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Effect of dormancy breaking chemicals on microtuber production potential under *in vivo* conditions of central India

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Abstract: The present study was carried out at ICAR -Central Potato Research Station, Gwalior during 2012-13 to assess the effect of dormancy breaking chemicals, their dip duration and microtuber size on growth and yield parameters. The three different size >4-6mm, >6-8mm and >8mm of variety Kufri Sindhuri were given dip treatment with six types of growth regulators/ dormancy breaking chemicals *viz* 1ppm gibberelic acid, 0.5 ppm gibberllic acid, 1% thiourea, 0.5 % thiourea, 1ppm gibberllic acid + 1% thiourea and 0.5 ppm gibberllic acid + 0.5 % thiourea along with water control for 30 min, 45 min and 60 min. All the treatments exhibited better growth and yield parameters over water control but significantly best at 5% was 0.5 ppm gibberllic acid treatment for growth parameters and 0.5 ppm gibberllic acid + 0.5 % thiourea treatment for yield parameters. All the dip duration 30 min, 45 min and 60 min had not significant at 5% level effect for both growth and yield parameters. The larger sized micro-tuber (>8 mm) showed significantly superior plant emergence, plant height, number of compound leaves per plant, number of stems per plant both at 50 and 75 days after planting followed by 4-8 mm grade and <4 mm grade micro-tubers. Similar trend was observed for all the yield parameters. The overall mean finding indicates that micro-tubers treated with 0.5 ppm gibberllic acid in combination with 0.5 % thiourea gave highest yield (226.0 q/ha tuber), among micro-tuber size of >8mm (295.0 q/ha tuber) and among dip duration 30 min (206.67q/ha) and 45 min (210 q/ha) resulted in significantly higher yield parameters under nucleus seed production in *in vivo* conditions of central India.

Keywords: Dormancy breaking chemicals, *In vivo* condition, Microtuber, Yield

INTRODUCTION

Microtubers are produced *in vitro* in a plethora of different growing systems with varying environment, media constituents, and storage intervals. Accordingly, microtubers come in different sizes, have different dormancy requirements, and differ widely in relative growth potential and productivity (Badoni and Chauhan, 2009). The tubers falling in between 4.0 and 6.0 mm considered as small, 6.1–8.0 mm as medium and above 8.1 mm as large. The average fresh weight of small, medium, and large microtubers were 0.18, 0.29, and 0.54 g, respectively (Park *et al*., 2009). Microtubers with 3 g weight sprout after 10 days, while microtubers with 375 and 750 mg sprout after 15 and 13 days respectively indicating dormancy requirement varies with size of the microtuber (Struik and Lommen., 1999). Microtubers can be used in the net house to produce minitubers in Generation-0 or they can be used directly in the field for nucleus seed production generation-1 in potato. In the micro-tuber based seed potato production system, about 30-40% micro-tubers are lost during storage (due to drying and rottage) and the emergence of remaining micro-tubers is only 45-50% (Venkatasalam *et al*., 2011). The function of minitubers resulting from microtubers is affected by many factors like species, microtuber size and length of its dormancy (Bolandi *et al*., 2011).

Minitubers dormancy period can be removed by setting the store temperature, cutting the tubers, and treating by chemicals. Treating minitubers with chemicals is a safe and confident method. Among the chemicals applied for breaking down the potato nodes dormancy, one can refer to GA_3, thiourea, ethylene, ethyl bromide, and carbon disulphide (Otroshy and Struik, 2006). Thiourea treatment is not only more efficient to break dormancy but it increases also sprouts number, comparing to other chemicals (IAA and GA_3) (Germchi *et al*., 2010). In addition, thiourea has great influence on yield and quality of potato tubers (Panah *et al*., 2007). Hassan-Panah *et al* (2007) reported a concentration of 1500 ppm and 5% thiourea to break dormancy and decreases dormancy period but showed that the tuber yield and its weight per plant declined after sowing seeds in the greenhouse

Components like micro-tubers tuber size, physiological

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age, green sprouting methods, size grading and crop husbandry techniques affect tuber yield (Struik and Lommen, 1999). Microtuber using about 250–500 mg size is the best for maximizing minituber yield compared to plantlet may be a feasible practice under the field condition (Hossain *et al*., 2017). All the grades of micro-tubers (<4 mm, 4-8 mm and >8 mm) of both the varieties Kufri Megha and Kufri Girdhari were able to produce mini-tubers albeit with different efficiencies distinctly showing the effect of micro-tuber size and genotypes (Srivastava *et al*., 2015).

