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Biochemical characterisation of induced variants of potato (*Solanum tuberosum* L.)

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Abstract

Gamma rays at dose rates of 20 Gy and 40 Gy were used for induction of variants for late blight resistance in two popular indian cultivars of potato (*Solanum tuberosum* L.) *viz.*, Kufri Jyoti and Kufri Chandramukhi. The field grown variants were assayed biochemically for the enzymes peroxidase, polyphenol oxidase and phenols. Peroxidase enzyme activity increased in the prospective mutants which showed resistance, whereas, polyphenol oxidase activity did not vary from the control, indicating that this enzyme was not linked to resistance phenomenon.

Key words : Potato, irradiation, mutant, peroxidase, polyphenol oxidase, phenols.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops of the world. A combination of *in vitro* techniques and radiation induced mutagenesis has been recommended to upgrade cultivars of vegetatively propagated crops, including potato [1]. Susceptibility to disease in plants depends on whether plant tissues have a sufficiently high concentration of substances essential for growth and nutrition of the pathogen and, therefore, the resistance was determined either by deficiency of such substances in plants or the presence of compounds inhibiting pathogen growth in host cells.

Biochemical studies have been done to correlate plant resistance with chemical composition in higher plants both before and after infection [2-8].

In the present investigation prospective mutants for late blight resistance were obtained by gamma irradiating two popular cultivars of potato *viz.*, Kufri Jyoti and Kufri Chandramukhi. Qualitative and quantitative estimation of enzymes related to disease resistance was done to correlate the activity of enzymes with the disease resistance.

Materials and methods

Two cultivars, Kufri Jyoti and Kufri Chandramukhi, were used for induction of mutants to late blight resistance.

(a) Shoot cultures were initiated from nodal sections of the plants raised from tubers in the pots. The nodal section consisting of a node and a leaf were surface sterilised in a mixture of 0.1% $HgCl_2$ and 0.1% sodium lauryl sulphate for 5-7 minutes. Thereafter, they were rinsed with sterile water and placed on MS basal medium [2]. Cultures were maintained under conditions of 16 hr photoperiod of 3000-4000 lux light intensity at day and night temperatures of 28±2°C and 25±2°C, respectively.

(b) *Irradiation and microtuberisation* : The *in vitro* raised plantlets were irradiated in test tubes at 5-6 nodal segment stage with gamma rays dose of 20 and 40 Gy. 6-8 weeks old *in vitro* plantlets was cut into 5-8 mm long segments each carrying one leaf with axillary bud and cultured on medium consisting of MS salts and 0.8% agar. The plantlets were incubated at 28 ± 1°C. (16 h light) and 25±1°C (8h dark) conditions for 60 days. They were then transferred to dark conditions at 20±2°C after pouring fresh liquid MS medium with BAP (10 mg/l) and sucrose (0.8% w/v). The microtubers obtained after irradiation were designated as M_1V_1 generation.

(c) *Screening procedure* : The microtubers were sown in the pots. The leaves from plants thus obtained were screened *in vitro* for disease resistance by placing them on toxic medium containing the culture filtrate of *Phytophthora infestans* in B-5 medium [3]. Observations were recorded after 5 days, and the plantlets were graded on scale of 1-5 and classified as resistant, moderately resistant and susceptible. For field evaluation, the plants-categorised as resistant and moderately resistant in M_1V_2 generation (second vegetative generation of mutagenised plants) were sown in the pots to obtain M_1V_3 (third vegetative generation of mutagenised plants). They were sprayed with sporangial inoculum of *P. infestans* and the plants were graded on a scale of 0-6.

(d) *Biochemical analysis* : Leaf samples at

flowering stage were weighed and grounded in prechilled pestle mortar using gel buffer at pH 8.65. The leaf extracts were centrifuged and the supernatant was stored at 4°C. Horizontal starch gel electrophoresis was used to study isoenzymes of peroxidase following the method of Smithies [4] with discontinuous buffer system of Poulick [5].

For quantitative assay, one gram of leaf was weighed and grounded in 1 ml of phosphate buffer in prechilled pestle and mortar. The extract was filtered and stored at 4°C. Peroxidase activity was measured by using the method of Shannon *et al.* [6]. For enzyme assay, 0.1 ml extract, 3.55 ml phosphate buffer, 0.25 ml O-dianisidine and 0.1 ml H_2O_2 were pipetted in cuvette. The change in absorbance was recorded every 15 seconds for 2 minutes. Polyphenol oxidase activity was measured by the modified method of Bateman [7]. For assay, 1 ml of extract was added to 3 ml catechol solution and placed in spectrophotometer. Change in absorbance was recorded every 30 seconds for 2 minutes.

