Potato J (2016) 43 (1): 38-44

INFLUENCE OF ANTIBIOTICS ON *IN VITRO* MORPHOLOGICAL CHARACTERS OF RECALCITRANT POTATO CULTIVAR KUFRI JYOTI

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ABSTRACT: The effect of three different antibiotics *viz*., carbenicillin (50, 75, 100 and 125 mg/l), cephotaxime (100, 150, 200 and 250 mg/l) and kanamycin (30, 40, 50 and 60 mg/l) against bacterial contaminants (*Bacillus pumilus*) and *in vitro* shoot growth of potato (*Solanum tuberosum* L.) recalcitrant cultivar Kufri Jyoti was evaluated. Efficacy of antibiotics to control bacterial contaminant differed greatly; cephotaxime and carbenicillin were found to be more effective as compared to kanamycin. The type of antibiotics and their concentrations affected the growth, morphological characters of the microplant and the bacterial contaminations. The medium containing carbenicillin @ 75 mg/l and cephotaxime @ 150 mg/l significantly accelerated the microplant height, number of leaves, nodes and roots; root length and fresh as well as dry mass. For most of the microplant growth parameters there was no significant difference between carbenicillin @ 50 mg/l and cephotaxime @ 100 mg/l and negligible bacterial contamination was observed at these concentrations. Higher concentration beyond this level however, had detrimental effect on the growth and morphology of microplants. The cost of incorporating carbenicillin was double as compared to cephotaxime per liter of culture medium.

KEYWORDS: Antimicrobial agents, growth parameters, micro-propagation

INTRODUCTION

Potato is a vegetatively propagated crop however, recent advance in tissue culture techniques especially *in vitro* micropropagation has facilitated the production, multiplication and maintenance of disease free potato clones. This technique has contributed towards the propagation of large number of plants from small piece of stock plant in relatively short period of time (Daniel and Linberge, 1998). Almost all micro- propagating laboratories around the world propagate plants with 'latent bacteria'; they are harmless to plants in normal culture conditions *in vitro* and are also often transferred to *in vivo* (Venkatasalam *et al*., 2013). *Pseudomonas*, *Flavobacterium* and *Blastobacter* have been reported on the wet surfaces of air conditioning systems. The laboratory walls and tables also harboured most of the contaminating microbes (Odutayo *et al*., 2007). These microbes can be transmitted through infected people and indoor air. The nutrient media on which the plant tissue is cultivated has all the conditions which are favorable for microbial growth. Micropropagation managers learned that it is not economically viable to eliminate all the microorganisms during micropropagation (Herman, 1989). The concerned microbes are those which are pathogenic or inhibit propagation efficiency *in vitro* by competing adversely with culture for nutrients. The presence of these microbes in cultures usually results in plant mortality, variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting (Kane, 2003). Surface sterilization could only kill the superficial microbes (George, 1993). Whereas, endophytic bacterial contamination is an important problem in plant tissue culture (Kneifel and Leonhardt, 1992) and cannot be eliminated with any surface sterilization technique,

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hence require antibiotic therapy (Mathias *et al*., 1987). The therapeutic chemicals particularly antibiotics capable of eradicating/ suppressing both epiphytic and endophytic bacterial contaminants are now available. Antibiotics also have been reported to control the growth of bacteria in cultures without eliminating them, so that contamination could reappear after transfer of plant material to media without antibiotics. However, antibiotics were incorporated in media to act as anticontaminants (Schaffner, 1979). Antibiotics *viz.* carbenicillin, cephotaxime and kanamycin are commonly used for controlling bacterial contamination in culture medium. Various authors have described the antibiotic sensitivity of bacteria isolated from plant-tissue cultures (Scortichini and Chiariotti, 1987; Chanprame *et al*., 1996; Estopa *et al*., 2001). Accordingly, the present study was carried out to investigate the effect of antibiotics in culture medium on growth and morphological characters of recalcitrant potato cultivar Kufri Jyoti.

