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Effect of plant growth promoting bio-agents (*Bacillus* sp) on the production of potato (*Solanum tuberosum*) mini-tubers in north-western Himalaya

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ABSTRACT

Two plant growth promoting bio-agents, viz. (*Solanum tuberosum* L.), *Bacillus cereus* (B4) and *Bacillus subtilis* (B5) were evaluated for production of potato mini-tubers from micro-plants two potato cultivars, viz. Kufri Giriraj and Kufri Kanchan (red skin tubers) under polyhouse conditions during the autumn (off) seasons of 2008 and 2009. Treatment of micro-plants with both the bio-agents (B4 and B5) proved significantly effective in improving the per cent ground cover, height of plants as well as haulms weight/plant in both the potato cultivars over control. However, no significant effect of these bio-agents was observed on establishment of micro-plants as well as on the number of shoots and compound leaves per plant. B4 bio-agent was found significantly superior in improving the ground cover and haulms weight/plant over B5. Number and yield of mini-tubers/m^2 varied significantly between the cultivars and were significantly better with both the bio-agents over control.

Key words: *Bacillus* sp, Bio-agents, Micro-plants, Mini-tubers, Potato

Potato (*Solanum tuberosum* L.) needs about 15% of its area to produce sufficient seed tubers (Lommen 1995, Struik and Wiersema 1999) while for cereals only one thirtieth of their area is necessary for seed production. In conventional systems of seed potato production, seed potato tubers are utilized for seed multiplication and production (Struik and Wiersema 1999). This method has a number of disadvantages like slow multiplication, inefficiency, high risk of diseases and pests, and it involves a large number of field multiplications (Beukema and Van der Zaag 1990, Struik and Wiersema 1999). Ease of planting, uniformity of tubers and vigorous plant growth are advantages of utilizing seed tubers for potato multiplication (Gopal 2004).

Recently, some new rapid multiplication techniques (micropropagation or tissue culture) have been developed and they are used all over the world for the production of seed potatoes (Knutson 1988, Lommen and Struik 1994). Rapid multiplication of seed tubers can solve some of the problems associated with the conventional multiplication system (Lommen 1995, Struik and Wiersema 1999). A large number of virus-free potato micro-plants can be obtained from a single healthy plant by the micro-propagation technique (Khurana *et al.* 2003).

Among the various techniques available under micro-propagation, direct *in vivo* planting of micro-plants in a soil medium under aphid proof net/polyhouses is a well established method for the production of potato mini-tubers. Mini-tubers are small tubers of 5 – 25 mm in diameter and weight between 0.1 – 10 g and sometime higher. The number of mini-tubers can be 2 – 10 per plantlet and sometimes it can be more, depending on the mother plant management. Mini-tubers can be planted directly in the field.

The state and central seed production agencies of India are able to meet only 20–25% requirement of quality seed potato (Kumar *et al.* 2007). For bridging this wide gap, large-scale integration of conventional and innovative methods like micro-propagation at commercial level is needed for producing enough quantity of healthy seed tubers in minimum period of time (Pandey 2006). On these lines, production of potato mini-tubers in the high hills of Shimla (Kufri/Fagu, 2700 m above mean sea level) under the polyhouse conditions is carried out in the main (*Kharif*) season as well as in autumn (off) season. During the autumn season, the vegetative growth/vigour of plants raised from micro-plants is significantly low as compared to main (*Kharif*) season and it results in early tuberization with higher proportions of small sized mini-tubers.

Seed treatment with plant growth promoting bio-agents of *Bacillus,* viz. B4 (*B. cereus*) and B5 (*B. subtilis*) have been reported to improve productivity in conventionally grown potato crop in subtropical plains (Sunaina 2001, Bharti *et al.* 2006); Gujarat (Nandekar *et al.* 2006); eastern plains (Singh 2006) and in main (*Kharif*) season of high hills (Sood and Sharma 2001). But, no information is available on the use of these bio-agents on micro-plants for

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the production of potato mini-tubers. Keeping in view this, an attempt was made for improving the plant vigour, number and yield of potato mini-tubers with optimum size by using above said bio-agents on the cut-ends of micro-plants before planting in polyhouses during the off (autumn) season in the high hills.