Hence in the present investigation, an attempt was made to evaluate the effect of different dormancy breaking growth regulators and their dip duration on different grades of microtubers for growth and yield parameters under aphid proof net house conditions of central India.

MATERIALS AND METHODS

The experiment was conducted at Central Potato Research Station, Gwalior (latitude of 26^0N, 78^0E) during 2012-13. 20 micro-tubers of three different size >4 -6mm, >6-8mm and >8mm of variety Kufri Sindhuri were given dip treatment with six types of growth regulators/ dormancy breaking chemicals *viz* 1ppm gibberelic acid, 0.5 ppm gibberllic acid, 1% thiourea, 0.5 % thiourea, 1ppm gibberllic acid + 1% thiourea and 0.5 ppm gibberllic acid + 0.5 % thiourea along with water control for 30 min, 45 min and 60 min. After dip treatment microtubers were planted in insect proof net house. The above said seed material was planted at 30 x 10 cm spacing in 2 m rows (0.6 M^2 plot size) during second week of November. The experiment was planted in split plot design and replicated three times. A light irrigation was given with the help of shower just after planting and subsequent irrigation was given after 10-12 days interval as per the need of the crop. Other packages of practices were followed as per the recommendation of seed potato crop. Data were pooled and analyzed statistically and means were separated according to the least significant differences (LSD) at 0.05 level of probability.

RESULTS AND DISCUSSION

Growth parameters: Emergence was significantly higher in all the dormancy breaking chemicals over water control (58.15%) and highest was recorded in 0.5% GA treatment (75.74%). 30 min dip duration showed non-significantly higher emergence (71.59%) over 45 and 60 min duration. >6-8mm size (78.73%) and>8mm size (69.68%) microtubers recorded significantly higher emergence at 5% level over >4mm size microtubers (59.52%) (Table 1).The micro-tuber survival was very good for the bigger sized micro-tubers *i.e.* in the grade of 4-8 mm and >8 mm for both Kufri Girdhari and Kufri Megha (>90%). Microtuber emergence was showed decreasing trend with decrease size

Layout plan of the experiment (treatment combinations)

Variety	Size of microtuber	Doses of dormancy breaking chemical	Dip duration	Size of microtuber	Doses of dormancy breaking chemical	Dip duration	Size of microtuber	Doses of dormancy breaking chemical	Dip duration
K. Sindhuri	>4 mm	1 ppm GA	30 min	>6 mm	1 ppm GA	30 min	>8 mm	1 ppm GA	30 min
			45 min			45 min			45 min
			60 min			60 min			60 min
		0.5 ppm GA	30 min		0.5 ppm GA	30 min		0.5 ppm GA	30 min
			45 min			45 min			45 min
			60 min			60 min			60 min
		1% Thiourea	30 min		1% Thiourea	30 min		1% Thiourea	30 min
			45 min			45 min			45 min
			60 min			60 min			60 min
		0.5% Thiourea	30 min		0.5% Thiourea	30 min		0.5% Thiourea	30 min
			45 min			45 min			45 min
			60 min			60 min			60 min
		1 ppm GA + 1% Thiourea	30 min		1 ppm GA + 1% Thiourea	30 min		1 ppm GA + 1% Thiourea	30 min
			45 min			45 min			45 min
			60 min			60 min			60 min
		0.5 ppm GA + 0.5% Thiourea	30 min		0.5 ppm GA + 0.5% Thiourea	30 min		0.5 ppm GA + 0.5% Thiourea	30 min
			45 min			45 min			45 min
			60 min			60 min			60 min

Table 1. Potato micro-tubers growth parameters as influenced by dormancy breaking chemicals, dip duration and microtuber size.

