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Short communication

Preliminary screening of *in vitro* raised ginger regenerants to soft rot and bacterial wilt diseases using electrolyte leakage method

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ABSTRACT

Two hundred and ninety six somaclones of ginger cvs. Maran and Rio-de-Janeiro regenerated through indirect organogenesis, indirect embryogenesis and *in vitro* mutagenesis were screened against soft rot and bacterial wilt diseases using toxic metabolites of *Pythium aphanidermatum* and *Ralstonia solanacearum* by electrolyte leakage method. Twenty four per cent of the clones exhibited low leakage of electrolytes to toxic metabolites of pathogens showing their tolerance to diseases. Regenerants of cv. Maran exhibited less leakage of electrolytes to the toxic metabolites. The regenerants derived through indirect embryogenesis showed higher tolerance to both the diseases.

Key words: Electrolyte leakage, bacterial wilt, ginger, soft rot, somaclones.

Soft rot caused by *Pythium* spp. and bacterial wilt caused by *Ralstonia solanacearum* are the major constraints in production of ginger (*Zingiber officinale* Rosc.). Attempts to isolate resistant clones using conventional breeding techniques were not successful in ginger as genetic variability available for disease resistance/ tolerance is low and all the available cultivars/ varieties were found to be susceptible to the diseases. Studies were conducted to manage soft rot and bacterial wilt diseases in ginger using cultural, chemical and biological methods by several workers and none of the methods gave absolute control for the two diseases (Kumar and Hayward, 1). Breeding through selection and hybridization are not possible in ginger due to lack of variability and absence of natural seed set. Therefore, broadening the genetic base through *in vitro* techniques like indirect organogenesis, indirect embryogenesis and *in vitro* mutagenesis would be of great significance in crop improvement programmes in ginger (Paul *et al*., 5).

Preliminary screening against soft rot and bacterial wilt diseases were carried out in regenerants of ginger cvs. Maran and Rio-de-Janeiro derived through indirect organogenesis, indirect embryogenesis and *in vitro* mutagenesis. Regenerants were produced through indirect organogenesis from shoot tip explants of cvs. Maran and Rio-de-Janeiro on half-strength MS basal medium supplemented with 3.00 mg l^{-1} BAP. Forty regenerants of Maran and 44 regenerants of Rio-de-Janeiro produced through indirect organogenesis were subjected to screening. Regenerants were produced through indirect embryogenesis from rhizome bud explants of the two cultivars on half strength MS basal medium supplemented with 0.50 mg l^{-1} 2,4-D and 1.00 mg l^{-1} BAP. Thirty four regenerants of Maran and 38 regenerants of Rio-de-Janeiro produced through indirect embryogenesis were subjected to screening. Morphogenic cultures derived from organogenic and embryogenic calli of the two cultivars were subjected to γ irradiation (10 and 20 Gy) selected after screening at dozes ranging from 10 to 50 Gy. After the irradiation, cultures were transferred on to half-strength MS medium supplemented with 3.00 mg l^{-1} BAP. One hundred and forty regenerants produced through *in vitro* mutagenesis were subjected to screening.

Regenerants produced through different routes were screened by electrolyte leakage method using toxic metabolites of *P. aphanidermatum* and *R. solanacearum.* The predominant species of *Pythium* causing soft rot of ginger in Kerala is *P. aphanidermatum*. The pathogen was isolated from naturally infected rhizomes. Pathogenicity of the isolated culture was tested by inoculating 7-d-old culture of the fungus on healthy surface sterilized ginger rhizomes. Inoculated rhizomes were kept in aseptic moist chamber and incubated at room temperature till rotting of the rhizomes was observed. Toxic metabolite(s) of *P. aphanidermatum* were produced as per the procedure reported by Paul and Shylaja (3). Five mm culture discs of 7-d-old culture of the fungus to Asparagine or synthetic mucor medium. The cultures were incubated for 15 days under shaking condition at 27°C with a shaking speed of 100 rpm. The culture filtrate was collected after filtering successively through a muslin cloth and Whatman No.1 filter paper. The filtrates were concentrated to one-tenth of its volume using a hot plate maintained at 100°C to produce concentrated culture filtrate (CCF).

