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In vitro induction of variability through radiation for late blight resistance and heat tolerance in potato

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Abstract. *In vitro* cultured shoots of potato, cvs. 'Kufri Jyoti' and 'Kufri Chandramukhi', were irradiated with 20 and 40 Gy gamma rays. Microtubers, obtained from M1V3 shoots multiplied *in vitro,* were planted in pots. The resulting plants were screened for resistance to late blight, using detached leaf method. In 'Kufri Chandramukhi', 42% plants and in 'Kufri Jyoti' 36% plants, obtained from 40 Gy treatment, showed resistance to late blight. The frequency of resistant plants was lower from 20 Gy treatment. The progenies of putatively resistant plants were grown in field, and inoculated with sporangial inoculum of late blight fungus. The field grown progeny segregated for disease resistance, and approximately 56% plants showed resistance. During the next propagation, the frequency of resistant plants increased to 72%. For developing heat tolerance, microtubers obtained from 20 and 40 Gy treatments and *in vitro* multiplied M_1V_3 shoots were cultured at high temperature of 28C. In both varieties, the number of the microtubers per plant was highly reduced and the resulting microtubers had distorted shape but showed better germination (62%), even in early sowing at relatively higher temperature. Of the two radiation doses, the higher dose of 40 Gy gave better results in both the varieties. Heat tolerance was also assessed from chlorophyll persistence. The progenies from putative heat-tolerant plants were tested in field by planting at higher temperature in two subsequent generations. The heat tolerant plants segregated in each generation, but the frequency of heat-tolerant plants increased.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important food crop in India. From an area of 0.935 million ha under potato, nearly 15.2 million ton tubers are produced annually. The main objectives of potato research in India include breeding for early maturity, high yield, and resistance to late blight and viruses. 'Kufri Jyoti' and 'Kufri Chandramukhi', the two most popular varieties, are susceptible to late blight. Also, the cropping pattern in North India demands an early planting variety in September, so that it matures by November and a following crop of wheat can be grown. However, the temperature during early potato planting period ranges between 20° to 32° C. The present project was initiated to induce variation for resistance to late blight and heat tolerance in the local potato varieties.

2. MATERIALS AND METHODS

Cultivar 'Kufri Jyoti' and 'Kufri Chandramukhi' were used as the experimental material. 'Kufri Jyoti' is a medium late maturing (100–110 days) variety and 'Kufri Chandramukhi' is an early maturing (90–100 days) variety. Both varieties are high yielding with slow rate of viral degeneration, wide adaptability and desirable commercial attributes, but are susceptible to late blight and are not suitable for early planting being heat susceptible.

2.1. Establishment of *in vitro* cultures and their multiplication

Healthy potato tubers were rinsed in 10% ethanol, and sprouted at 20°C in dark. Sprouted tubers were planted in pots. Nodal sections were excised from stems, and surface sterilized for 5 min. in a solution of 0.1% HgCl2 and 0.1% Sodium lauryl sulphate, and washed three times in sterile water. Nodal sections, 0.5–1.0 cm, were cultured on basal MS medium [1]. The cultures were maintained at 28±2°C under 16/8 light/dark (3000–4000 lux). After 5 to 6 weeks, the shoots were cut into 0.5–1.0 cm long nodal segments, and sub-cultured on fresh medium to produce a second generation of plants. The *in vitro* grown plants with 5 to 6 nodes each were irradiated with 20 and 40 Gy gamma rays. The irradiated plants were cut into nodal sections, and cultured on basal MS medium for 4–5 weeks.

2.2. Microtuber formation

In vitro cultured, 4 to 5 weeks old plants, were used for obtaining microtubers. The plants were cut into 0.5–1.0 cm long segments, each with one leaf and an axillary bud, and transferred to MS medium with 8% sucrose. The cuttings were placed in 300 ml jam jars with 40 ml medium or 120 ml Watson Module containers with 25 ml medium. The Watson Module system consists of disposable pre-sterilized clear plastic containers with snap on lids. Cultures were kept at 28±2°C (16hr light) and 25±2°C (8 hr dark) for 60 days. They were then transferred to dark at 20±1°C after pouring liquid MS medium supplemented with 10 mg/l BAP and 8 % sucrose. This protocol was tested for 25 cultivars, and was found suitable for most varieties.

