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INFLUENCE OF GELLING AGENTS AND NODES ON THE GROWTH OF POTATO MICROPLANT

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ABSTRACT: The objective of this study was to identify the suitable gelling agent and size of nodal segment in order to enhance *in vitro* multiplication rate of different Indian potato genotypes. The results showed that MS medium solidified with agar increased the number of leaves, nodes and roots as well as root length, whereas phytagel enhanced the microplant height, internodal length, fresh and dry weight of microplants of potato. Media solidified with agar enhanced the *in vitro* multiplication rate in Kufri Bahar, Kufri Chandramukhi, Kufri Chipsona-1, Kufri Giriraj, Kufri Himsona, Kufri Kanchan, Kufri Lauvkar, Kufri Pukhraj and Kufri Sutlej, whereas phytagel was better for Kufri Anand and Kufri Chipsona-3. However, genotypes Kufri Badshah and Kufri Himalini performed equally in both the gelling agents. It is clear from the present findings that agar is better than phytagel for increasing the *in vitro* multiplication rate in majority of Indian potato cultivars. Though there was a significant difference among type of nodes, double node significantly enhanced the microplant height, number of roots and fresh as well as dry weight. But there was no significant difference on morphological character like number of nodes and internodal length which would influence the multiplication rate. Hence, single node can be used for accelerating the multiplication rate.

INTRODUCTION

In vitro responsiveness is a heritable character and the existence of wide genetic variation for this trait is now well documented in many plant species (3, 4). Tissue culture response is affected by many factors. In the present study, we studied the effect of genotypes, gelling agent and size of nodal segment on micropropagation performance of thirteen potato genotypes. Two types of gelling agents are used normally in tissue culture. One is agar which is most frequently used solidifying agent. It is commercially extracted from red algae genera *Gelidium gracillaria, plerocladia.* The main reasons for its wide use are stability, high clarity, non-toxicity and metabolic inactiveness (13). However, some investigators have raised doubts about the non-toxic nature of agar (1, 12). Other gelling agent used is phytagel (Gelidium), a gellan gum isolated from the bacterium *Pseudomonas elodea*. The size of nodal segment used is either with single or double node. Objective of our study was to identify suitable gelling agent and type of node for different genotypes in order to enhance the *in vitro* multiplication rate.

MATERIAL AND METHODS

The study was undertaken at Central Potato Research Institute, Shimla during the year 2008-2009. Thirteen potato (*Solanum tuberosum* L.) genotypes were used in the present study. These represent a wide genetic base and were selected because of their contrasting *in vitro* response. Already maintained disease-free plantlets were used for experimentation (16). The experiment was conducted with single and double nodes which were essentially derived from middle part of the microplants in order to maintain node homogeneity.

The basic Murashige and Skoog (15) medium supplemented with sugar 30 g/l and 4.19 µM D-calcium pantothenate was used. The growth regulators used were NAA 0.05 µM and GA_3 0.29 µM. Before autoclaving the pH of medium was adjusted to 5.8 using 3 M NaOH and the medium was solidified with 0.7 % agar (Himedia, India) or 0.2 % phytagel (Gaisson,

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USA). The medium was poured in test tubes, sealed with cotton plugs and autoclaved at 121° C for 22 min. Three cuttings of either single or double node segments were cultured per tube (25 × 150 mm) containing 13 ml of MS medium. Cultures were incubated in 16 h photoperiod under cool florescent lamp at 22 ± 1 °C (50-60 µ mol/ m^2/ s light intensity) in culture room.

After twenty eight days of culturing, observations were recorded on microplant height, number of leaves, nodes and roots as well as root length, fresh and dry weight. As there were three microplants per culture tube, data were recorded and averaged. All the microplants from the culture tube were taken out with the help of forceps, agar was removed with water and excess moisture of plantlets was absorbed with tissue paper. In case of number of roots, primary roots were counted, as there was secondary branching too. Root length was recorded for the longest root. Fresh weight of all the three plantlets was taken by using electronic balance and these were dried at 80°C for 48 h in the hot air oven and dry weight was taken after bringing it to room temperature.

Experimental design and statistical analysis: The experiment was conducted in a factorial (13 × 2 × 2) completely randomized design. Each treatment comprised four replicate test tubes and the experiment was repeated once. As the experiment was conducted twice, data were pooled over individual experiments. The three-way analysis of variance was done using the software AGRES and means were separated according to the least significant differences (LSD) at 0.05 level of probability.

