*Indian Phytopath.* 52 (2) : 169-171 (1999)

# *In vitro* induction and selection for late blight resistance in potato

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**Key words** : Gamma rays, *Phytophthora infestans*, culture filtrate, late blight, potato, resistance

Potato is a model crop for tissue culture studies as it is amenable to a wide range of tissue culture techniques from micropropagation to regeneration of whole plants from cultured protoplasts (3,5). Improvement of potato cultivars by irradiation of *in vitro* cultures offers several advantages over conventional methods of plant breeding which are laborious and time consuming. Plants and calli irradiated with gamma rays have been used to induce variability for tolerance to salinity and resistance to pathotoxins (8). Late blight caused by *Phytophthora infestans* (Mont) de Bary is a devastating disease of potato and causes tremendous losses in its yield. Attempts were made to use gamma rays for irradiating the *in vitro* cultures of potato to induce variability for late blight resistance.

Shoot cultures were initiated using explants from nodal sections of grown up plants of the two cultivars of potato, viz., Kufri Jyoti and Kufri Chandramukhi. The nodal sections consisting of a node and a leaf were surface sterilized in a mixture of 0.1 per cent mercuric chloride and 0.1 per cent sodium lauryl sulphate for 5 min. These were rinsed with sterilized distilled water and placed on Murashige and Skoog (6) basal medium with 3 per cent sucrose. Cultures were maintained under light conditions of 16 h photo period (3000-4000 lux light intensity) at day and night temperatures of 28±2°C and 25±2°C, respectively. *In vitro* raised plantlets at four to five nodal segment stage were irradiated with gamma rays at the doses of 20 Gy and 40 Gy. The irradiated plantlets were transferred to fresh medium and kept for two weeks before subculturing for microtuberisation.

Six to eight weeks old *in vitro* plantlets were used as explants for microtuberisation. For initial two months, the cultures were kept on MS basal medium with eight per cent sucrose and incubated at 28±2°C (16 h day) and 25±2°C (8 h night) under light conditions. They were then transferred to dark conditions (3) at 20±2°C (optimum temperature for microtuberisation) after pouring fresh MS medium having benzyl amino purine (10 mg/l) and sucrose (80 g/l). The microtubers obtained were sown in the pots to obtain second generation. The surface sterilized leaves of the full grown plants were placed on Gamborg B-5 media (4) with 3 per cent culture filtrate of *P. infestans*. The culture filtrate was sterilized with filters of size 0.2 µm to remove the fungal spores before adding to the medium. The medium was autoclaved and the filter sterilized culture filtrate was added to the medium under the sterile conditions. The observations were recorded after 5 days on leaf area damaged on a 1-5 rating scale (1: healthy; 2: one or two lesions on the leaf; 3: few lesions on the leaves with browning areas; 4: 25 per cent area of leaves covered with lesions; 5: more than 50% of the leaves covered with lesions with browning areas; 1,2: Resistant; 3: Moderately resistant; 4,5: Susceptible).

For evaluating resistance under natural conditions, the microtubers of the plants categorized as highly resistant and moderately resistant in second generation were sown in pots during the successive year to obtain the third generation. The mature plants were inoculated by spraying with aqueous sporangial inoculum ($4.5\times10^5$ sporangia/ml) of *P. infestans*. The inoculum was prepared by collecting spores from the infected leaves by adding distilled water. The pots were covered with wet polythene bags for 24 h. The disease reaction was recorded six days after inoculation. The plants were graded on a rating scale of 0-5 with 0 indicating no infection and 5 indicating more than 80 per cent leaf area damaged and stems brown (9).

Leaves of the plants originating from microtubers when placed on the media containing 3 per cent culture filtrate of *P. infestans*, varied in reaction. Susceptible reaction (grade S) was observed on all the control plants. In most of the cases, the colour of leaves was

**Table 1.** Disease reaction of *in vitro* screened plantlets for late blight resistance using detached leaf method after irradiation

| Variety | Treatment | Total plant tested | Plants with disease reaction (%) R | MR | S |
|---|---|---|---|---|---|
| Kufri | 40 Gy | 76 | 36 | 20 | 44 |
| Chandramukhi | 20 Gy | 62 | 12 | 8 | 80 |
| | Control | 6 | 0 | 0 | 100 |
| Kufri Jyoti | 40 Gy | 68 | 20 | 20 | 60 |
| | 20 Gy | 74 | 8.6 | 30.4 | 61 |
| | Control | 8 | 0 | 0 | 100 |

R=Resistant; MR=Moderately resistant; S=Susceptible.