Total phenols were estimated using the Swain and Hillis [8] method. 200 mg of dry material was extracted with 10 ml of 80% acetone. Extract was centrifuged at 3000 rpm. The supernatant was discarded and the pellet was dissolved in 3 ml of 0.3 N HCl in methanol and centrifuged. The supernatant was evaporated to dryness and the dry residue was dissolved in 5 ml distilled water and centrifuged at 3000 rpm. The supernatant was used for determination of phenols. To 1 ml of phenol extract 0.5 ml offolin ciocalteau reagent was added. After 3 minutes 6.5 ml distilled water was added. Later 1 ml of sodium carbonate solution was added and the volume adjusted to 10 ml. The above mixture was kept for 1 hr. for colour development and absorbance was recorded at 630 nm.

Results and discussion

The peroxidase activity was studied in leaf samples. The activity varied from 0.005 to 0.086 OD/min/mg (Table 1). The resistant variants had 0.084, 0.072, 0.077, 0.078, 0.072, 0.064 and 0.086 OD/min/mg. The moderately resistant cultivars had 0.056, 0.058, 0.062, 0.044 and 0.046 OD/min/mg, whereas, the susceptible cultivars had the values ranging from 0.005 to 0.025 OD/min/mg. Peroxidase enzye activity was thus directly correlated with the degree of resistance in the variants.

The susceptible cultivars had much less peroxidase activity as compared to the resistant variants. Peroxidase activity in the susceptible variant was comparable to the peroxidase activity in the control plants. Thukral *et al.* [9] have correlated higher activity of peroxidase with resistance to downy mildew in pearl millet. Sherif *et al.* [10] also reported that wheat cultivars resistant to *Erysiphe graminis* f. sp. *tritici* showed higher peroxidase and polyphenol oxidase activities. According to Arora *et al.* [11], increase in peroxidase activity of the infected plant of susceptible cultivars than healthy plants during pathogenesis of pearl millet by downy mildew was due to acceleration of host senescence by the pathogen.

Table 1. Peroxidase and polyphenol oxidase activity and phenol content in the variants of potato varieties Kufri Chandramukhi and Kufri Jyoti isolated after gamma irradiation in M_1V_3 generation

Variety	Treatment (Dose)	Disease Reaction	Enzyme activity		Phenol content (mg)
			Peroxidase (OD/min/mg)	Polyphenol oxidase (OD/min/mg)	
Kufri Chandramukhi					
	20 Gy	R	0.072	0.021	48.2
		R	0.084	0.020	56.5
		MR	0.012	0.024	36.3
		S	0.012	0.012	10.3
		MR	0.091	0.017	33.8
		S	0.025	0.014	14.6
	40 Gy	MR	0.056	0.016	32.3
		R	0.028	0.017	41.4
		S	0.014	0.015	15.5
		R	0.091	0.019	41.4
	Control	S	0.025	0.013	11.6
		S	0.020	0.012	10.8
Kufri jyoti					
	20 Gy	R	0.077	0.020	40.6
		R	0.078	0.021	48.2
		MR	0.046	0.019	39.8
		R	0.082	0.016	-
		S	0.019	0.012	-
		S	0.025	0.012	11.8
	40 Gy	MR	0.086	0.016	33.6
		R	0.086	0.019	35.9
		R	0.082	0.019	51.3
		R	0.086	0.018	56.8
		R	0.055	0.014	39.2
	Control	S	0.005	0.013	14.2
		S	0.021	0.012	10.6

R: Resistant, MR : Moderately resistant, S : Susceptible

The polyphenol oxidase activity was also studied in the leaf samples. The activity ranged from 0.012 to 0.020 OD/min/mg in the resistant variants. The moderately resistant variants showed 0.016, 0.024, 0.017, 0.016, 0.019 and 0.014 OD/min/mg whereas the values in the susceptible cultivars were 0.012, 0.015, 0.013 and 0.014 OD/min/mg. The polyphenol oxidase activity was not significantly different in the resistant and susceptible variants. Therefore the polyphenol oxidase activity could not be correlated with resistance in the present study. Thukral *et al.* [9] reported positive correlation between polyphenol oxidase activity and disease resistance.

Total phenols were assayed in the late blight resistant and susceptible cultivars along with control. The total phenol content varied from 10.6 to 56.8 mg. The total phenolic content was 56.8, 57.8, 56.5, 48.2, 41.4, 48.2 to 40.6 mg in the different resistant variants. In the moderately resistant variant, the values were 33.8, 32.3, 36.3, 39.8, 35.9 to 39.3 mg whereas the susceptible cultivars had phenol content of 10.6, 10.8, 14.2, 11.8, 12.6 and 14.6mg.

The amount of phenols was higher in the resistant genotypes as compared to susceptible ones. Analysis for total phenols showed that the resistant cultivars, which were least affected in field conditions when artificially inoculated had a high content of phenols and the susceptible cultivars (most severely affected under the field conditions) had the lowest phenol content. The susceptible plants had the total phenol content comparable to the control. Prashar and Sindhana [12] reported that quantity of total phenols was high in the stem and leaves in pea varieties resistant to powdery mildew as compared to the susceptible ones, but Seever and Doly [13] reported no significant differences in total phenol compounds between the healthy or inoculated resistant and susceptible plant at any stage of disease development.

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