RESULTS

The analysis of variance showed that genotypes and gelling agents had a major effect on all the characters studied, while that due to node was significant (p = 0.05) only for microplant height, number of roots, fresh and dry weight. Two way interactions between genotype x gelling agent was significant (p = 0.05) for all the characters, suggesting that the effect of gelling agent was not uniform over the genotypes. Whereas the interaction of genotype x node was significant only for number of roots, root length and fresh weight. This indicated that the effect of node on these characters was not consistent. However, there was no significant differences for any of the morphological characters in two-way interaction between gelling agent x node and three-way interaction between genotype x gelling agent x node.

Microplant height: Microplant height was significantly influenced by genotype, gelling agent, node and interaction of genotype with gelling agent. Phytagel enhanced the microplant height in Kufri Anand (8.2 cm), Kufri Bahar (10.5 cm), Kufri Chandramukhi (6.7 cm), Kufri Chipsona-3 (6.7 cm) and Kufri Himalini (9.5 cm) whereas, agar did so in Kufri Pukhraj (9.0 cm) (**Fig. 1**).

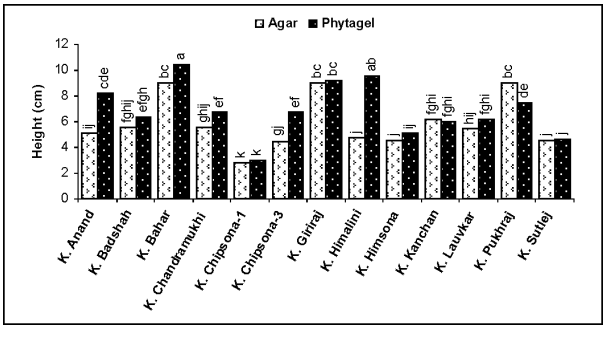

Fig. 1. Microplant height in genotypes on medium with agar and phytagel.

Number of leaves: Number of leaves per microplant was affected by genotype, gelling agent and their interaction. Agar significantly increased the number of leaves in Kufri Bahar (6.6), Kufri Chipsona-1 (6.3), Kufri Giriraj (6.7) and Kufri Pukhraj (6.6) (**Fig. 2**).

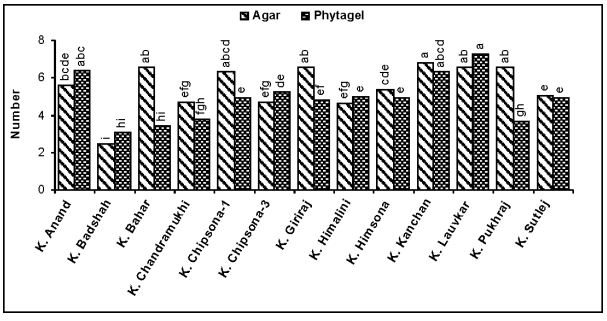

Fig. 2. Number of leaves in genotypes on medium with agar and phytagel.

Number of nodes: Number of nodes differed due to genotype, gelling agent and their interaction. Phytagel statistically increased the nodes in Kufri Anand (6.5), whereas agar in Kufri Bahar (6.4), Kufri Chandramukhi (4.8), Kufri Chipsona-1 (6.3) and Kufri Pukhraj (6.4) (**Fig. 3**).

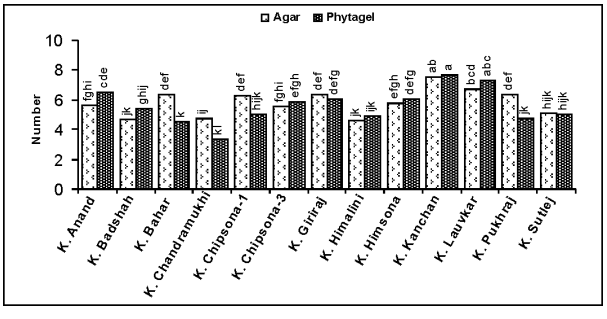

Fig. 3. Number of nodes in genotypes on medium with agar and phytagel.

Inter-nodal length: Significant differences were observed due to genotype, gelling agent and their interaction for inter-nodal length of microplants. Phytagel significantly increased the inter-nodal length in Kufri Anand (1.3 cm), Kufri Bahar (2.3 cm), Kufri Chandramukhi (2.1 cm), Kufri Chipsona-3 (1.2 cm) and Kufri Himalini (2.0 cm) (**Fig. 4**).

