IN VITRO INDUCTION OF VARIATION THROUGH RADIATION FOR LATE BLIGHT RESISTANCE AND HEAT TOLERANCE IN POTATO

J.L. MINOCHA, A. DAS, J.GOPAL, S.S. GOSAL
Biotechnology Centre
Punjab Agricultural University
Ludhiana, Punjab, India

Abstract

In vitro plants were obtained from nodal sections of sprouts of cvs. 'Kufri Jyoti' and 'Kufri Chandramukhi' of potato cultured on MS medium with 3% sucrose. Callus from leaves of *in vitro* cultured plantlets was induced on modified Linsmaier and Skoog medium supplemented with 5 mg/l NAA. The obtained shoots and calli were irradiated with 20 and 40 Gy gamma rays. Irradiated shoots were transferred to MS medium with 8% sucrose for multiplication, and then to MS medium with 8% sucrose and 10 mg/l BAP to induce microtuber formation, which gave on average 1.3 microtubers per plant. The microtubers were planted in pots and variation was observed in plant morphology and tuber characters. To study variation for late blight resistance, irradiated calli were kept on Gamborg B-5 medium with culture filtrate of *Phytophthora infestans*. To induce variation for heat tolerance, *in vitro* shoots from irradiated material were mass-propagated and allowed to produce microtubers at high temperature.

1. INTRODUCTION

The main objectives of potato breeding in India include high yield, late blight resistance, virus resistance and early maturity. '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 normal crop of wheat can be raised. The temperature during this period ranges between 20 to 32°C. The present project was initiated with the objective to induce *in vitro* variation for resistance to late blight and heat tolerance in cvs. 'Kufri Jyoti' and 'Kufri Chandramukhi' through radiation.

2. MATERIALS AND METHODS

Cv. 'Kufri Jyoti' and 'Kufri Chandramukhi' were used as the experimental material. 'Kufri Jyoti' is a medium late maturing (100-110 days) and 'Kufri Chandramukhi' an early maturing (90-100 days) variety. Both 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.

Micropropagation:

Micropropagation allows rapid multiplication of clones in a short duration under disease free, controlled environment and on a year round basis. For producing *in vitro* plantlets, healthy potato tubers were rinsed with 10% ethanol and incubated at 20°C in dark. When the sprouts had 6-10 nodes, the tubers were transferred to light for 7 days to harden the sprouts. The

sprouts were surface sterilized in a mixture of 0.1% $HgCl_2$ and 0.1% sodium laurel sulphate for 5 minutes, and rinsed three times with sterilized distilled water. The nodal sections (0.5-1.0 cm long) were cultured on Murashige and Skoog [1] medium without vitamins and hormones. The above methodology was being used for producing *in vitro* plants of potato in this laboratory, but it did not work satisfactorily with 'Kufri Chandramukhi' and 'Kufri Jyoti', because the cultures showed very high incidence of fungal infection. It was probably from internal contamination of the explants, which could not be removed by surface sterilization. To overcome this, the sprouted tubers were planted in pots, and nodal sections from the stems of the plants were used as explants which gave better results than the nodal sections from sprouts. The cultures were kept under 16 hr photoperiod (3000-4000 lux, at 28 ± 2°C) and 8 hr dark, (25 ± 2°C). After 5 to 6 weeks, the plantlets were cut into 0.5-1.0 cm long segments. Each segment carried one axillary bud and usually one leaf. They were cultured on fresh medium and allowed to produced a second generation of plantlets. The *in vitro* grown plants with 5 to 6 nodes were irradiated with 20 and 40 Gy gamma-rays. The irradiated plants were cut into nodal sections, and cultured on MS medium for 4 to 5 weeks.

Microtuberisation

Plantlets, 4 to 5 week old, were used for microtuberisation. The plants were cut into 0.5-1.0 cm long segments, each with one leaf and an axillary bud, and transferred for microtuberisation to MS medium with 8% (w/v) sucrose. The cuttings were placed in jam bottles (40 ml medium per 300 ml bottle) or Watson Module containers (25 ml medium per 120 ml container). The Watson Module system consists of disposable pre-sterilized clear plastic containers with snap on lids. Cultures were kept at 28 ± 2°C (16 hr light) and 25 ± 2°C (8 hr dark) for 60 days. They were then transferred to dark conditions at 20 ± 1°C after pouring liquid MS medium containing 10 mg/l BAP and sucrose 8% sucrose. This protocol was tested for 25 cultivars, and was found suitable for all except 3 to 4 varieties (data not shown).

Callus induction

Leaf cuttings from *in vitro* plants were cultured on Linsmaier and Skoog [2] modified medium by omitting cytokinins and adding 5 mg/l NAA. Callus initiation and subsequent proliferation was obtained in dark at 28°C. The calli thus obtained 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 B-5 medium [3].

