (10.0 cm) as well as standard control sucrose (9.5 cm), whereas, galactose recorded the minimum (2.1 cm). Sulphur less sugar as well as commercial sugar significantly increased the number of leaves as well as nodes as compared to other carbon sources. However, sugar cubes also exhibited at par results with respect to number of nodes (Figure 1). In general, galactose significantly reduced the number of leaves and nodes.

Almost all the carbon sources except galactose had statistically similar effect on inter-nodal length. This may be due to the increased availability of carbon in the form of purified sucrose that increases the intracellular sucrose concentration and it also has been reported to stimulate the *in vitro* growth of different crop species as a result of more negative water potential in the medium (Riek et al., 1997; Ebrahim et al., 1999). In addition to this, sucrose has been considered as one of the most common carbon source used in plant tissue culture due to its efficient uptake across the plasma membrane (Shimon et al., 2000; Sima and Desjardins, 2001; Yu et al., 2000). While, *Mentha piperita* cultured on media prepared with tap water + commercial sugar and double distilled water + tissue culture grade sucrose did not show any difference (Sunandakumari et al., 2004). Blanc et al (2002) reported that, rapid hydrolysis of sucrose could increase the content of hexoses and storage compounds, directing the cells of embryogenic callus of *Hevea brasiliensis* to proliferate fast. Besides this, commercial sugar is impure sucrose and they may contain some other substances which may not suitable for tissue culture (Hossain et al., 2005). Our results are also in agreement with the earlier findings. Except dextrose and galactose, all other carbon sources exhibited same effect on number of roots as well as root length. This may be due to the decreased availability of carbon source in the medium that increased the number of roots and root length. Therefore, commercial sugar can be easily used for root proliferation and reduction of cost during micropropagation. Among different carbon sources sulphur less sugar (295 mg) recorded significantly maximum fresh weight in comparison to hexose and pentose carbon sources used in this study which was found to be at par with sucrose (292 mg), commercial sugar (280 mg) and sugar cubes (280 mg). Sucrose (22.4 mg) resulted in production of significantly maximum dry weight, however, it was found to be at par with sulphur less sugar (20.7 mg) (Table 1).

Solidifying agents

The analysis of variance showed that solidifying agents significantly ($P \leq 0.05$) influenced the microplant height, number of leaves and nodes, inter nodal length and fresh as well as dry weight. Gelrite (9.4 cm) significantly enhanced the microplant height in comparison to agar purified (standard) followed by bacteriological grade agar

Table 1. Effect of different carbon sources on morphological characters of potato microplants.

Characters/carbon sources	C_1	C_2	C_3	C_4	C_5	C_6	C_7	$LSD_{0.05}$
Number of leaves	5.5^{a}	5.2^{b}	5.4^{ab}	4.3^{c}	4.4^{c}	5.2^{b}	1.9^{d}	0.27
Inter-nodal length (cm)	1.9^{a}	1.8^{ab}	1.8^{ab}	1.5^{c}	1.7^{bc}	1.8^{ab}	1.2^{d}	0.16
Number of roots	5.4^{a}	5.5^{a}	5.2^{a}	5.3^{a}	4.1^{b}	5.5^{a}	0.0^{c}	0.39
Root length (cm)	7.6^{a}	7.5^{a}	7.4^{a}	5.9^{c}	6.7^{b}	7.3^{a}	0.0^{d}	0.45
Fresh weight (mg)	295.0^{a}	292.0^{a}	280.0^{a}	192.0^{b}	202.0^{b}	280.0^{a}	61.0^{c}	18.81
Dry weight (mg)	20.7^{ab}	22.4^{a}	19.2^{b}	14.9^{c}	14.2^{c}	19.3^{b}	7.5^{d}	2.69

C_1: Sulphur less sugar; C_2: Sucrose; C_3: Commercial sugar; C_4: Fructose; C_5: Dextrose; C_6: Sugar cubes; C_7: Galactose. Values are mean of four cultivars, three microplantlets and six replicates (test tubes). Values superscripted with the same letter in each column are not significantly different on the basis of least significant difference (P≤0.05).