Number of roots: Significant variation in number of roots per microplants was observed for genotype, gelling agent and size of nodal segment, interaction of genotype with gelling

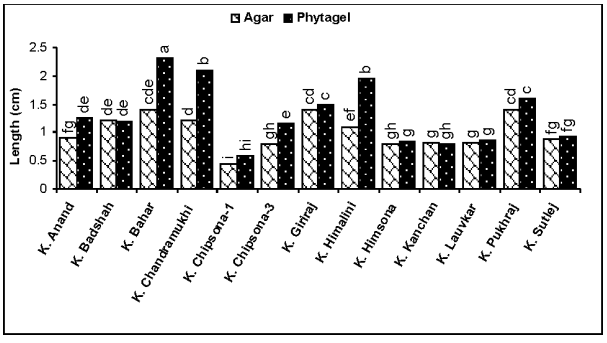

Fig. 4. Internodal length in genotypes on medium with agar and phytagel.

agent and genotype with size of node also affected number of roots. Agar significantly increased the number of roots in Kufri Bahar (8.5), Kufri Chandramukhi (10.7), Kufri Giriraj (8.4), Kufri Kanchan (9.7) and Kufri Pukhraj (8.5) (**Fig. 5**). Double node accelerated number of roots in Kufri Bahar (7.0), Kufri Chandramukhi (11.3), Kufri Giriraj (8.5) and Kufri Himalini (10.1) (**Fig. 6**).

Root length: Root length of potato microplants differed due to genotype, interaction of genotype with gelling agent and genotype with type of node. Phytagel significantly increased the root length in Kufri Himalini (6.9 cm), whereas agar did so in Kufri Sutlej (5.7 cm) (**Fig. 7**). Root length was significantly enhanced by single node in Kufri Bahar (7.9 cm) and double node in Kufri Chandramukhi (6.0 cm) (**Fig. 8**).

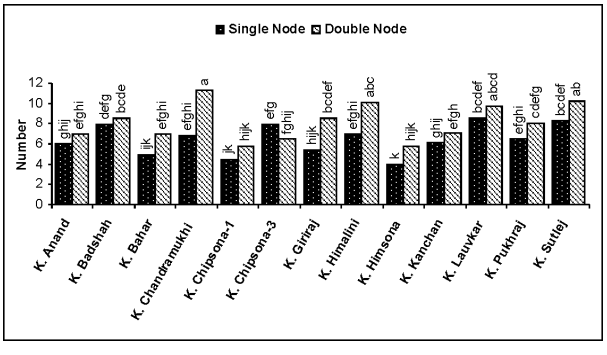

Fig. 5. Number of roots in genotypes on medium with agar and phytagel.

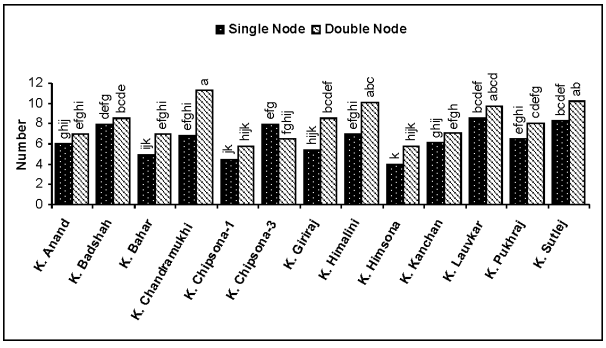

Fig. 6. Number of roots in genotypes in single and double nodal segment.

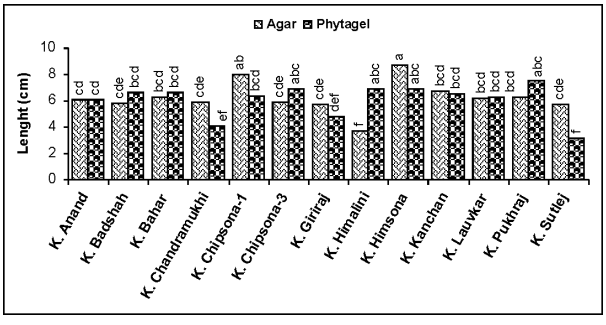

Fig. 7. Root length in genotypes on medium with agar and phytagel.

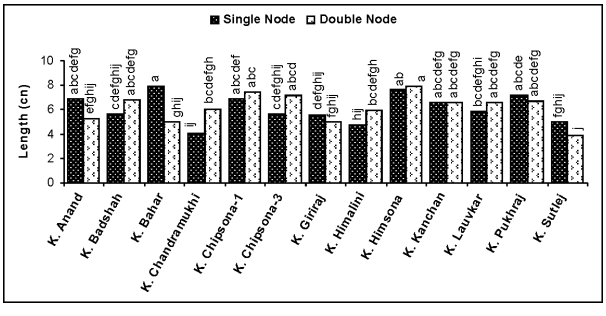

Fig. 8. Root length in genotypes in single and double nodal segment.