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Ginger plants showing symptoms of bacterial wilt disease were collected and subjected to ooze test to confirm the presence of bacterium. Such pieces with profuse bacterial ooze were cut into small bits and surface sterilized with 0.1% mercuric chloride solution for 1 min. and then washed free off the sterilant. These bits were crushed on a sterilized glass slide with a few drops of sterile distilled water to obtain a bacterial suspension. One loopful of suspension was streaked on triphenyl tetrazolium chloride (TZC) medium to get well-isolated colonies of the bacterium. The cell free culture filtrate of *R. solanacearum* was produced as described by Paul and Shylaja (3). *R. solanacearum* was cultured in peptone casamino acid broth for 5 d in a shaker cum incubator maintained at 27°C with a shaking speed of 100 rpm. The bacterial broth was autoclaved at 15 psi for 20 min. and then filtered. Toxin was precipitated by adding acetone to the extract. The precipitate was allowed to settle overnight and then separated by centrifugation at 5,600 rpm for 20 min. The precipitate was then washed with acetone and kept for evaporation. The toxic metabolite thus obtained was dissolved in distilled water for bioassay. Screening by electrolyte leakage using toxic metabolites of *P. aphanidermatum* and *R. solanacearum* was conducted as per the procedure reported by Paul and Shylaja (3). Leaves for electrolyte leakage studies were collected from three-month-old healthy regenerants growing in shaded net house. The leaves were cut into small pieces (1 cm long and 5 mm wide) and random samples (200 mg) were enclosed in muslin cloth and placed in test tubes. Three millilitres of diluted toxin preparation (10% v/v) was infiltered into the leaf sections in vacuum for 10 min. The leaf sections were then rinsed with 4 ml of distilled water. Conductance of ambient solution was measured in µ Siemens (µS) with a high precision conductivity meter (Systronics 20). Experiments were repeated thrice.

Electrolyte leakage induced by toxic metabolites of pathogens, 10 min. after infiltration was used for comparison. Leakage of electrolytes induced by toxic metabolite(s) of *P. aphanidermatum* ranged from 14.10 to 64.05 µS in the various regenerants screened (Table 1). The regenerants of cv. Rio-de-Janeiro exhibited more leakage of electrolytes (14.85 - 64.05 µS) than regenerants of cv. Maran (14.10 - 55.25 µS). The regenerants were grouped into three categories based on their leakage values. Regenerants, which came in the first two classes of frequency table (< 24.50 µS) were designated as regenerants of low leakage group, next four classes (24.50 - 52.49 µS) as of medium leakage group and last two classes (> 52.50 µs) as of high leakage group. Of the regenerants screened, 26% came in the low leakage group, 68% in the medium leakage group and six per cent in the high leakage group. Similar observations were made by Shylaja *et al*. (6), when they used electrolyte leakage method for screening black pepper calliclones against *Phytophthora* foot rot disease. Loss of electrolytes was much lower in the tolerant cv. Cheriakanyakkadan as compared to susceptible cv. Karimunda. Regenerants of two cultivars when compared, somaclones of cv. Maran exhibited low leakage of electrolytes than clones of cv. Rio-de-Janeiro. Leakage of electrolytes was low in 28% regenerants of Maran as compared to 23% of Rio-de-Janeiro. Hence, the regenerants of Maran exhibited more tolerance to soft rot. The tolerance reaction of cv. Maran to soft rot disease was reported by Paul *et al*. (4). Majority of the regenerants of both cultivars (68-69%) exhibited leakage of electrolytes in the medium range. More number of regenerants of Rio-de-Janeiro (9%) exhibited high leakage of electrolytes as compared to regenerants of Maran (3%).

Plantlets regenerated through indirect organogenesis/ embryogenesis and *in vitro* mutagenesis of two cultivars showed significant variations in

Table 1. Frequency distribution of electrolyte leakage induced by toxic metabolites of *P. aphanidermatum* and *R. solanacearum* in ginger regenerants.

P. aphanidermatum			*R. solanacearum*		
Electrolyte leakage (µS)	Frequency (%)		Electrolyte leakage (µS)	Frequency (%)	
	Maran	Rio-de-Janeiro		Maran	Rio-de-Janeiro
10.50-17.49	6.80	3.31	25.20-30.10	8.16	7.28
17.50-24.49	21.09	19.87	30.20-35.10	12.93	13.25
24.50-31.49	27.21	26.49	35.20-40.10	31.29	39.07
31.50-38.49	24.49	17.22	40.20-45.10	26.53	19.87
38.50-45.49	8.84	11.92	45.20-50.10	13.61	8.61
45.50-52.49	8.84	11.92	50.20-55.10	6.12	4.64
52.50-59.49	2.72	7.28	55.20-60.10	1.36	6.62
59.50-66.49	-	1.99	60.20-65.10	-	0.66