**Table 2.** Infection type and disease reaction of the progenies sown under field condtions

| Variety | Irriadiation Treatment 40 GY Progeny No. | 40 GY Infection grade | 20GY Progeny No. | 20GY Infection grade |
|---|---|---|---|---|
| Kufri | 1A | 0(R) | 23C | 5(S) |
| Chandramukhi | 1B | 0(R) | 18A | 5(S) |
| | 3A | 3(MR) | 18B | 2(MR) |
| | 3C | 1(R) | 18C | 1(R) |
| | 14A | 2(MR) | 19A | 0(R) |
| | 14C | 2(MR) | 19C | 0(R) |
| | 16A | 3(MR) | 21A | 0(R) |
| | 17A | 5(S) | 21B | 1(R) |
| | 17B | 2(MR) | 21C | 1(R) |
| | 17C | 1(R) | | |
| | 22A | 5(S) | | |
| | 23A | 5(S) | | |
| Kufri Jyoti | 30A | 2(MR) | 5A | 0(R) |
| | 30C | 1(R) | 5C | 0(R) |
| | 31A | 5(S) | 6A | 0(R) |
| | 31C | 5(S) | 6C | 0(R) |
| | | | 7A | 1(R) |
| | | | 7C | 5(S) |
| | | | 8A | 4(S) |

0 = Healthy resistant (R); 1 = Few specks on leaves only resistant (R); 2 = One or two lesions on few leaves only moderately resistant plants green (MR); 3 = One or two lesions clearly visible on leaves with specks on stem but plants giving green look (MR); 4 = lesions uniform on leaves with few leaves showing drooping, lesions present on stem, plants giving greenish brown look (MS); 5 = >80% of the leaf area damaged with brown stem (S).

yellow and brownish whereas some of the leaves remained unaffected. When irradiated with a dose of 20 Gy, 12 per cent highly resistant and 8 per cent moderately resistant plants were observed in Kufri Chandramukhi whereas 8.6 per cent highly resistant and 30.4 per cent moderately resistant plants were observed in Kufri Jyoti. A dose of 40 Gy induced 3 times more resistant plants in Kufri Chandermukhi and 2.3 times more in Kufri Jyoti (Table 1). The resistant plants tested under *in vitro* conditions were found to be resistant under field conditions as well except in a few cases where resistant as well as susceptible reaction was observed in the progeny of the same plant (Table 2).

The exploitation of *in vitro* selection for the improvement of resistance to pathogens depends on the positive correlation between resistance to toxin or culture filtrate, which can be used as selective agents at cellular level and the tolerance to pathogens at plant level (10). When detached leaves of second generation were placed on *P. infestans* culture filtrate, they exhibited varying degree of resistance. The percentage of resistant plants was more in case of irradiation dose rate of 40 Gy, in both the varieties. Pot sown sprayed with *P. infestans* inoculum also exhibited varying disease reaction. The reaction varied from susceptible, where the plants were completely damaged after sporulation, to completely healthy plants where not even a speck had appeared on the leaves. Cerato *et al.* (2) screened potato plants for late blight resistance and observed that the percentage of clones selected agreed with the known field resistance to foliage blight of the cultivars from which they were derived. In the present study, all the plants obtained from the tubers of single resistant plant in the second generation were not found to be consistent for disease reaction. Lu *et al.* (7) observed that there were differences in the skin colour expression among the vegetatively propagated progenies of single potato plant. This indicates that there was segregation of the mutant character. Based on the segregation, mutants could be differentiated between solid and chimeric mutants (1). Further studies to isolate solid mutants for late blight resistance in potato are in progress.

Financial support to the senior author in the form of Junior Research Fellowship from the Council of Scientific and Industrial Research is gratefully acknowledged.

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Received for publication November 1, 1997