MATERIALS AND METHODS

The experiment was conducted at Central Potato Research Station – Kufri (Fagu Unit) Shimla, HP, India, 2700 m above mean sea level during the autumn (off) seasons of 2008 and 2009 under polyhouse conditions in completely randomized block design (RBD 2 factorial) with three replications. Disease-free micro-plants of two potato varieties suited for hills, viz. Kufri Giriraj and Kufri Kanchan (red skin tubers) maintained and multiplied through shoot cuttings under micro-propagation (16/8 hr photoperiod) were used. Micro-plants with an age of 21 days in culture room followed by 10 days hardening under polyhouse conditions were used for planting. After cutting just above the media level, the cut ends of micro-plants were dipped for 10 minutes in 0.25% solution of B4 (*B. cereus*) and B5 (*B. subtilis*) preparations containing a potency of 10^8 colony farming units per gram. After surface drying under shade, two plantlets were planted per hill in 2.0 m long beds at 20 × 10cm spacing during the last week of September. The temperature range inside the polyhouse during the crop season varied from 1°C to 27°C.

Data on establishment of micro-plants at 30 days after planting (DAP), per cent ground cover at 50 DAP, plant height, number of shoots and compound leaves per plant at 75 DAP and haulms weight at 90 DAP, were recorded. Ground cover (%) was estimated with the help of a 50 × 50 cm grid with 100 equal compartments at two locations in each plot as described by Burstall and Harris (1983). Haulms weight per plant was also recorded. Mini-tubers obtained were graded in four grades, viz. >20g, 10-20g, 3-10g and <3g.

The average of two years data was analyzed statistically by applying the technique of analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Mean values were calculated and separated using F-test at 5% level of significance.

RESULTS AND DISCUSSION

Per cent establishment of micro-plants

Per cent establishment of micro-plants was significantly different among the potato cultivars (Table 1). Among the cultivars, average per cent establishment was significantly higher in Kufri Giriraj (96.8) than Kufri Kanchan (88.8). In Kufri Giriraj, per cent establishment of micro-plants was statistically on par with both the bio-agents but in Kufri Kanchan, B4 supported higher establishment of micro-plants (91.7%) than B5 (88.4%). The interaction between the cultivars and bio-agents for per cent establishment was positive indicating that the response of bio-agent varied with the cultivar. The varying response of bio-agents on the different potato cultivars can be attributed to the differences in their genetic make up. Poor response of bio-agent treatment in Kufri Giriraj over control can be attributed to the fact that this cultivar had better establishment even without the treatment of growth promoting bio-agents (96.7%) and thus the scope for further improvement in establishment becomes limited. Nandekar *et al.* (2006) have also reported no improvement in potato emergence with same bio-agents used on potato tubers in Satpura plateau of India.

Plant height

Both the bio-agents proved effective in improving the plant height significantly over control (Table 1). In potato cultivar, Kufri Giriraj both the bio-agents (B4 and B5) resulted in a significant increase in the plant height but in Kufri Kanchan only B4 bio-agent was found to be slightly superior over control. Plant height also varied significantly between the two cultivars and was more in Kufri Giriraj (16.2 cm) than Kufri Kanchan (13.8 cm). The interaction between the cultivars and bio-agents for plant height was also found to be significant.

Increase in plant height with the bio-agent treatment of micro-plants can be attributed to the colonization of developing roots of potato plants by the respective bacterial strains and resulted into higher root volume and root mass as observed by Sunaina *et al.* (2001) and increased root length by Bharti *et al.* (2006) in the potato crop raised with B4 and B5 treated seed tubers in sub-tropical plains.

In the present study, improvement in plant height with bio-agent treated micro-plants seems due to the same reasons for enhanced growth in roots leading to more absorption of nutrients from the rhizosphere.

Number of shoots and compound leaves per plant

The number of shoots per plant varied significantly between the cultivars with more shoots in Kufri Giriraj (2.14) than Kufri Kanchan (1.88), whereas, both the bio-agents could not improve the number of shoots/plant significantly (Table 2).