Growth regulator/Dip duration/Tuber size	Emergence %	Growth parameters					
		Plant height (cm)		No. of stem		No of Compound leaves	
		50 DAP	75DAP	50 DAP	75DAP	50 DAP	75DAP
Water control	58.15	7.6	47.7	1.2	1.2	8.8	18.6
1ppm gibberllic acid	71.30	8.0	51.3	1.4	1.4	11.9	21.4
0.5 ppm gibberllic acid	75.74	8.3	49.7	1.5	1.5	11.0	21.5
1% Thiourea	73.70	7.9	57.4	1.4	1.2	11.0	22.0
0.5% Thiourea	64.26	7.4	56.4	1.3	1.3	9.4	20.1
1ppm gibberllic acid+1% thiourea	72.22	8.1	55.7	1.8	1.4	12.1	20.7
0.5 ppm gibberllic acid+ 0.5% thiourea	70.74	9.4	55.2	1.4	1.4	10.0	20.5
SEm (±)	1.83	0.18	0.68	0.09	0.29	0.69	0.72
CD	5.62	0.57	2.11	0.29	NS	2.13	NS
30 min	71.59	7.7	54.2	1.5	1.4	10.5	21.2
45min	68.18	8.2	52.3	1.4	1.4	10.5	21.0
60min	68.57	8.4	53.5	1.5	1.3	10.8	19.9
SEm (±)	1.20	0.24	0.68	0.06	0.04	0.35	0.45
CD	NS	NS	NS	NS	NS	NS	NS
>4mm	59.92	5.1	45.8	1.2	1.1	7.0	17.7
>6-8mm	78.73	8.1	54.5	1.3	1.2	9.7	18.8
>8mm	69.68	11.1	59.7	1.9	1.7	15.1	25.6
SEm (±)	1.20	0.24	0.58	0.06	0.04	0.35	0.45
CD	3.36	0.66	1.61	0.15	0.11	0.98	1.27
SEm (±) Growth regulator x Dip duration	3.17	0.62	1.52	0.15	0.10	0.92	1.20
CD Growth regulator x Dip duration	8.89	1.75	4.26	0.41	NS	2.59	3.37
SEm (±) Growth regulator x Microtuber size	3.17	0.62	1.52	0.15	0.15	0.92	1.20
CD Growth regulator x Microtuber size	8.88	1.75	NS	0.41	NS	2.59	3.37
SEm (±) Dip Duration x Microtuber size	2.08	0.41	1.00	0.10	0.07	0.61	0.79
CD Dip Duration x Microtuber size	NS	NS	2.79	NS	0.19	NS	2.21
SEm (±) Growth regulator x Dip Duration x Microtuber size	5.49	1.08	2.64	0.25	0.18	1.60	2.08
CD Growth regulator x Dip Duration x Microtuber size	NS	3.03	NS	NS	0.50	4.48	5.83

97 % in 3-5g and lowest 82.0% in 0.3-0.5g microtubers (Kawakami and Iwama, 2012). As the size grade decreases to <4 mm, the potential of the micro-tuber survival decreased significantly (79.4%) Srivastava *et al.* (2015). Désiré *et al.* (1995) The growth and survival/ vigor of potato tubers is supported by the food material stored in the tubers particularly carbohydrates and could thus be conducive to plant development and hence relatively bigger micro-tubers have distinct advantage as far as emergence is concerned and confirms the present study. Present findings are also in agreement with Somani and Venkatasalam (2012) who reported the role of both size and genotype on microtuber emergence (%) and survival where emergence *per cent* of microtubers ranged from 27.4 (Kufri Kanchan) to 73.6 (Kufri Lauvkar) with an overall average of 64.6%. Cultivar Kufri Lauvkar (73.6%) resulted maximum emergence where the average weight of microtubers planted was highest (0.13 g) and minimum in Kufri Kanchan (27.4%) with average weight of microtubers planted was 0.08 g. Only 46% of microtubers with <3mm diameter are able to sprout, while this amount for microtubers with 3-5mm and > 5mm diameter was 75% and 98% respectively (Bolandi *et al.*, 2011). Alsadon *et al* (1988) reported that functionality of plants glands produced by small micro tubers is less than larger microtubers. Larger sizes give better emergence and a better early vigour, and produce a higher yield and more tubers per plant (Struik and Wiersema, 1999).

