Potato J (2012) 39 (2): 166-172

EFFECT OF CARBON SOURCES AND EXPLANTS ON *IN VITRO* MULTIPLICATION OF POTATO

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ABSTRACT: In order to reduce the *in vitro* production cost, a study was conducted to evaluate the efficacy of two carbon sources *viz*., sucrose and commercial sugar on *in vitro* multiplication rate of fifteen potato cultivars using two types of explants. Significant response of carbon sources, explants and cultivars was observed on different morphological characters of *in vitro* plantlets. Sucrose as a carbon source resulted in a significant increase in the number of nodes in Kufri Badshah, Kufri Chipsona-3, Kufri Giriraj, Kufri Himsona and Kufri Pukhraj which ultimately influences the multiplication rate. However, number of nodes was not affected significantly with the source of carbon in a majority of other potato cultivars *viz.,* Kufri Anand, Kufri Bahar, Kufri Chandramukhi, Kufri Chipsona-1, Kufri Girdhari, Kufri Himalini, Kufri Kanchan, Kufri Lauvkar, Kufri Pushkar and Kufri Sutlej. Use of double node cuttings as explant source resulted in a significant increase in the number of nodes in Kufri Himalini and Kufri Himsona only, whereas, in all other cultivars both the explants *viz*., double as well as single node performed equally. Thus, in the majority of potato cultivars commercial sugar can be used successfully for reducing the cost of production of *in vitro* potato plantlets and single node as explants for increasing the multiplication rate without affecting the quality.

KEY WORDS: carbon source, cultivars, explant, *in vitro*, potato.

INTRODUCTION

Potato is an important food crop in India as well as in the world (Scott and Suarez, 2012). India has seen a revolution in potato production during recent years (Scott and Suarez, 2011). However, we still need to do a lot in the front of enhancing potato productivity in the country. Unavailability of healthy seed potato has largely been reported as the principal reason for low potato productivity in India. This problem can be alleviated by adoption of hi-tech seed potato production through micro-propagation since one of the advantages of micropropagation is the faster multiplication of healthy seed material (Coleman *et al*., 2001). *In vitro* growth is affected by many factors (Miller *et al*., 1985), one of which is the type of exogenous carbon source added to the medium which acts as energy source and the other is type of explant, which serves as base for the development of plantlet. Micropropagation has become a routine procedure, but the high cost involved has prevented laboratories for benefiting from tissue culture technology (Kodym and Zapata-Arias, 2001). Sucrose has been considered the most common carbon source used in plant tissue culture due to its efficient uptake (Shimon *et al.,* 2000; Yu *et al*., 2000; Sima and Desjardins, 2001).

In micropropagation media, chemicals and gelling agents accounts for 85% of cost while, sucrose alone accounts for about 14% of total cost. The cost of analytical/laboratory grade sucrose is much higher than the commercial grade sugar. To benefit from direct use of tissue cultured material in developing countries, the cost of commercial micropropagation is to be reduced drastically without compromising on the quality and multiplication rate of propagules (Miller *et al*., 1985). To cut down the cost of micropropagation, alternatives to

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chemicals of high purity in media preparation are used in various laboratories, Chandra *et al.* (1992) used ordinary sugar during *in vitro* microtuber production while Ganapathi *et al.* (1995) used the commercial grade sugar in place of analytical grade sucrose during mass multiplication of banana without any significant change in the frequency of shoot formation.

Although during autoclaving, both dissociate into glucose and fructose, even then during mass multiplication of disease free stock we observed different responses of potato genotypes towards carbon sources. However, in potato micropropagation no attempt has been made till date against this economic backdrop. Therefore, in the present study, an attempt was made to identify the efficient source of carbon to reduce the cost of media as well as to use single node segments as explants for the effective utilization of healthy and precious mother plantlets during subsequent sub-culturing to hasten the multiplication rate.