Screening procedures

To screen for late blight resistance, microtubers were sown in pots with mixture of soil and farm yard manure in the ratio of 1:1. The microtubers were planted at a depth of ½ to 1 inch. The leaves from plants so produced were surface sterilized with 0.1% $HgCl_2$ and 0.1% Sodium laurel sulphate for 7 minutes. Plantlets were also raised *in vitro* from these plants. The sterile leaves were then placed on the toxic medium containing culture fluid of one pathotype of *Phytophthora infestans* and normal ingredients of Gamborg medium [3]. The culture filtrate was prepared by inoculating the fungal cultures on the Pea's extract medium. The cultures were checked for infection by microscopical observations and afterwards cultured in fluid Pea's extract. The culture fluid was filtered after 3 weeks to remove sporangia. Batches of 20 pieces

of calli (ca. 2 mm) were placed on the toxic media in a Petri dish. In the other case, young sprouts were transferred to culture medium containing filtrate. During selection, cultures were illuminated 14 hr a day by white light ca. 3000-4000 lux.

To screen for heat tolerance, the plantlets were grown at 28 ± 2°C, and allowed to microtuberise at this temperature. Plantlets were produced from irradiated calli cultured on normal B5 medium, and transferred to MS medium, and allowed to produce microtubers at 28-30°C.

3. RESULTS AND DISCUSSION

Micropropagation protocol had broad applicability as all the 25 genotypes tested responded well. The nodal explants from the plants obtained from sprouted tubers planted in soil gave better results than those from sprouted tubers. A total of 501 *in vitro* plantlets (253 in 'Kufri Chandramukhi' and 248 in 'Kufri Jyoti') were irradiated. The plantlets after irradiation with 2 and 4 kr gamma rays were used for microtuberisation. The number of microtubers per plant varied for different genotypes (data not shown). In case of 'Kufri Jyoti' and 'Kufri Chandramukhi', the total number of microtubers produced till now was 517 (Table I). Microtubers were usually 2 to 10 mm in diameters and originated as aerial structures from microstems, although, a few were also formed in the medium. All microtubers had cream to white skin.

TABLE I. MICROTUBERIZATION OF IRRADIATED *IN VITRO* PLANTS

Variety	Dose (Gy)	No. of plants irradiated	No. of plants for micro-tuberisation	No. of microtubers
Kufri Chandramukhi	20	133	384	129
	40	120	278	110
Kufri Jyoti	20	133	315	152
	40	115	350	126

Callus was induced on two media: 1. MS + 5 mg/l NAA + 5 mg/l IAA + Kin 0.5 mg/l KIN, and 2. LS + 5 mg/l NAA + 2 mg/l IAA + 0.3 mg/l KIN. The efficiency of callus induction was much more on the second medium. In case of Linsmaier and Skoog medium the success was 83.9 and 86.7% for 'Kufri Chandramukhi' and 'Kufri Jyoti', respectively; on MS medium, the success was 42.8 and 39.2% for 'Kufri Chandramukhi' and 'Kufri Jyoti', respectively (Table II).

TABLE II. CALLUS IRRADIATION AND REGENERATION

Variety	Dose (Gy)	No. of tubers irradiated	No. of plants regenerated
Kufri	20	25	18/56
Chandramukhi	40	36	0/62
Kufri	20	38	10/62
Jyoti	40	22	1/40

The microtubers obtained from the *in vitro* plantlets were planted on two different dates. Data were obtained on plant morphology and tuber characteristics to study genetic variation. The data were recorded on the irradiated as well as the control (uniradiated) plants. Variation was present for different morphological characters such as number of nodes, leaf ratio, lateral leaf ratio, tuber number and tuber weight (data not shown).

Screening for resistance to late blight was done by using the detached-leaf method. The leaves of plants obtained from microtubers were sterilized and put on the toxic medium containing the fungal-culture filtrate. Plants gave different reaction. There was yellowing and browning of leaves in most cases, but some leaves remained unaffected. The leaves of the *in vitro* produced plants were also placed on the toxic medium and disease reaction was observed. Lesions appeared on the leaves. The number of lesions varied for different plants. The plants were graded on the scale of 1-10 with 10 being the most susceptible plant. The plants were thus classified into resistant, moderately resistant and susceptible types (Table III). All control plants were susceptible. Resistance was observed in both 'Kufri Chandramukhi' and 'Kufri Jyoti', irradiated with 40 Gy and moderate resistance was observed at 20 Gy dose (Table III).

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

Variety	Treatment	Plants with disease reaction			
		R (%)	MR (%)	S (%)	Total plants listed
KCM	50 Gy	36	20	44	39
	30 Gy	12	8	80	40
	Control	-	-	100	6
KJ	40 Gy	20	20	60	40
	20 Gy	8.6	30.4	61	47
	Control	-	-	100	8

The irradiated calli were also placed in Petri dishes containing the toxic medium; but all the calli turned brown and died. When organogenic calli were irradiated and placed on the toxic medium, all the calli from 40 Gy dose died after turning brown/black. However, a few calli from 20 Gy survived and showed greenish sectors. For heat tolerance, 29% of the irradiated calli showed regeneration.

Future work plan

Screening for late blight

a) The plantlets regenerated from resistant calli will be screened for late blight resistance by detached leaf method.

b) The tubers obtained from potted plants will be used in field trial to select for late blight resistance and agronomically important traits.

Screening for heat tolerance

a) Microtubers obtained at 28 ± 2°C and 20°C will be planted in field at different times (September 1, 15, 30 and 15th October 1995).

b) Plantlets regenerated from irradiated calli will be used for microtuber formation at 28 ± 2°C, and tubers thus obtained will be planted in the field.

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