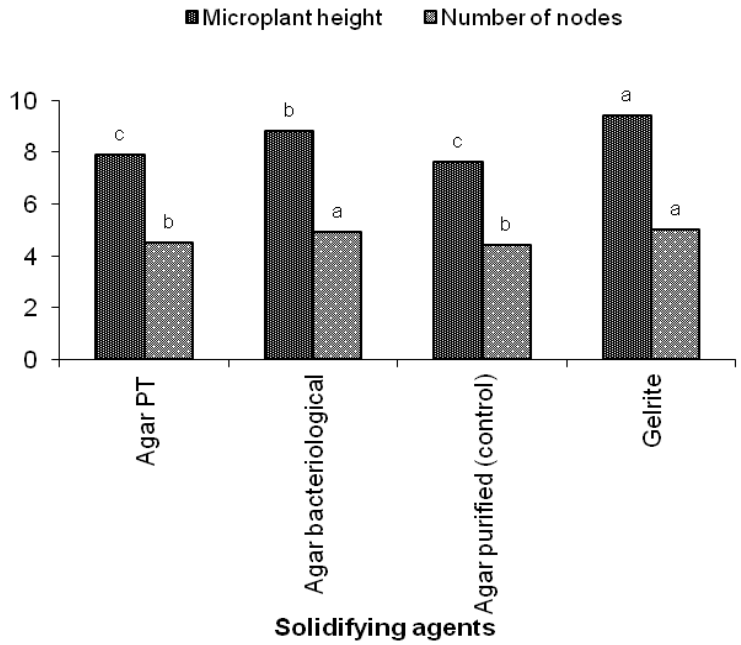

Figure 2. Effect of solidifying agents on micro-plantlet height and number of nodes.

(8.8 cm). Among the solidifying agents, gelrite significantly increased the number of leaves (4.6) and nodes (5.0) as compared to standard check however, it was found to be at par with bacteriological grade agar (Figure 2). Medium solidified with gelrite significantly increased the inter-nodal length (1.9 cm) followed by Agar PT (1.8 cm) and bacteriological agar (1.7 cm). Gelrite significantly increased the fresh (308.3 mg) as well as dry weight as compared to other solidifying agents (Table 2).

The accelerated growth in our study in the medium solidified with gelrite may be due to more availability of water in the media with gelrite, which was used in the lower concentration (Beruto et al., 1999). But Klimaszewska et al. (2000) reported that such effects were due to the physico-chemical characteristics of solidifying agent. The most prominent distinction among the solidifying agents which influences the *in vitro* growth characters is the water retention capacity of the gels and the availability of nutrients to the cultured tissue. Gelrite has been reported to yield better results than agar by many authors for regeneration and shoot multiplication (Henderson, 1987; Goldfarb et al., 1991; Van Ark et al., 1991; Welander and Maheswaran, 1992; Sharma et al., 2011). In addition to this, it was reported that agar from different sources contains various amounts of contaminants, whereas phytagel is free from phenolic compounds but has higher ash content than agar (Scherer et al., 1988). This may also be one of the reasons for reduced microplant growth with agar.

Table 2. Effect of different solidifying agents on morphological characters of potato microplants.

Characters/solidifying agent	S_1	S_2	S_3	S_4	$LSD_{0.05}$
Number of leaves	4.17^{c}	4.45^{ab}	4.22^{bc}	4.56^{a}	0.25
Inter-nodal length (cm)	1.77^{bc}	1.82^{b}	1.71^{c}	1.91^{a}	0.08
Number of roots	5.69	5.64	5.91	5.32	NS
Root length (cm)	5.71	5.99	5.94	5.83	NS
Fresh weight (mg)	221.72^{c}	260.86^{b}	223.26^{c}	308.25^{a}	21.09
Dry weight (mg)	16.43^{c}	18.73^{b}	16.04^{c}	21.74^{a}	1.90

S_1: Agar (PT); S_2: Agar (Bacteriological); S_3: Agar (Purified); S_4: Gelrite. Values are mean of four cultivars, three microplantlets and six replicates. Values superscripted with the same letter in each column are not significantly different on the basis of least significant difference (P≤0.05).

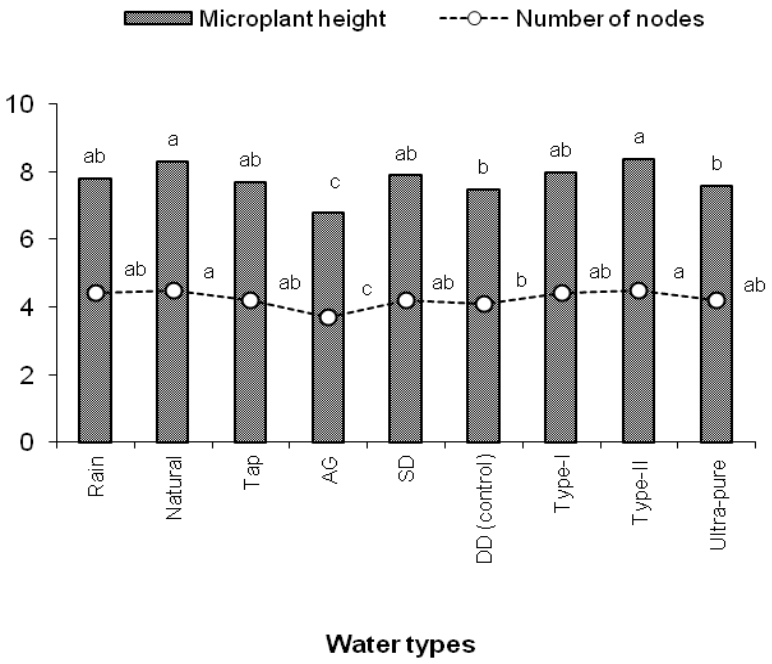

Figure 3. Effect of water types on micro-plantlet height and number of nodes.