electrolyte leakage values induced by toxic metabolites of pathogens. When electrolyte leakage induced by toxic metabolite(s) of *P. aphanidermatum* was compared, regenerants derived through indirect embryogenesis exhibited low leakage of electrolytes in the range of 14.60-64.05 µS with mean leakage of 31.30 µS and were more tolerant to the disease (Table 2). In regenerants from irradiated calli, the mean leakage of electrolytes was 32.56 µS and in plantlets regenerated through indirect organogenesis, the mean leakage of electrolytes observed was 34.58 µS. The leakage of electrolytes was low in 18% regenerants derived through somatic embryogenesis, 14% regenerants derived through *in vitro* mutagenesis and 8% regenerants derived through indirect organogenesis (Table 3). Majority of the regenerants derived through somatic embryogenesis exhibited leakage of electrolytes in the lower range indicating tolerance of the clones to soft rot disease. Electrolyte leakage was in the medium range in 20% regenerants derived through indirect organogenesis, 17% regenerants derived through *in vitro* mutagenesis and 14% regenerants derived through indirect embryogenesis. Leakage of electrolytes was in the high range in five per cent regenerants derived through somatic embryogenesis and two per cent regenerants each derived through indirect organogenesis and *in vitro* mutagenesis. Liu *et al.* (2) reported that doubled haploid lines of rapeseed derived through somatic embryogenesis exhibited greater resistance to stem rot disease (*Sclerotinia sclerotiarum*) than the donor lines and the resistant control Zhongyou 821.

The cultivar response within each group of regenerants was also analysed. In all the three groups, the regenerants of cv. Maran exhibited less leakage of electrolytes (30.77 µS) to toxic metabolite(s) of *P. aphanidermatum* as compared to regenerants of cv. Rio-de-Janeiro. The regenerants of Maran derived through irradiation (20 Gy) of embryogenic calli exhibited low leakage of electrolytes (26.19 µS) indicating more tolerance of the regenerants to *P. aphanidermatum* as compared to other groups of regenerants. Leakage of electrolytes induced by toxin of *R. solanacearum* varied between 26.45-63.90 µS in the regenerants of two cultivars (Table 1). The regenerants of Rio-de-Janeiro exhibited more leakage (26.80-63.90 µS) than the regenerants of cv. Maran (26.45-57.15 µS). Regenerants, which came in the first two classes of frequency table (< 35.20 µS) were considered as regenerants of low leakage group, next four classes (35.20-55.10 µS) as of medium leakage group and last two classes (> 55.10 µS) as of high leakage group. Of the regenerants screened, 21% came in the low leakage group, 75% in the medium leakage group and four per cent in the high leakage group. In regenerants of the two cultivars screened, 21% each of the regenerants of cvs. Maran and Rio-de-Janeiro came in the low electrolyte leakage group indicating more tolerance of the regenerants to bacterial wilt disease. Electrolyte leakage was in the medium range in 77% regenerants of cv. Maran and 72% in regenerants of cv. Rio-de-Janeiro. Only one per cent regenerants of cv. Maran came in the high leakage group, while seven per cent regenerants of cv. Rio-de-Janeiro came in the group, indicating the high susceptibility of regenerants of cv. Rio-de-Janeiro to bacterial wilt. When electrolyte leakage induced by toxic metabolite(s) of *R. solanacearum* was compared between regenerants derived through different routes, regenerants through somatic embryogenesis exhibited low leakage of electrolytes (36.86 µS) and were more tolerant to the bacterial wilt disease (Table 2). In regenerants produced through indirect organogenesis and *in vitro* mutagenesis, the mean leakage of electrolytes was 41 µS. The leakage of electrolytes was low in 23% regenerants derived through somatic embryogenesis and seven per cent regenerants each derived through indirect organogenesis and *in vitro* mutagenesis (Table 3).

Table 2. Electrolyte leakage in regenerants of ginger produced through various *in vitro* routes.