MATERIALS AND METHODS

The experiment was carried out at Central Potato Research Institute, Shimla with the objective to control bacterial contaminants from the *in vitro* culture in order to improve the response of plantlets of recalcitrant cultivar Kufri Jyoti. Double node cuttings derived from bacteria contaminated *in vitro* microplants were used as explant for this study. The basic MS medium (Murashige and Skoog, 1962) was modified and supplemented with 0.1 M sucrose and 4.19 µM D-calcium pantothenate. In order to control the bacterial growth, hasten the plantlet growth and rate of multiplication, the explants were cultured on above said medium supplemented with three different antibiotics *viz*., carbenicillin, cephotaxime and kanamycin (HiMedia). Each antibiotic was used in different concentrations; i) carbenicillin (50, 75, 100 and 125 mg/l), ii) cephotaxime (100, 150, 200 and 250 mg/l) and iii) kanamycin (30, 40, 50 and 60 mg/l). The pH of the medium was adjusted to 5.8 with 0.1 N NaOH and 0.2% phytagel was used for solidification of the medium. The medium was transferred to one litre bottles @ 800 ml per bottle and autoclaved at 121°C (15 psi) for 38 min. The sterilized medium was cooled down, required quantity of carbenicillin and kanamycin were added in the autoclaved medium under aseptic conditions after filter sterilization whereas, cephotaxime was added without filter sterilization, as it could not be passed through 0.22 µm syringe filter. After through mixing, the medium was poured in sterile test tubes @ 13 ml per test tube. Henceforth, two infected double-node explants were inoculated per tube and culture tubes were incubated at 22 ± 1°C under 16 h photoperiod of approx. 50 µmol/ m^{-2}/s light intensity in the non-hermetic culture room. After twenty-eight days of culturing, observations were recorded on microplant height (cm), number of leaves, nodes and roots, inter-nodal and root length (cm) as well as the fresh and dry mass (mg). As there were two microplants per culture tube, data was recorded for each microplant and averaged. In case of number of roots, only primary roots were counted. Root length was recorded for the longest root. Fresh and dry mass was taken for all the plantlets. For dry mass, micro-plants from each test tube were dried at 80°C for 48 h in the hot air oven and dry mass was recorded after bringing to room temperature.

Experimental design and statistical analysis

The experiment was conducted using completely randomized design (CRD). Each treatment comprised of five replicates and each replicate consisted of four test tubes.

As the experiment was conducted twice, data were pooled over two environments and were statistically analyzed using analysis of variance and means were separated according to the least significant differences (LSD) at 0.05 level of probability using the software AGRES.

RESULTS AND DISCUSSION

Before experimentation, the presence of endophytic bacteria in the experimental material was confirmed on potato-dextrose-agar medium and the same was identified as *Bacillus pumilus* (unpublished data). Analysis of variance showed that mean squares were significant due to different antibiotics and its concentrations for the morphological characters like height, number of leaves and roots, root and internodal length as well as fresh and dry matter of microplants of recalcitrant potato cultivar Kufri Jyoti after 28 days of sub-culturing. The microplant height was significantly influenced by the antibiotics and its concentrations. The number of leaves was significantly influenced by the antibiotics and its concentrations. Among different antibiotics, maximum number of leaves per microplant was observed with the amendment of antibiotic carbenicillin @ 75 mg/l (6.0) in the culture medium, which was at par with cephotaxime @ 100 and 150 mg/l (4.8 and 5.0) and carbenicillin @ 50 mg/l (4.6). Increasing the concentration beyond this significantly reduced the number of leaves. However, in rest of the concentration of different antibiotic number of leaves was found to be at par with control. Antibiotic carbenicillin @ 75 mg/l slightly promoted the inter-nodal length (1.0 cm) in comparison to control (0.8 cm) which was at par to most of the test concentrations of different antibiotics. The fresh and dry matter of microplants was statistically influenced by different antibiotics and its concentrations. The fresh and dry matter was significantly higher in medium supplemented with 150 mg/l (647.86 and 62.36 mg) cephotaxime which was at par with cephotaxime @ 100 mg/l (534.52 and 55.8 mg). Carbenicillin @ 75 mg/l significantly increased the fresh and dry matter of microplants as compared to control whereas; kanamycin in different concentrations significantly reduced the fresh and dry matter of microplants as compared to control (**Table 1**).

Among different antibiotics, carbenicillin (86-100%) and cephotaxime (88-100%) significantly reduced the contamination percent as compared to kanamycin (20-80%). Antibiotic carbenicillin @ 75 mg/l significantly increased the microplant height (4.8 cm) and number of nodes (4.8) which was at par with cephotaxime @ 150 mg/l. Increasing the concentration beyond this significantly reduced the microplant height and number of nodes. However, different antibiotics and their concentrations were at par with control with respect to both micro-plant height and number of nodes except carbenicillin @ 50 mg/l (3.5 cm) with respect to only micro-plant height (**Fig. 1**).