Water types

Water sources significantly (*P*≤0.05) influenced the microplant height, number of leaves, number of nodes, inter nodal length, number of roots, root length and fresh as well as dry weight. Among different types of water, Type-II water recorded significantly maximum microplant height (8.4 cm) which was found to be at par with natural, rain, tap water, single distilled and Type-I water (Figure 2). Type-II recorded significantly maximum number of leaves (4.4) which was found to be at par with almost all other types of water apart from double distilled (standard) and aqua guard water. Type-II and natural water recorded significantly maximum number of nodes (4.5) which was found to be at par with rain, Type-I, tap, single distilled and ultra pure water (Figure 3).

Among different types of water, most of the water types resulted equal inter-nodal length. Ultra pure water recorded significantly maximum number of roots (7.1) in comparison to double distilled water (standard) whereas, aqua guard the minimum (4.4). Type-II water recorded significantly maximum fresh (230 mg) as well as dry weight (19.6 mg) whereas, rain water the minimum (Table 3). This may be due to the presence of bicarbonate and chlorides of calcium as well as magnesium in comparison with the distilled water where these elements are present in very trace amounts. Sunandakumari et al. (2004) also observed non significant differences in *M. piperita* cultured on media prepared with tap water + commercial sugar and double distilled water + tissue culture grade sucrose. However, we have not faced any problem of high rate of

Table 3. Effect of different water types on morphological characters of potato microplants.

Characters/water type	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	$LSD_{0.05}$
Number of leaves	4.30^{ab}	4.24^{ab}	4.05^{ab}	3.57^{c}	4.11^{ab}	4.00^{b}	4.20^{ab}	4.38^{a}	4.11^{ab}	0.36
Inter-nodal length (cm)	1.78^{ab}	1.73^{bc}	1.73^{abc}	1.65^{c}	1.81^{ab}	1.83^{a}	1.66^{c}	1.81^{ab}	1.82^{ab}	0.10
Number of roots	5.40^{b}	6.17^{b}	5.60^{b}	4.42^{c}	6.18^{a}	6.13^{b}	5.66^{b}	5.93^{b}	7.14^{a}	0.79
Root length (cm)	5.46	5.56	5.08	4.49	5.09	5.95	4.83	5.53	4.79	NS
Fresh Weight (mg)	193.4^{d}	218.2^{ab}	198.1^{cd}	192.5^{d}	202.2^{bcd}	210.1^{bcd}	216.1^{abc}	230.2^{a}	206.2^{bcd}	19.6
Dry Weight (mg)	15.88^{d}	18.69^{ab}	17.47^{bc}	18.13^{ab}	15.78^{d}	16.39^{cd}	18.71^{ab}	19.60^{a}	18.85^{ab}	1.57

W_1: Rain water; W_2: Natural water; W_3: Tap water; W_4: Aqua-guard water; W_5: Single distilled water; W_6: Double distilled water; W_7: Reverse osmosis /Type-I water; W_8: Electronically de-ionized/Type-II water; W_9: Ultra pure water. Values are mean of four cultivars, three microplantlets and six replicates. Values superscripted with the same letter in each column are not significantly different on the basis of least significant difference (P≤0.05).

Figure 4. Growth of potato micro-plantlets on standard and low cost carbon source.

precipitation in the solution before or after adding agar or solidifying agent for preparation of medium as observed by Das and Gupta (2009) therefore, boiling of tap water was not required.

The cost of production for one litre of micro propagation medium using different components that was attributed to the cost of plantlet production was compared. The results stated that the cost of production of plantlets in micro propagation medium could be reduced from 59 to 68% by using different substitutes. The highest reduction of cost (68%) could be noticed when all the three components tap water, commercial sugar and agar (bacteriological) were together being substituted in place of double distilled water, laboratory grade sucrose and agar (purified), respectively.

From the present study, it was inferred that the analytical grade (AR) sucrose can be successfully replaced by an ordinary commercial sugar, which is 5 to 6 times cheaper than the sucrose (Figure 4). Similarly, for preparation of media, we can use clean tap water instead of double distilled/ultrapure water which will reduce the investment on costly apparatus as well as on electricity (Figure 5). For solidifying the media, we can replace the Agar (purified) with agar ((bacteriological) or gelrite/phytagel, though the unit cost of gelrite/phytagel is more than agar but the quantity of gelrite/phytagel used

Figure 5. Growth of potato micro-plantlets on standard and low cost water source.

Figure 6. Growth of potato micro-plantlets on standard and low cost solidifying agents.

for solidifying unit quantity of media is much less (25%) and will lead to save 43 to 52% cost on solidifying agent (Figure 6). In short, easily available low cost alternatives presented in this work allow a low cost strategy for successful micropropagation of potato without compromising on quality of plants.

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