Plant height at 50 days after planting was significantly higher in 0.5 ppm gibberllic acid+ 0.5% thiourea treatment (9.4cm) followed by 0.5 ppm gibberllic acid (8.3cm) over water control (7.6cm). No significant difference was recorded in dip duration. Plant height was significantly lowest in >4mm size microtubers (5.1cm) over >6-8mm size (8.1cm) and >8mm size microtubers (11.1 cm) at 50 days after planting. Increase in plant height with increase in microtuber size is due higher vigour in the microtuber of large size. At 75 days after planting, all the growth regulator treatment were significantly higher at 5% level over water control (47.7cm) table 1. There was increase in plant height with increase in the GA_3 doses from 0 to 160 ppm in variety Morphona (Shekari *et al*., 2010). No significant difference was recorded in dip duration. Plant height was significantly lower in >4mm size microtubers (48.8cm) than >6-8mm size (54.5cm) and

Table 2. Potato micro-tubers yield parameters as influenced by dormancy breaking chemicals, dip duration and microtuber size.

Growth Regulator/Dip duration/Tuber size	Yield parameters					
	Yield/plant		<3g Yield/ha		Total	yield/h
	No (000/ ha)	Weight (Kg)	No (000/ ha)	Weight (q/ha)	No (000/ha)	Weight (q/ ha)
Water control	18	0.15	967	18.33	2067	181.67
1ppm gibberllic acid	19	0.19	1100	20.00	2350	218.33
0.5 ppm gibberllic acid	23	0.18	1183	20.00	2500	205.00
1% Thiourea	21	0.19	1083	20.00	2350	208.33
0.5% Thiourea	19	0.20	967	20.00	2150	203.33
1ppm gibberllic acid+1% thiourea	20	0.15	1450	25.00	2667	196.67
0.5 ppm gibberllic acid+ 0.5% thiourea	21	0.21	1183	20.00	2467	226.67
SEm (±)	1.2	0.012	38	0.83	48	8.50
CD	NS	0.036	118	2.67	148	26.00
30 min	21	0.18	1150	20.00	2500	206.67
45min	22	0.18	1133	21.67	2367	210.00
60min	19	0.19	1117	20.00	2233	201.67
SEm (±)	0.9	0.008	38	0.83	52	5.00
CD	NS	NS	NS	NS	143	NS
>4mm	16	0.11	983	15.00	1850	113.33
>6-8mm	19	0.19	1150	21.67	2417	208.33
>8mm	26	0.25	1267	23.33	2833	295.00
SEm (±)	0.9	0.008	38	0.83	52	5.00
CD	2.5	0.022	108	2.50	143	14.17
SEm (±) Growth regulator x Dip duration	2.3	0.021	102	2.33	135	13.33
CD Growth regulator x Dip duration	NS	0.059	NS	NS	NS	37.33
SEm (±) Growth regulator x Microtuber size	2.3	2.3	102	2.33	135	13.33
CD Growth regulator x Microtuber r size	NS	0.059	287	6.50	380	37.33
SEm (±) Dip Duration x Microtuber size	1.5	0.014	67	1.50	88	8.67
CD Dip Duration x Microtuber size	NS	NS	NS	NS	NS	NS
SEm (±) Growth regulator x Dip Duration x Microtuber size	4.1	0.037	177	4.00	235	23.17
CD Growth regulator x Dip Duration x Microtuber size	NS	NS	NS	NS	NS	NS

>8mm size microtubers (59.7 cm) at 75 days after planting. Increase in micro-tuber size from <4mm to >8mm size there was a corresponding increase in plant height when reported after 60 days after planting (Srivastava *et al*., 2015). This confirms the present findings of increase in plant height with increase in tuber size.