MATERIAL AND METHODS

The study was carried out at Central Potato Research Institute, Shimla during 2009-10 and 2010-11 with fifteen tetraploid (2n = 4x = 48) potato (*Solanum tuberosum* L. ssp. *tuberosum*) cultivars belonging to different maturity groups. The cultivars used for the study were Kufri Anand (KAN), Kufri Badshah (KBD), Kufri Bahar (KBR), Kufri Chandramukhi (KCM), Kufri Chipsona-1 (KC1), Kufri Chipsona-3 (KC3), Kufri Girdhari (KGD), Kufri Giriraj (KGR), Kufri Himalini (KHM), Kufri Himsona (KHS), Kufri Kanchan (KKN), Kufri Lauvkar (KLV), Kufri Pushkar (KPK), Kufri Pukhraj (KPR) and Kufri Sutlej (KST). Three single or double node cuttings dissected essentially from middle portion of the micro-plants were cultured per test tube (25 × 150 mm) containing 13 cm^3 semi-solid (7.0 g/dm^3) Murashige and Skoog (1962). The MS medium supplemented with 30 g/dm^3 either sucrose or commercial sugar, 4.19 µM D-calcium pantothenate, NAA 0.05 µM and GA_3 0.29 µM. The experiment was carried out in a factorial (2 × 2 × 15) completely randomized design (CRD) with fifteen genotypes over a period of 28 days. Each treatment comprised of three replicates and each replicate consisted of four test tubes. The culture tubes were incubated under a 16 h photoperiod (irradiance of 60 µmol/m^2/s) 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) and fresh as well as dry mass (mg). 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, 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

The analysis of variance showed that cultivar had a major effect on all the characters studied. Carbon source significantly ($P \leq 0.05$) influenced the number of nodes, number of roots, root length and dry weight, whereas, nodal segment influenced the microplant height, number of nodes, number of roots and

fresh as well as dry weight significantly. Two way interaction between cultivar × carbon source was significant ($P \leq 0.05$) for all the characters suggesting that the effect of carbon source was not uniform over the cultivars. Whereas, the interaction of cultivar × type of nodal segment was significant only for number of leaves, number of nodes and inter nodal length. This indicated that the effect of explant source on these characters was cultivar dependent. In addition to this, two-way interaction between carbon source × type of nodal segment was significant only for inter nodal length and number of roots. However, there was no significant difference for any of the morphological characters in three-way interaction between cultivar × carbon source × type of explant.

Sucrose significantly enhanced the microplant height in Kufri Badshah (8.4 cm), Kufri Chipsona-3 (6.6 cm), Kufri Himalini (6.8 cm) and Kufri Lauvkar (6.8 cm), whereas, sugar did so in Kufri Bahar (9.0 cm) and Kufri

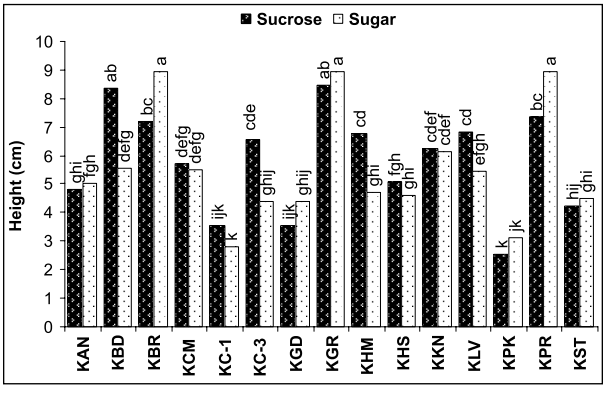

Fig. 1. Microplant height of different cultivars on MS medium with sucrose and sugar.

Pukhraj (9.0 cm) (**Fig. 1**).

Sucrose significantly increased the number of leaves in Kufri Chipsona-3 (6.5) and Kufri Himalini (5.7), whereas, sugar did so in Kufri Bahar (6.6), Kufri Kanchan (6.8) and Kufri

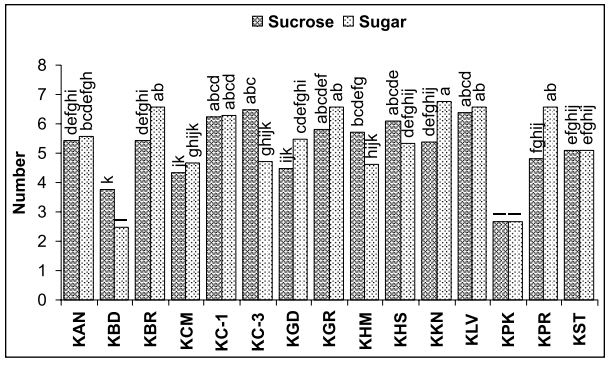

Fig. 2. Number of leaves of different cultivars on MS medium with sucrose and sugar.