2.3. Callus induction

Leaf cuttings from *in vitro* plants were cultured on modified LS medium [2] without cytokinins but supplemented with 5 mg/l NAA. Callus initiation and subsequent proliferation was obtained in dark at 28°C. The obtained calli were irradiated with 20 and 40 Gy gamma rays. Regeneration was obtained by transferring calli to modified LS medium containing 50 ml coconut milk, 0.1 mg NAA and 5 mg BAP per litre. Young regenerated sprouts were transferred to B5 medium [3].

2.4. Screening for late blight resistance

The microtubers were planted 1.2–2.5 cm deep in a mixture of soil and farm yard manure (1:1) in pots. To screen for late blight resistance, leaves were surface sterilized with 0.1% $HgCl_2$ and 0.1% Sodium lauryl sulphate for 7 min. or obtained from *in vitro* grown plantlets from these plants. The sterile leaves were then placed on the Gamborg medium [3], containing toxin from a single pathotype of *Phytophthora infestans*. The toxin filtrate was prepared from fungal cultures grown on pea extract medium. The cultures were checked microscopically for infection, and cultured in fluid pea extract. After three weeks, the toxin fluid was filtered to remove sporangia.

Tubers from plants classed as resistant or moderately resistant to late blight in 1995 were grown in pots on 15 October 1996, and kept outdoors. Late blight inoculum was prepared by collecting spores from a mixture of pathogen races, and was sprayed on the plants at a concentration of 10–15 spores/drop in distilled water. The plants were covered with polythene bags to provide humidity. The bags were removed after 24 hr, and disease reaction was observed after five days. The plants were graded on a scale of 1 to 6 (Table I). The same method was used for screening for disease resistance in 1997.

TABLE I. SCALE TO CLASSIFY INFECTION OF FIELD GROWN PLANTS

Infection type	Description	Class*
0	Healthy	R
1	Few specks on the leaves only.	R
2	One or two lesions on few leaves, plants green.	HR
3	One or two lesions clearly visible on leaves with specks on stem but plants green.	MR
4	Lesions uniform on leaves with few leaves drooping, lesions present on stem, plants greenish brown.	MS
5	Most leaves with brown lesions, up to half of stem showing brown discoloration, plants mostly brownish with few green areas.	S
6	Plants dead with dead leaves, brown stem and leaves.	S

*R- resistant; HR- Highly resistant; MR- Moderately resistant; MS- Moderately susceptible; S- susceptible

2.4. Selection for tolerance to high temperature

During 1995–96, microtubers were produced at 20°C and 28°C. *In vitro* irradiated plants were cut into nodal sections and cultured on medium for microtuber formation. After proliferation of the shoots, liquid medium was added to culture vessels, which were maintained at 20°C and 28°C. Non-irradiated control plants were propagated in each experiment.

The microtubers obtained from irradiated and control plants at 20°C were planted on 1 and 15 September, and 1 and 15 October for germination under high temperature, and screened for heat tolerance. To study heat tolerance, leaf discs were taken from fully expanded leaves, and placed in Petri dishes lined with wet cotton, sealed with parafilm, and maintained in a growth chamber at 25°C. After four days, leaf discs were scored for damage. A disc was considered damaged when more than 50% area was yellow. The ratio of damaged disc was used as a relative measure of injury.

The nodal sections from plants that showed resistant reaction were used as explant for micropropagation to multiply the mutated sectors. The nodal sections were sterilized with 0.1% $HgCl_2$ and 0.1% SDS, and rinsed three times in sterile distilled water. The explants were then cultured on MS basal medium without hormones. The micropropagated plants were irradiated second time with 20 Gy dose.