Number of stem /plant at 50 days after planting were significantly higher in 1ppm gibberllic acid+1% thiourea (1.8) followed by 0.5 ppm gibberllic acid (1.5) over water control (1.2). No significant difference was recorded in dip duration. Significantly higher stem/ plant was recorded >8mm size microtubers (1.9) followed by >6-8mm size (1.3) over >4mm size microtubers (1.2). At 75 days after planting, no significant differences were recorded among growth regulators and dip duration. Significantly higher stem/plant was recorded >8mm size microtubers (1.7) followed by >6-8mm size (1.2) over >4mm size microtubers (1.1) table 1. Present findings are also in agreement with Srivastava *et al*. (2015) who reported that larger sized micro-tubers (>8 mm and 4-8 mm) did not exhibited significant (α = 5%) difference in terms of stems/plant but were superior to those in the smallest size micro-tuber (<4 mm).

Number of compound leaves/plant at 50 days after planting were significantly higher at 5% level in 1ppm gibberllic acid+1% thiourea (12.1) followed by 1ppm gibberllic acid (11.9) over water control (8.8). No significant difference was recorded in dip duration. Significantly higher number of compound leaves/plant was recorded >8mm size microtubers (15.1) followed by >6-8mm size (9.7) over >4mm size microtubers (7.0). At 75 DAP no significant differences were recorded among growth regulators and dip duration. Significantly higher number of compound leaves/plant was recorded >8mm size microtubers (25.6) followed by >6-8mm size (18.8) over >4mm size microtubers (17.7) table1. The number of leaves/plant followed similar pattern to plant height. As the micro-tuber size increased, the number of leaves/plant also significantly increased from 39 in <4mm to 47.5 in >8mm microtuber size (Srivastava *et al*., 2015). Similar pattern among plant height and number of leaves/plant was expected due to the fact that increase in plant height may result due to an increase in the number of nodes leading to increased number of leaves/plant. A smaller LAI was observed in plants from small microtubers than from large microtubers at the beginning of the growing season, and no difference was observed in Leaf area index after the start of flowering about 30 days after emergence (Kawakami *et al*.2006; Kawakami and Iwama 2012). The initial smaller LAI in the plants from small microtuber suggests that a narrow planting distance could decrease the initial difference in LAI among plants grown from seed tuber of small size (Kawakami and Iwama, 2012).

Yield parameters: Non-significantly higher tuber number/plant were recorded in all the treatments over water control and highest was recorded in 0.5 ppm gibberllic acid treatment (23). The average number of tubers per plant was greatest in the GA_3 treatment (3.6); almost double that of other treatments that were not different from the control (Habib, 1999). GA_3 application significantly influenced tuber number per plant. GA_3 increased the mean tuber number per plant from 4 in control to 7.5 at 160 ppm GA_3 application (Shekari *et al*., 2010). In dip durations there were non-significant difference. Significantly higher tuber number were recorded in >8mm size microtubers (26) followed by >6-8mm size (19) over >4mm size microtubers (16). Significantly higher tuber weight was recorded in all the treatments except 1ppm gibberllic acid+1% thiourea over control (0.15 kg) table 2. Treatment with GA_3 (1000 ppm for 1 hour) yielded highest tuber number/plant (Rehman *et al,* 2001). Mani *et al* (2013) reported that applying at least 250 mM solution of thiourea increased yield up to 810 g/plant, which equivalent to 28t/ha. This value is significantly superior over control (630 g/plant). No significant difference was recorded in dip duration. Significantly higher weight of tuber/plant was recorded >8mm size microtubers (0.25kg) followed by >6-8mm size (0.19kg) over >4mm size microtubers (0.11kg).There is a direct and significant relationship between used microtuber size and weight and number of produced minitubers in each plant, so that total weight of produced minituber by each plant which is produced from microtubers<5mm is 29.48 g and for microtubers>10mm is 49.08 g (Bolandi *et al*. 2011). This confirms the present study and indicates that higher size of microtuber yields more than smaller one.