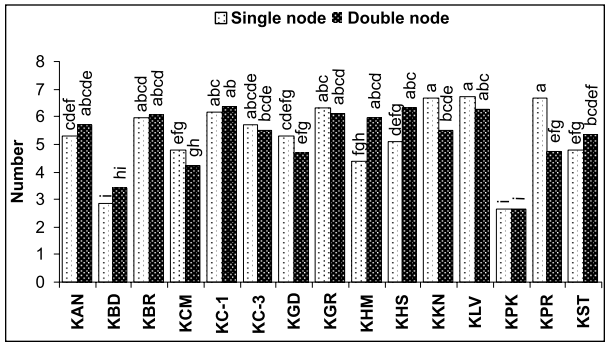

Fig. 3. Number of leaves of different cultivars grown with single and double node explants.

Pukhraj (6.6) (**Fig. 2**). Double node explant accelerated number of leaves in Kufri Himalini (6.0) and Kufri Himsona (6.3), while, single node did so in Kufri Kanchan (6.7) and Kufri Pukhraj (6.7) (**Fig. 3**). However, response of the carbon source and type of nodal segment was statistically at par in rest of the cultivars with respect to microplant height and number of leaves.

Sucrose proved beneficial in increasing the number of nodes in Kufri Badshah (5.7), Kufri Chipsona-3 (7.3) Kufri Giriraj (7.7), Kufri Himsona (7.0) and Kufri Pukhraj (7.4) in comparison to sugar (**Fig. 4**), while, other cultivars responded equally to both the carbon sources. Double node explant accelerated the number of nodes in Kufri Himalini (6.0) and Kufri Himsona (7.0) only (**Fig. 5**).

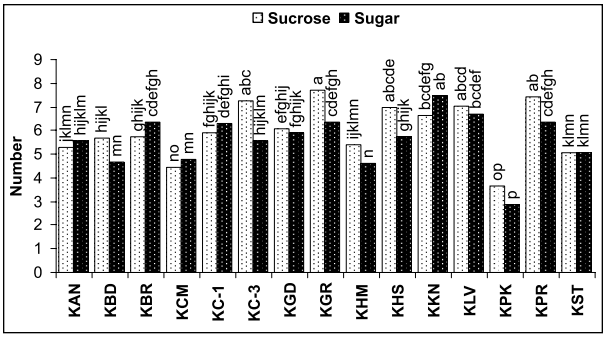

Fig. 4. Number of nodes of different cultivars on MS medium with sucrose and sugar.

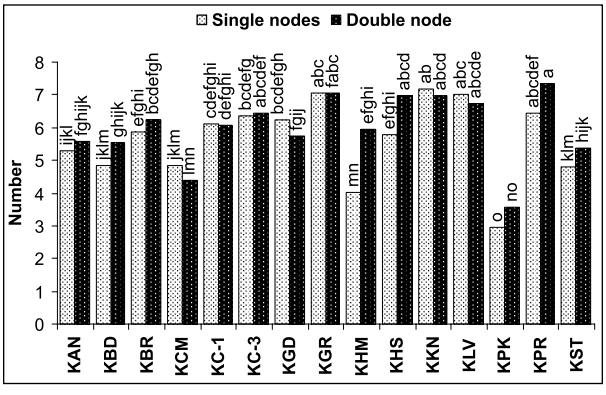

Fig. 5. Number of nodes of different cultivars grown with single and double node explants.

Sucrose significantly increased the inter-nodal length in Kufri Badshah (1.5 cm) and Kufri Himalini (1.4 cm), whereas, sugar did so in Kufri Giriraj (1.4 cm), Kufri Pushkar (1.1 cm) and Kufri Pukhraj (1.4 cm) (**Fig. 6**).

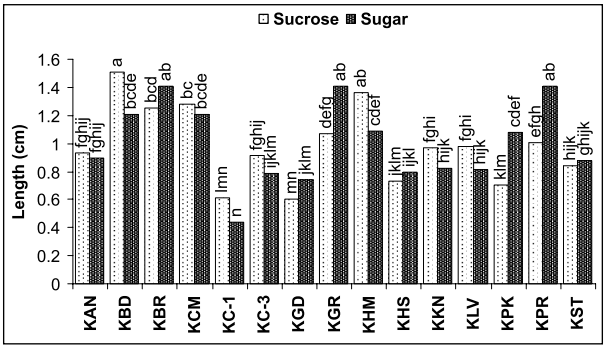

Fig. 6. Inter-nodal length different cultivars on MS medium with sucrose and sugar.