3. RESULTS AND DISCUSSION

3.1. *In vitro* culture and microtuber production

The nodal segments from plants obtained from sprouted tubers planted in soil gave better results than directly from sprouted tubers. A total of 176 explants of 'Kufri Chandramukhi' and 162 of 'Kufri Jyoti' were cultured. By subculture of 547 *in vitro* grown plantlets, 400 plantlets were obtained in 'Kufri Chandramukhi', and from 481 plantlets, 328 plants were obtained in 'Kufri Jyoti'. Not all the cultured plants survived and thin segments failed to grow into plantlets. The *in vitro* plantlets of both the varieties were irradiated at 20 and 40 Gy. When the plantlets were transferred to medium with 8% sucrose, there was excessive proliferation of shoots, and the leaf-size was greatly reduced.

Microtubers initiated after 10 days of transfer of cultures to dark. Microtubers originated as aerial structures from microstems, although a few microtubers were also formed in the medium. Microtuber size varied from 2 to 15 mm in diameter. The number of microtubers ranged from 1 to 3 per plant. In M_1V_3 generation of 'Kufri Chandramukh', 368 microtubers were obtained from 384 plantlets derived from 20 Gy treatment and 278 plants from 40 Gy treatment produced 214 microtubers. In 'Kufri Jyoti', 315 cultured explants from 20 Gy dose gave 298 microtubers and 350 explants from 40 Gy gave 296 microtubers (Table II).

TABLE II. *IN VITRO* PLANT IRRADIATION AND INDUCTION OF MICROTUBERS AT 20°C

Variety	Dose GY	No. of plants irradiated	No. of plants micro-tuberised	No. of microtubers
Kufri	20	133	384	368
Chandramukhi	40	120	278	214
Kufri Jyoti	20	133	315	298
	40	115	350	296

3.2. Isolation of mutants

Microtubers were sprouted a month before sowing. Sprouting of microtubers improved their emergence. Without pre-sprouting, the microtubers either failed to germinate or germinated very late. Leaves from microtuber derived plants were placed on medium with toxic filtrate. Some of the leaves turned yellow and brownish whereas others remained totally unaffected. When the leaves from the *in vitro* cultured plantlets were placed on the toxic medium, lesions appeared on leaf-surface after 4 days, as expected and reported by others [4]. When toxin filtrate was used at 30 ml/l in the medium, more than 50% of the leaf area was covered with lesions. All the control plants were susceptible. Plants resistant to late blight were observed in both cultivars from the two doses of 20 and 40 Gy.

TABLE III. REACTION OF *IN VITRO* SCREENED PLANTLETS TO LATE BLIGHT DISEASE

Variety	Dose Gy	Disease score*			Plants tested No.
		R	MR	S	
Kufri Chandramukhi	20	36	20	44	39
	40	12	8	80	40
	Control	0	0	100	6
Kufri Joyti	20	20	20	60	40
	40	9	30	61	47
	Control	0	0	100	8

z* Per cent plants: R- resistant; MR- Moderately resistant; S- susceptible

The progeny of the plants, classed as resistant during 1995, gave resistant reaction. In some cases though, variation was observed, i.e. tubers from the same plant showed varying disease reaction. In case of moderate resistance, progeny included susceptible types. This suggests that chimeras were present and the mutant sectors could be multiplied by rapid *in vitro*

propagation as proposed by Lu et al. [5]. The plants, which gave resistant reaction in 1996, gave resistant reaction in 1997 also. Although, variability was observed in the progeny of the same plant, the frequency of segregation was less as compared with that in the previous year. Whereas in 1996, the segregation was up to nearly 50%, in 1997, the maximum segregation was only 27.7%, thereby confirming that the lines were becoming stable.

3.3. Isolation of heat tolerant mutants

The normal temperature for microtuberisation was 20±1°C, hence screening for heat tolerance was done by sub-culturing the irradiated material at 28°C. The plantlets, irradiated with 20 and 40 Gy formed mictubers, but the response was drastically different compared with the control (Table IV). The control plants degenerated and no microtuber were obtained, even after two months of culture. The difference between controls and irradiated material was dramatically less between microtubers produced at 20°C. The effect of high temperature was also reflected in tuber shape.