Number of compound leaves/plant followed a trend almost similar to the number of shoots. Compound leaves /plant were not influenced significantly neither with the genotype nor the bio-agents. Interactions between the cultivars and bio-agents for number of shoots and compound leaves/plant were positive (Table 2).

Similar, non-significant influences on the number of stems and compound leaves/plant with the application of same bio-agents on seed potato tubers of Kufri Badshah in conventionally grown potato crop have also been reported by Nandekar *et al.* (2006).

Per cent ground cover

Per cent ground cover was affected significantly by both the factors, viz. the bio-agents as well as the genotype. Perusal of data in Table 3 reveals that treatment with B4 bio-agent resulted in maximum ground cover in both the

Table 1 Effect of bio-agents (B4 and B5) on establishment of potato micro-plants and plant height

Variety	% Establishment				Height / plant (cm)			
	B4	B5	Control	Mean	B4	B5	Control	Mean
K. Giriraj	96.6	97.0	96.7	96.8	16.4	21.2	11.1	16.2
K. Kanchan	91.7	88.4	86.4	88.8	15.1	13.4	13.0	13.8
Mean	94.2	92.7	91.5		15.7	17.3	12.1	
CD (P=0.05)								
Cultivar ©		4.7				1.9		
Bio-agent (B)		N.S.				2.3		
C × B		8.2				3.3		

Table 2 Effect of B4 and B5 treatment of potato micro-plants on number of shoots and compound leaves per plant

Variety	No. of shoots/ plant				No. of compound leaves/plant			
	B4	B5	Control	Mean	B4	B5	Control	Mean
K. Giriraj	2.00	2.23	2.20	2.14	12.4	14.9	14.5	13.9
K. Kanchan	1.97	1.90	1.77	1.88	16.8	15.8	13.9	15.5
Mean	1.98	2.07	1.98		14.6	15.4	14.2	
CD(P=0.05)								
Cultivar ©			0.25			N.S.		
Bio-agent (B)			N.S.			N.S.		
C × B			0.44			3.9		

Table 3 Effect of B4 and B5 treatment of micro-plants on per cent ground cover and haulms weight per plant.

Variety	% Ground cover (50 DAP)				Haulms weight/plant (g)			
	B4	B5	Control	Mean	B4	B5	Control	Mean
K. Giriraj	100.0	95.8	71.9	89.2	16.6	12.6	6.5	11.9
K. Kanchan	66.9	64.6	60.8	64.1	16.2	10.1	12.1	12.8
Mean	83.5	80.2	66.3		16.4	11.3	9.3	
CD(P=0.05)								
Cultivar ©			6.2			N.S.		
Bio-agent (B)			7.6			2.7		
C × B			10.8			3.8		

cultivars which was closely followed and statistically at par with B5 bio-agent. Per cent ground cover was significantly higher in Kufri Giriraj (89.2%) than Kufri Kanchan (64.1%). Interactions between the cultivars and bio-agents for per cent ground cover were significant.

Improvement in ground cover with plant growth promoting bio-agents can be attributed to the same reasons as for plant height. It seems due to the better expansion of leaf lamina in the bio-agent treated crop than control as the number of leaves and compound leaves did not increase with the treatment of bio-agents in the present study. Differences between the cultivars for per cent ground cover with the bio-agent treatment again indicate the differential response of different genotypes to such bio-agents. Bharti *et al.* (2006) have also reported better development of crop canopy (leaves) in the potato cultivar Kufri Ashoka raised from B4 and B5 treated tubers during the winter season of sub-tropical plains. The exact mode of action of these bio-agents used in the study is not known but it is likely that they produce growth promoting hormones as well as mobilize nutrients from the rhizosphere for better crop canopy.

Haulms weight/plant

Weight of haulms/plant was significantly influenced by the bio-agents but was at par between the cultivars (Table 3). Similar to ground cover, haulms weight per plant was significantly higher with B4 bio-agent in both the cultivars. B5 bio-agent was found to be superior for the haulms weight over control in Kufri Giriraj only. Interactions between the cultivars and bio-agents for haulms weight per plant were positive.