The number of roots was significantly influenced by different antibiotics and its concentrations. Statistically, higher number of roots was observed in the microplantlets grown on the media supplemented with cephotaxime @ 100 mg/l (10.4) which was at par with cephotaxime @ 150 mg/l (9.4) and carbenicillin @ 7 5 mg/l (7.0). However, kanamycin in different concentrations inhibited the root development of microplant. The root length was statistically influenced by different antibiotics and its concentrations. Significantly longer root was observed in the media supplemented with carbenicillin @ 75 mg/l (14.9 cm) which was at par with carbenicillin @ 50 mg/l (14.6 cm) and cephotaxime @ 100 and 150 mg/l (14.04 and 14.46 cm) (**Fig. 2**).

Table 1. Effect of antibiotics on growth and morphological characters of microplants of recalcitrant potato cultivar Kufri Jyoti.

S. No.	Name of antibiotic	Dose (mg/l)	Microplant growth parameters averages			
			No. of leaves	Inter-nodal length (cm)	Fresh mass (mg)	Dry mass (mg)
1.	Carbenicillin	50	4.6	0.8	372.06	35.10
2.	Carbenicillin	75	6.0	1.0	452.12	41.96
3.	Carbenicillin	100	3.8	0.9	267.96	25.40
4.	Carbenicillin	125	3.2	0.7	161.36	16.96
5.	Cephotaxime	100	4.8	0.7	534.52	55.80
6.	Cephotaxime	150	5.0	0.8	647.86	62.36
7.	Cephotaxime	200	3.8	0.6	333.20	33.14
8.	Cephotaxime	250	3.8	0.6	306.62	32.00
9.	Kanamycin	30	3.6	0.8	169.06	18.88
10.	Kanamycin	40	3.8	0.7	222.82	26.24
11.	Kanamycin	50	3.2	0.9	266.22	35.36
12.	Kanamycin	60	3.0	0.7	176.80	20.62
13.	Control		3.2	0.8	300.00	34.44
CD (0.05)			1.4**	0.2*	125.57**	16.15**

* Significant at pÝ0.05** Significant p≤0.01

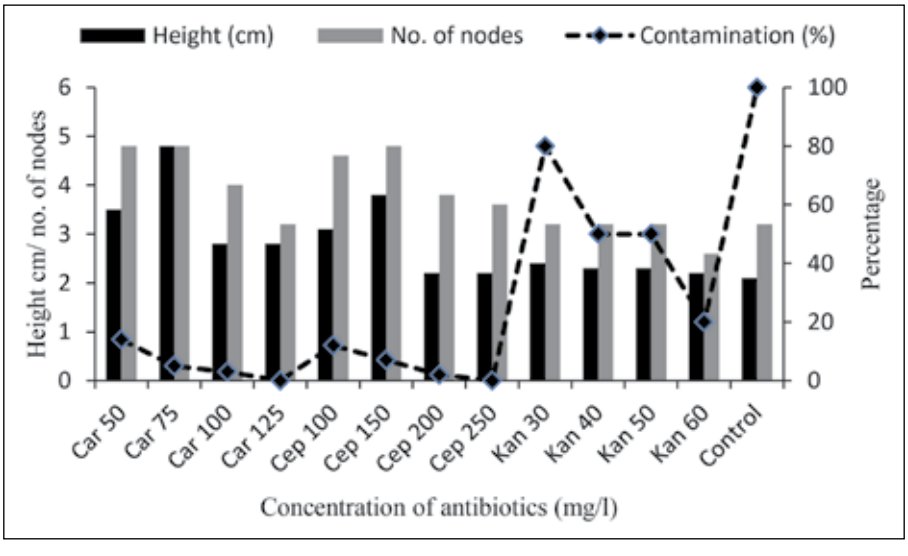

Fig.1. Effect of antibiotics on microplant height, number of nodes and contamination percent of recalcitrant potato cultivar Kufri Jyoti.

It was observed that out of three antibiotics used, cephotaxime and carbenicillin at different concentrations controlled the bacterial contaminants *in vitro* cultures. Maximum control of bacterial growth to the tune of 90-100% was noticed with carbenicillin @ 50-75 mg/l and cephotaxime @ 100-150 mg/l. Similar results were also obtained by Wojtania *et al*. (2005). In antibiotic sensitivity test, antibiotics inhibit the bacterial cell wall synthesis, bacterial protein synthesis and DNA replication (Pollock *et al*., 1983; Quesnel and