TABLE IV. MICROTUBER PRODUCTION FROM IRRADIATED AND CONTROL PLANTS AT 28°C

Variety	Dose Gy	No. of Culture Vessels	No. of Microtubers
Kufri Chandramukhi	20	10	6
	40	10	14
Control	0	5	0
Kufri Jyoti	20	10	4
	40	10	11
Control	0	5	0

Microtubers, planted on various dates, showed marked difference in their germination (Table V). In early planting, when temperature was high, tuber germination was better in the irradiated ones than the controls (Table VI). Based on chlorophyll persistence [6], plants showing leaf-area damaged less than 40% were categorized as heat tolerant. In 'Kufri Chandramukhi' from 40 Gy treatment, 76 of the 164 plants tested showed less than 50 % damaged leaf-area. From 20 Gy treatment, 70 of 250 plants showed less than 50% leaf injury. During 1997, chlorophyll persistence showed that nearly 50% of the plants in both varieties had less than 20% damaged leaf-area, indicating that these included variants for heat tolerance (Tables VII and VIII). Thus, late blight and heat tolerant variants have been obtained in both the varieties. However, more vegetative generations are required to obtain stable mutants.

TABLE V. EFFECT OF IRRADIATION AND PLANTING DATE ON MICROTUBER GERMINATION, 1996

Date of planting	Variety	Treatment	No. of microtubers	No. of plants	Germination (%)
1st Sept. 1996	Kufri	20 Gy	105	58	55
	Chandramukhi	40 Gy	54	31	57
		Control	10	3	30
	Kufri Jyoti	20 Gy	68	29	43
		40 Gy	68	32	47
		Control	10	5	50
15 Sept. 1996	Kufri	20 Gy	115	74	64
	Chandramukhi	40 Gy	64	42	66
		Control	10	5	50
	Kufri Jyoti	20 Gy	72	42	54
		40 Gy	74	43	58
		Control	10	6	60
1 Oct. 1996	Kufri	20 Gy	69	69	72
	Chandramukhi	40 Gy	35	35	67
		Control	8	8	80
	Kufri Jyoti	20 Gy	72	56	72
		40 Gy	70	54	77
		Control	10	8	80
15 Oct. 1996	Kufri	20 Gy	52	49	88
	Chandramukhi	40 Gy	44	39	89
		Control	10	10	100
	Kufri Jyoti	20 Gy	86	75	87
		40 Gy	84	78	93
		Control	10	10	100

TABLE VI. GERMINATION OF MICROTUBERS OBTAINED FROM IRRADIATED AND CONTROL PLANTS PLANTED ON DIFFERENT DATES, 1997

Planting date	Variety	Dose Gy	No. of microtubers planted	No. of plants obtained	Germination %
1 Sept. 1997	Kufri Jyoti	40	25	8	32
		20	20	1	5
		Control	10	0	0
	Kufri Chandramukhi	40	22	4	19
		20	10	5	50
		Control	10	0	0
15 Sept. 1997	Kufri Jyoti	40	42	38	90
		20	26	16	61
		Control	10	6	60
	Kufri Chandramukhi	40	42	22	52
		20	36	32	88
		Control	10	7	70

TABLE VII. CHLOROPHYLL PERSISTENCE IN IRRADIATED PLANTS, 1996

Variety	Dose Gy	No. of plants tested	No. of plants with damaged leaves				
			20%	40%	60%	80%	100%
Kufri Chandramukhi	40	164	28	48	56	20	12
	20	250	46	24	66	58	56
	Control	26	-	-	10	8	5
Kufri Jyoti	40	207	46	26	54	40	41
	20	202	22	40	64	48	28
	Control	28	-	-	12	10	5

TABLE VIII. CHLOROPHYLL PERSISTENCE IN IRRADIATED PLANTS, 1997

Variety	Dose Gy	No. of plants tested	No. of plants with damaged leaves				
			20%	40%	60%	80%	100%
Kufri Chandramukhi	40	22	10	5	5	2	-
	20	37	15	10	4	6	2
	Control	10	-	-	-	3	7
Kufri Jyoti	40	38	16	4	7	5	6
	20	16	6	5	1	2	2
	Control	10	-	-	-	-	10

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