More haulms weight per plant with the bio-agents indicate better plant vigour in the form of size and thickness of leaf laminas over control as the number of stems and compound leaves were not affected significantly in same treatments. Similar to our findings, Bharti *et al.* (2006) have also reported that dry matter content in stems and leaves is significantly higher in the potato plants raised from B4 and B5 treated seed tubers. They also found that pattern of dry matter allocation was influenced by the bio-agent treatments as a result of which the leaf/stem dry weight ratio was remarkably lower in B4 and B5 treated plants compared with untreated control.

Number of mini-tubers/m²

Number of mini-tubers /m^2 was significantly influenced by the bio-agents as well as the genotype. Treating micro-plants with both the bio-agents resulted in a significant increase in the number of mini-tubers per unit area over control (Table 4). Improvements in number of mini-tubers were almost same with both the bio-agents. Irrespective of bio-agent, number of mini-tubers was more (269.8 /m^2) in potato cultivar Kufri Kanchan than 207.5/m^2 in Kufri Giriraj (Table 4). Interactions between the cultivars and bio-agents for number of mini-tubers per m^2 were significant.

Increase in number of mini-tubers with the application of bio-agents can be attributed to the better foliage growth (ground cover/ haulms weight). It must have resulted in increased photosynthetic rates coupled with higher nutrient uptake compared to control. Similar improvements in the number of potato tubers in conventional grown crop with the bio-agents treated seed tubers have also been reported (Kumar *et al.* 2001). Saikia and Deka (2006) have also reported non-significant differences between both the bio-agents (B4 and B5) for the number of potato tubers when bio-agent (B4 and B5) treated seed tubers were planted.

Yield of mini-tubers/m²

Yield of mini-tubers was significantly higher with both the bio-agents over control (Table 4). Within bio-agents, significantly more yield was recorded with B4 (2.60 kg / m^2), than B5 (2.18 kg /m^2) which was also superior to control (1.59 kg /m^2). B4 bio-agent resulted in significantly higher yield of mini-tubers/m^2 in both the cultivars. Within cultivars, yield of mini-tubers was more in Kufri Giriraj (2.61 kg /m^2) than Kufri Kanchan (1.63 kg /m^2). Interactions between the cultivars and bio-agents for yield of mini-tubers were significant.

Higher potato yields with bio-agent treated micro-plants can be attributed to the corresponding higher number of mini-tubers obtained with the same treatments over control. Superiority of B4 (*B. cereus*) for the better potato productivity in the present study can be attributed to the corresponding higher ground cover and haulms weight produced by this bio-agent, which must have resulted in higher production of food material per unit area. Earlier workers, viz. Singh (2006) for Kufri Jyoti; Bharti *et al.* (2006) for Kufri Ashoka and Sunaina *et al.* (2001) for Kufri Bahar have also demonstrated the better efficacy of B4 over B5 for the potato productivity when grown from treated seed tubers during winters in sub-tropical plains, whereas, Saikia and Deka (2006) reported significantly higher potato productivity with *B. subtilis* (B5) over *B. cereus* (B4) in potato cultivar Kufri Anand grown from treated seed tubers during winter. Our results for potato yields are in confirmation to maximum number of earlier studies.

Number and yield of large size (>20g) mini-tubers

Both the bio-agents resulted in a significant increase in the number and yield of large size (>20g) mini-tubers. Between bio-agents, B4 (*B. cereus*) was found to be significantly superior than B5 (*B. subtilis*) in improving the number as well yield of large size mini-tubers in both the potato cultivars (Fig 1). Between the cultivars, number and yield of >20g mini-tubers /m^2 was significantly more in Kufri Giriraj (46.2 and 1.53 kg /m^2 respectively) than Kufri Kanchan (20.8 and 0.67 kg /m^2 respectively).

Superiority of *B. cereus* (B4) in improvement of size of mini-tubers can be attributed to the higher potato yields produced by this bio-agent over B5. Better ground cover with B4 might have resulted in higher photosynthetic activity and ultimately in higher proportions of large sized mini-tubers in the produce.