Fresh weight: Fresh weight of potato microplant differed due to genotype, gelling agent, node, interaction of genotype with gelling agent and genotype with size of nodal segment. Phytagel increased the fresh weight in Kufri Badshah (345.73 mg), Kufri Bahar (399.98 mg), Kufri Chipsona-3 (299.52 mg) and Kufri Himalini (322.16 mg) (**Fig. 9**). Single node enhanced the fresh weight in Kufri Bahar (346.67 mg), whereas double node did so in Kufri Chandramukhi (265.18 mg), Kufri Kanchan (391.96 mg) and Kufri Lauvkar (356.22 mg) (**Fig. 10**).

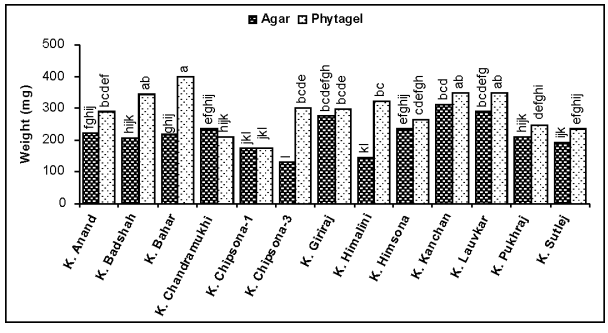

Fig. 9. Microplant fresh weight in genotypes on medium with agar and phytagel.

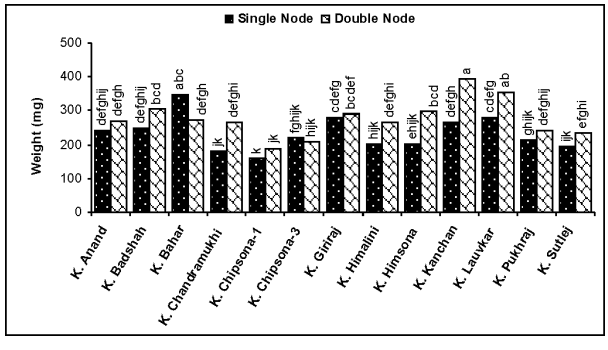

Fig. 10. Microplant fresh weight in genotypes in single and double nodal segment.

Dry weight: Dry weight of potato microplants differed due to genotype, gelling agent, node and interaction of genotype with gelling agent. Phytagel significantly increased the dry weight in Kufri Anand (34.30 mg), Kufri Badshah (34.90 mg), Kufri Bahar (35.24 mg), Kufri Chipsona-3 (33.98 mg), Kufri Himalini (35.88 mg), Kufri Kanchan (33.55 mg) and Kufri Lauvkar (35.27) (**Fig. 11**).

DISCUSSION

Phytagel resulted in production of longer shoots, internodes and increased the fresh

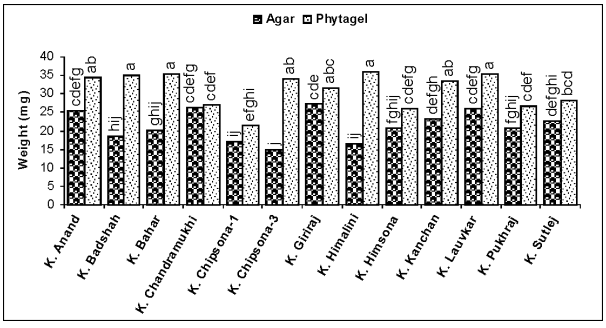

Fig. 11. Dry weight in genotypes on medium with agar and phytagel.

and dry weight of microplantlets as compared to agar. This may be of great advantage in slow growing genotypes having shorter inter-nodal length that restricts the *in vitro* multiplication rate. The accelerated microplant growth and inter-nodal length may be due to more availability of water in the media with phytagel, which was used in the lower concentration (2). But Klimaszewska *et al*. (11) reported that such effects were due to the physico-chemical characteristics of gelling agent. The most prominent distinction among the gelling 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 in case of regeneration and shoot multiplication (10, 5, 18, 20). 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 (17). This may also be one of the reasons for reduced microplant growth in agar. The media solidified with agar enhanced the number of leaves, nodes, roots and root length which was in contrast to the results obtained by Veramendi *et al.* (19). This is of advantage to the fast growing genotypes. The increase in number of roots and root length may be due to less availability of water and nutrients in the media solidified with agar. Double nodal cutting increased microplant height, number of 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 upper auxillary bud above the medium. In case of single-nodal cutting, the root development is from inter-nodal segment which delays the root initiation. The increased number of roots in double nodal cutting might also help to enhance the nutrient uptake by microplants and that resulted in improved morphological characters of plantlets. The interaction effect of genotype with gelling agent was noticed for many morphological characters including microplant height, number of leaves, number of nodes, number of roots, inter-nodal length, root length, fresh and dry weight. Some genotypes performed better in phytagel, some in agar and some in both. Genetically controlled *in vitro* response in potato has also been reported previously (1, 8, 14, 9).

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