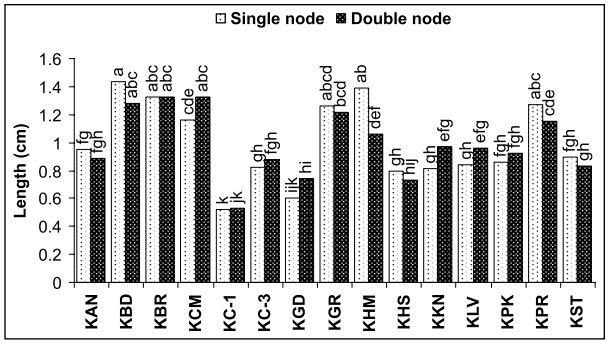

Fig. 7. Inter-nodal length different cultivars grown with single and double node explants.

Single node explant accelerated the inter-nodal length in Kufri Himalini only (1.4 cm) (**Fig. 7**). Differential response of cultivars on different carbon sources was observed with respect to microplant height, number of nodes and inter-nodal length. This may be due to genotypic effect which has already been reported in potato (Gopal *et al.*, 2001; Sharma *et al.*, 2011; Venkatasalam *et al.*, 2011).

The interaction effect of genotype with carbon source was noticed for many morphological characters, some cultivars performed better in sucrose, some in sugar and some in both. Better response of some cultivars in sucrose, may be due to the increased availability of carbon source in the form of purified sucrose that increases the intracellular sucrose concentration. Sucrose is also reported to stimulate the *in vitro* growth of different crop species as a result of more negative water potential in the medium (Lipavska and Verugdenhil, 1996; Riek *et al.*, 1997; Ebrahim *et al.*, 1999). Beside 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). Blanc (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. While, Sunandakumari *et al.* (2004) reported that *Mentha piperita* L cultured on media prepared with tap water and commercial sugar vs. double distilled water with tissue culture grade sucrose did not show any difference. Besides this, commercial sugar is impure sucrose and may contain some other substances which may be suitable for tissue culture (Hossain *et al.*, 2005).

Sucrose significantly increased the number of roots in Kufri Badshah (10.6), whereas, sugar did so in Kufri Bahar (8.5), Kufri Chandramukhi (10.7), Kufri Giriraj (8.4),

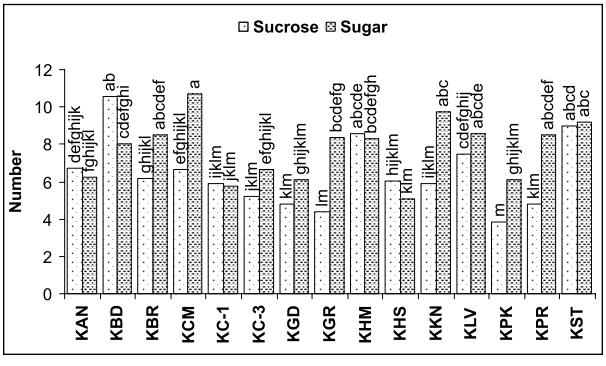

Fig. 8. Number of roots of different cultivars grown on MS medium with sucrose and sugar.

Kufri Kanchan (9.7) and Kufri Pukhraj (8.5) (**Fig. 8**).

Sugar significantly influenced the root length in Kufri Anand (6.1 cm), Kufri Bahar (6.3 cm), Kufri Chandramukhi (5.9 cm), Kufri Girdhari (6.1 cm), Kufri Himsona (8.7 cm), Kufri Pushkar (5.6 cm) and Kufri Sutlej (5.7 cm), whereas, sucrose did so in Kufri Himalini (6.8 cm) only (**Fig. 9**). In our study, sugar accelerated the number of roots as well as root length in several cultivars as compared to sucrose. This may be due to the decreased availability of carbon source with sugar in the medium that increased the number of

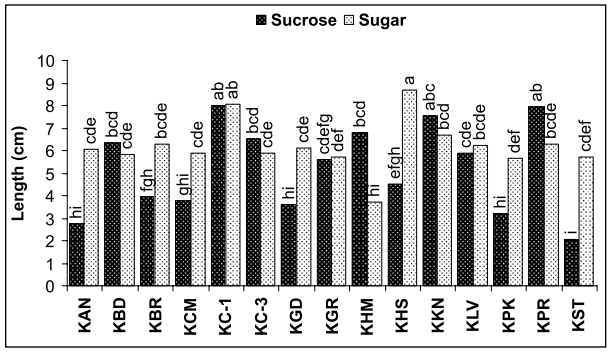

Fig. 9. Root length of different cultivars on MS medium with sucrose and sugar.

roots and root length. Inhibitory effect of higher concentration of sucrose, fructose and glucose as well as prolific rooting at lower concentration has also been reported earlier (Shatnawi *et al.*, 2006; Younas *et al.*, 2008).