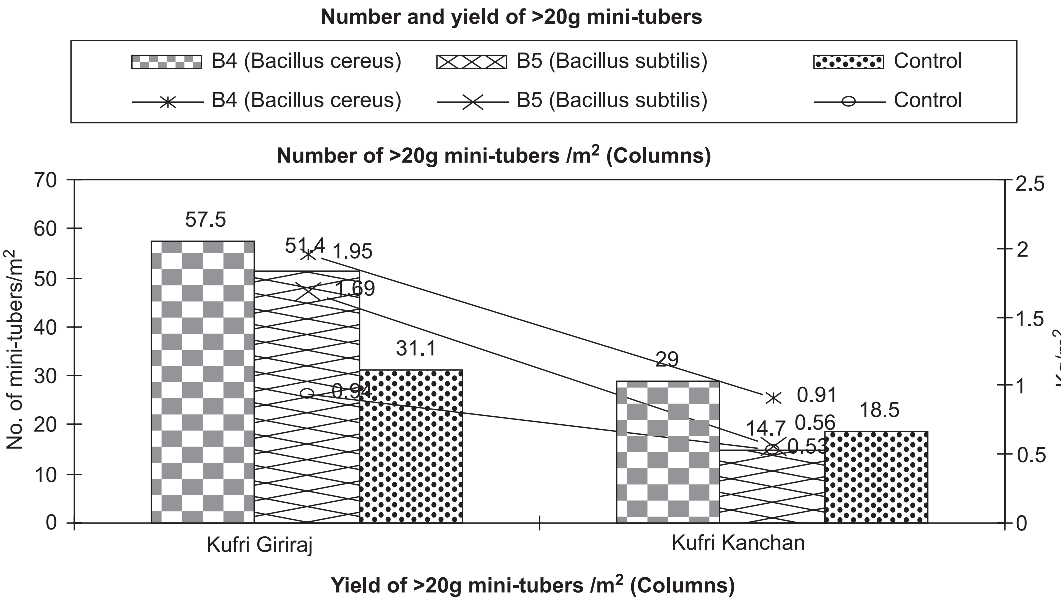
Number and yield of under size (<3 g) mini-tubers

Number and yield of under size (<3 g) mini-tubers followed a trend just opposite to the large sized (>20g) mini-tubers (Fig 2). Both the bio-agents though resulted in an increase in the number and yield of small sized (<3g) mini-tubers/m^2 over control but the differences were significant with B5 (*B. subtilis*) only.

Between the cultivars, number and yield of <3g mini-tubers were significantly more in Kufri Kanchan (148.7 and 0.131 kg /m^2 respectively) than Kufri Giriraj (48.1 and 0.036 kg /m^2 respectively).

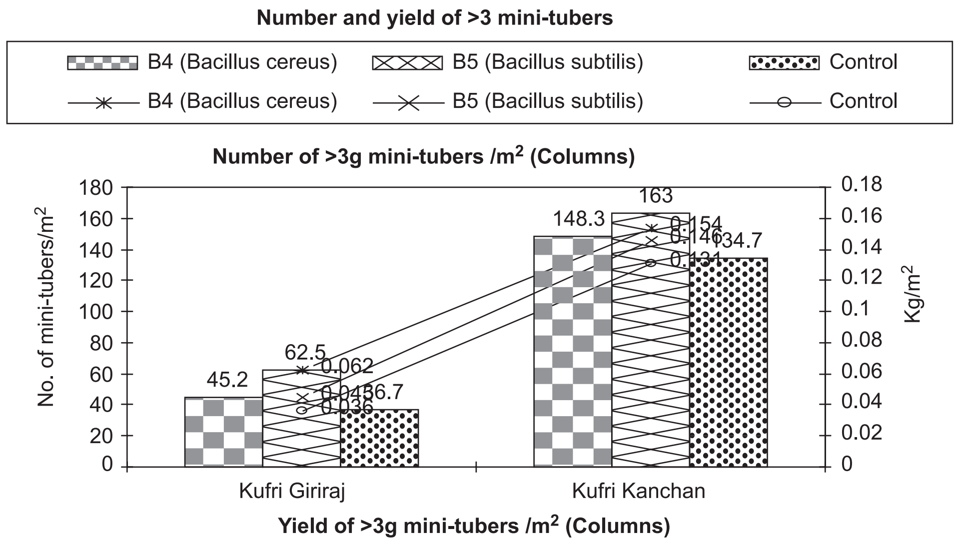
Table 4 Effect of treating the micro-plants with B4 and B5 on the number and yield of potato mini-tubers