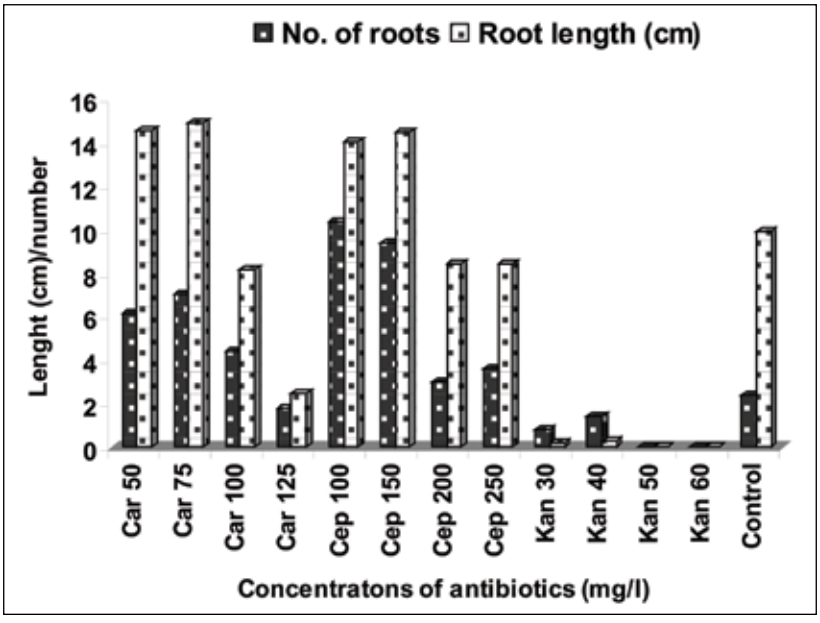

Fig. 2. Effect of antibiotics on number of roots and root length of recalcitrant potato cultivar Kufri Jyoti.

Russell, 1983). Carbenicillin and cephotaxime are extensively used to eliminate *Agrobacterium* from the culture medium (Tang *et al*., 2000; Alsheikh *et al*., 2002).

It was found that cephotoxime and carbenicillin were not only effective in exhaling the bacteria but also augmented the growth of the culture. It may be due to cephotaxime in culture being converted by cell metabolism to an unknown compound which has phytohormonal activity (Borelli *et al*., 1992). Cephotaxime has also been reported to interfere with ethylene (Pius *et al*., 1993). It has been demonstrated by Holford and Newbury (1992) that carbenicillin breaks down to give physiologically active auxin phenylacetic acid as a result the normal endogenous auxin/cytokinin balance in the explants would be changed. Yu and Wei (2008) found more vigorous growth of plantlets on medium supplemented with cephotaxime as compared to control. Mathias and Boyd (1986) also observed that callus growth and organogenesis was promoted in wheat cultured on the media supplemented with cephotaxime and carbenicillin. In apple, cephotaxime successfully promoted regeneration and stimulated shoot growth and development (Yepes and Aldwinckle, 1994). Carbenicillin has been reported to enhance multiplication in *Anthirrhinum majus* (Holford and Newbury, 1992).

In addition to this, carbenicillin @ 75 mg/l positively influenced all the morphological characters whereas, at higher concentration a drop in the growth pattern of plantlets was observed. Yu and Wei (2008) reported that 100 mg dm^{-3} carbenicillin strongly inhibited plant regeneration, with regeneration capacity decreasing to 31.4% while in in case of cephotaxime @ 150 mg/l it was found to be the optimum. Both antibiotics have been known to have plant hormone-like effects on cultured plant tissues (Nauerby *et al*., 1997). In the present study, it was found that carbenicillin produced more healthy plants as compared to cephotaxime. Shoots grown in the presence of cephotaxime tend to be shorter and have yellow leaves more often than shoots grown in the presence of

carbenicillin (Wojtania *et al*., 2005). This may be because carbenicillin was less toxic than the cephotaxime.

From the study, it can be concluded that antimicrobial agents like carbenicillin and cephotaxime can be incorporated in the micropropagation medium of potato to minimize microbial contaminants as well as for improving the vigour of microplantlets. In general, it was clear that carbenicillin up to 75 mg/l and cephotaxime up to 150 mg/l were the optimum and safe doses for *in vitro* potato micro-propagation. Cost of incorporating carbenicillin is ₹ 90 and cephotaxime ₹ 170 per litre of culture medium. Further studies on the effect of these agents on reduction of microbial contamination is required to be carried out. In general, all the concentrations of kanamycin showed destructive effect on almost all the morphological characters studied. This may be due to toxic effect of the concentrations used and it needs further investigation with lower concentration (Venkatasalam *et al*., 2013).

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MS received: 08 December 2014; Accepted: 21 June 2016