Sucrose resulted in a significant increase

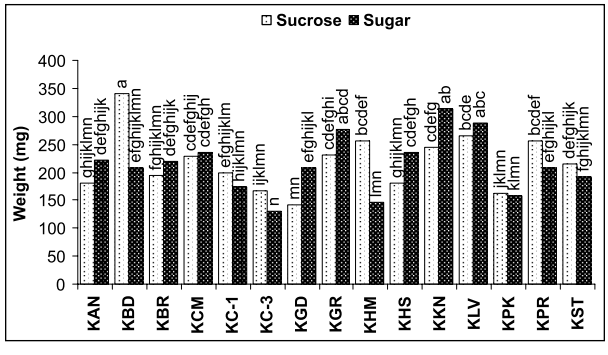

Fig. 10. Fresh weight of different cultivars in MS medium with sucrose and sugar.

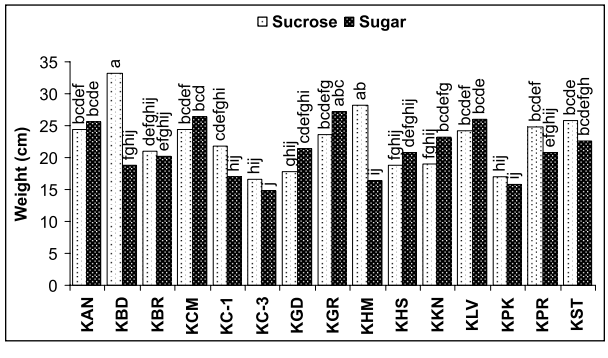

Fig. 11. Dry weight of different cultivars on MS medium with sucrose and sugar.

in the fresh weight in Kufri Badshah (340 mg) and Kufri Himalini (257 mg), while, sugar did so in Kufri Girdhari (209 mg) and Kufri Kanchan (313 mg) (**Fig. 10**). Sucrose significantly influenced the dry weight in Kufri Badshah (33 mg) and Kufri Himalini (28 mg) (**Fig. 11**).

In general, double node explants increased microplant height, number of nodes, roots and fresh as well as dry weight of the plantlets. This might be due to the early initiation of roots from lower node that is inserted inside the medium and development of shoots from the other upper axillary bud above the medium. In case of single node cutting, the root development is from inter nodal segment which must be resulting in the delayed root initiation. The increased number of roots in double nodal cutting might also enhance the nutrient uptake by plantlets and that resulted in improved morphological characters (Sharma *et al.,* 2011). However, in specific the interaction effect of cultivar with type of explant was noticed only for number of leaves, number of nodes and inter-nodal length. Accordingly, few cultivars performed better in double node explant, few in single node explant and majority in both which may be due to genetic effect.

The interaction effect of cultivar with carbon source was noticed for all the morphological characters like microplant height, number of leaves, nodes, roots, inter-nodal length, root length, fresh and dry weight. Some cultivars performed better in sucrose, some in sugar and some in both. Genetically controlled *in vitro* response has already been reported previously (Amirouche *et al.,* 1985; Miller *et al.,* 1985; Gopal *et al.,* 2001). Therefore, the results showed that the carbon sources and its uptake by the cultivars differ and selection of carbon source does play an important role in regulation of growth.

CONCLUSIONS

In conclusion, the results showed that some of the potato cultivars were sensitive to type of carbon source as well as type of explant. Some of the cultivars performed better in sucrose, few in sugar and majority in both. As said earlier, in case of direct organogenesis, optimum number of nodes with optimum inter nodal length is one of the most advantageous character which ultimately decides the rate of multiplication. In most of the cultivars, above said characters were not affected by type of carbon source and explant. Therefore, sugar and single node explants can be used during *in vitro* multiplication of majority of the potato cultivars which will also reduce the cost of multiplication without affecting the multiplication rate and single node explant will double the multiplication rate. The approximate cost of carbon source per liter can be reduced from ₹ 18 to 1.5 by using commercial sugar taking current market price of sucrose ₹ 600/kg and ₹ 50/kg for commercial grade sugar. However, sucrose can be used for accelerating the *in vitro* multiplication rate in Kufri Badshah, Kufri Chipsona-3, Kufri Giriraj, Kufri Himsona and Kufri Pukhraj and double node as explant in Kufri Himalini and Kufri Himsona.

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MS received: 18 October 2012; Accepted: 6 November 2012