There is general tendency of production <3g minitubers from microtubers/ microplants. In the present study, significantly higher tuber number/ha was recorded in all the treatment except 0.5% thiourea over water control (967 thousand/ha) and highest was recorded in 1ppm gibberllic acid+1% thiourea (1450 thousand/ha). No significant difference was recorded in dip duration. Significantly higher <3g tuber number/ha were recorded in >8mm size microtubers (1267 thousand) followed by >6-8mm size (1150 thousand) over >4mm size microtubers (983 thousand). For weight, only 1ppm gibberllic acid+1% thiourea (25 q/ha) recorded significantly higher <3g tuber weight. No significant difference was recorded in dip duration. Significantly higher <3g tuber weight (q/ha) were recorded in >8mm size microtubers (23.33) followed by >6-8mm size (21.67) over >4mm size microtubers (15.00) table 2. Srivastava *et al*., (2015) reported that the small sized micro-tubers (<4 mm) produced relatively less number of small sized tubers per plant as compared to the larger sized micro-tubers (4-8 mm and >8 mm). Thus maximum yield per plant was produced by >8 mm grade micro-tubers followed by 4-8 mm and <4 mm grade micro-tubers. Similar yield pattern was observed for number of tubers/m2 and yield/m2. The difference for tuber size distribution can be influenced by the micro-tuber size as well as the genotype (Ranalli *et al.* 1994). Total yield variation may also be accounted to the initial growth and survival rate at the time of planting (Kumar *et al.*, 2007).

Significantly higher tuber number (thousand/ha) was recorded in all the treatment except 0.5% thiourea over water control (2067 thousand) and highest was recorded in 1ppm gibberllic acid+1% thiourea (2667 thousand) table 2. GA_3 was the most active compound for early as well as multiple sprouting; it produced the highest number of tubers with lowest total fresh weight compared with the other treatments (Habib, 1999). Among dip durations, 30 min dip treatment (2500 thousand) recorded significantly higher tuber number (thousand/ha) over 60 min dip treatment (2233 thousand). Significantly higher tuber number (thousand/ha) were recorded in >8mm size microtubers (2833 thousand) followed by >6-8mm size (2417 thousand) over >4mm size microtubers (1850 thousand).

Significantly higher tuber yield (q/ha) was recorded in 1ppm gibberllic acid (218.33q) and 0.5 ppm gibberllic acid+ 0.5% Thiourea (226.67 q) over control (181.67 q). Mani *et al.* (2013) reported that application of 250 mM of thiourea excelled over other doses for growth plant parameters, tuber yield and quality attributes. It gives an increase in yield of 26% with tubers rich in starch and in soluble proteins due to an increase in total sugars and sucrose measured in growing leaves of treated plants. No significant difference was recorded in dip duration. Significantly higher weight of tuber (q/ha) was recorded >8mm size microtubers (295 q) followed by >6-8mm size (208.33 q) over >4mm size microtubers (113.33 q) table 2. Minitubers functionality was increased by microtubers size and reaches to 20, 27 and 40 tons/ha for microtubers with <3mm, 3-5mm and >5mm diameter (Bolandi *et al.,* 2011).No significant difference in fresh or dry tuber yield was observed among the MT classes, suggesting that the use of the MT of even 0.3 g may be a feasible practice under the field condition. The tuber fresh yield of about 41 to 66 t ha-1 observed in the plants from MT demonstrates the high yield potential of MT as seed tubers in the field, although the yearly variation in tuber yield was higher in plants from MT than in plants from CT (Kawakami and Iwama, 2012). According to Singh *et al.* (2001) the rate of micro-tuber multiplication increased with the increase in size. This confirms the present study where seed multiplication rate was 26 times in >8mm microtubers and 16 times in <4mm microtubers leading to the higher tuber number and yield than small sized tubers.

Conclusion

The present study concluded that potato micro-tubers can be used as initial planting material for nucleus seed production in potato. The micro-tubers of >8mm size treated with 0.5 ppm gibberllic acid alone or in combination with 0.5 % thiourea for 30 min resulted in higher growth and yield parameters. The minitubers produced from microtubers can be used for further multiplication for producing high quality seed material under G-1 and G-2 thereby helping in attaining self-sufficiency for seed potato.

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