Variety	Total number of mini-tubers/m²				Total yield of mini-tubers/m² (Kg)			
	B4	B5	Control	Mean	B4	B5	Control	Mean
K. Giriraj	227.3	235.6	159.6	207.5	3.17	2.88	1.77	2.61
K. Kanchan	294.6	272.5	242.2	269.8	2.03	1.47	1.40	1.63
Mean	261.0	254.0	200.9		2.60	2.18	1.59	
CD(P=0.05)								
Cultivar ©			19.8			0.20		
Bio-agent (B)			24.3			0.25		
C × B			34.3			0.35		



CD 0.05		
Cultivar ©	3.2	0.16
Bio-agent (B)	3.9	0.20
C X B	5.5	0.28

Fig 1. Effect of treating the potato micro-plants with B4 and B5 bio-agents on the number and yield of large sized (>20g) mini-tubers.



CD 0.05		
Cultivar ©	17.9	0.019
Bio-agent (B)	22.0	0.023
C X B	31.1	0.032

Fig. 2. Effect of B4 and B5 treatment of potato micro-plants on the number and yield of under sized (<3g) mini-tubers.

Average number of mini-tubers /plant

Number of mini-tubers per plant followed a trend just similar to total number of mini-tubers /m^2. Average number of mini-tubers per plant was significantly more with both the bio-agents, i.e. B4 and B5 over control and the differences between the bio-agents were not significant (Table 5). Between the cultivars, average number of mini-tubers/plant was significantly higher in Kufri Kanchan (9.30) than Kufri Giriraj (4.29). Interactions between the cultivars and bio-agents for the average number of mini-tubers/plant were significant (Table 5).

Average yield of mini-tubers/plant

Both the bio-agents resulted in a significant increase in

Table 5 Effect of B4 and B5 treatment of potato micro-plants on the average number and average yield of mini-tubers per plant

Variety	Average number of mini-tubers/ plant				Average yield of mini-tubers/ plant (g)			
	B4	B5	Control	Mean	B4	B5	Control	Mean
K. Giriraj	4.67	4.87	3.33	4.29	65.6	59.3	36.6	53.8
K. Kanchan	9.87	9.43	8.60	9.30	68.0	50.9	49.4	56.1
Mean	7.27	7.15	5.97		66.8	55.1	43.0	
CD(P=0.05)								
Cultivar ©			0.95		N.S.			
Bio-agent (B)			1.16		7.9			
C × B			1.64		11.2			

the yield of mini-tubers per plant over control, i.e. a trend similar to total yield /m^2. Between the bio-agents, average yield of mini-tubers was significantly better with B4 bio-agent than B5 (Table 5). In potato cultivar Kufri Giriraj, average yield of mini-tubers was significantly higher with both the bio-agents, viz. B4 and B5 over control, but in Kufri Kanchan, only B4 bio-agent resulted in a significant increase in the average yield of mini-tubers. Yield of mini-tubers per plant was at par between both the potato cultivars. The interactions between cultivars and bio-agents were significant for yield of mini-tubers per plant.

CONCLUSION

The results reveal that a dip application of the cut-ends of potato micro-plants with plant growth promoting bio-agents of *Bacillus* sp., viz. B4 (*B. cereus*) and B5 (*B. subtilis*) at a concentration of 0.25% for 10 minutes prior to planting in poly-houses is quite effective in improving the number and yield of potato mini-tubers/m^2 as well as in improving the proportions of large sized (>20g) mini-tubers during the off (autumn) season in high hills of north-western Himalaya. The technique will be an aid in successful raising of potato mini-tubers during the second (off) season in high hills and thus in rapid multiplication of elite planting material.

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