Group	No. of plants screened	Electrolyte leakage (µS)-range		Mean electrolyte leakage (µS)	
		P. aphanidermatum	*R. solanacearum*	*P. aphanidermatum*	*R. solanacearum*
MC	40	16.00-51.15	29.25-57.15	34.72	40.15
RC	44	19.20-60.65	26.80-63.90	34.43	42.54
Mse	34	14.60-51.90	26.45-51.10	27.31	37.20
Rse	38	14.85-64.05	26.85-54.05	35.29	36.52
MC 10 Gy	40	15.45-55.25	29.10-54.95	34.85	39.64
RC 10 Gy	35	16.75-58.45	26.95-58.75	34.99	41.23
Mse 20 Gy	32	14.10-36.25	29.80-55.85	26.19	43.27
Rse 10 Gy	33	16.85-63.65	30.20-56.20	34.19	40.04
$CD_{0.05}$				4.93	3.03

Table 3. Frequency distribution of electrolyte leakage induced by toxic metabolites of *P. aphanidermatum* and *R. solanacearum* in regenerants of ginger produced *in vitro*.

Electrolyte leakage (µS)	*P. aphanidermatum*							Electrolyte leakage (µS)	*R. solanacearum*						
	Frequency (%)								Frequency (%)						
	MC	RC	Mse	Rse	MC 10 Gy	RC 10 Gy	Mse 20 Gy	Rse 10 Gy		MC	RC	Mse	Rse	MC 10 Gy	RC 10 Gy	Mse 20 Gy	Rse 10 Gy
10.50-17.49	5.00	-	8.82	7.89	7.50	2.86	6.25	3.03	25.20-30.10	7.50	6.82	14.71	15.79	7.50	5.71	3.13	3.03
17.50-24.49	10.00	18.18	35.29	21.05	17.50	22.86	31.25	18.18	30.20-35.10	7.50	6.82	23.53	26.32	15.00	8.57	6.25	9.09
24.50-31.49	15.00	36.36	29.41	18.42	20.00	20.00	43.75	30.30	35.20-40.10	37.50	29.55	29.41	39.47	30.00	42.86	28.13	48.48
31.50-38.49	40.00	13.64	14.71	21.05	20.00	14.29	18.75	18.18	40.20-45.10	27.50	27.27	20.59	10.53	35.00	17.14	18.75	21.21
38.50-45.49	17.50	11.36	5.88	2.63	10.00	22.86	-	12.12	45.20-50.10	15.00	9.09	5.88	5.26	7.50	11.43	28.13	9.09
45.50-52.49	12.50	11.36	5.88	10.53	15.00	11.43	-	15.15	50.20-55.10	2.50	9.09	5.88	2.63	5.00	-	12.50	6.06
52.50-59.49	-	6.82	-	15.79	10.00	5.71	-	3.03	55.20-60.10	2.50	9.09	-	-	-	14.29	3.13	3.03
59.50-66.49	-	2.27	-	2.63	-	-	-	-	60.20-65.10	-	2.27	-	-	-		-	-

MC = Plantlets – indirect organogenesis – cv. Maran; RC = Plantlets – indirect organogenesis – cv. Rio-de-Janeiro; Mse = Plantlets – indirect embryogenesis – cv. Maran; Rse = Plantlets – indirect embryogenesis – cv. Rio-de-Janeiro; MC 10 Gy = Plantlets – indirect organogenesis – cv. Maran – irradiated at 10 Gy; RC 10 Gy = Plantlets – indirect organogenesis – cv. Rio-de-Janeiro – irradiated at 10 Gy; Mse 20 Gy = Plantlets – indirect embryogenesis – cv. Maran – irradiated at 20 Gy; Rse 10 Gy = Plantlets – indirect embryogenesis – cv. Rio-de-Janeiro – irradiated at 10 Gy.

Majority of the regenerants derived through somatic embryogenesis exhibited leakage of electrolytes in the lower range indicating tolerance of the clones to bacterial wilt disease. Electrolyte leakage was in the medium range in 20% regenerants each derived through indirect organogenesis and *in vitro* mutagenesis and 15% regenerants derived through indirect embryogenesis. None of the regenerants derived through somatic embryogenesis exhibited electrolyte leakage in the high range while three per cent regenerants each derived through indirect organogenesis and *in vitro* mutagenesis exhibited higher leakage of electrolytes.

Preliminary screening of 296 regenerants against soft rot and bacterial wilt diseases by electrolyte leakage method revealed that 24% of the clones exhibited low leakage of electrolytes showing their tolerance to both the diseases. Regenerants of cv. Maran exhibited less leakage of electrolytes to toxic metabolite(s) of *P. aphanidermatum* and *R. solanacearum*. The regenerants derived through indirect embryogenesis showed higher tolerance to diseases in the screening trials which need further confirmation in field evaluation and detailed screening studies.

REFERENCES

1. Kumar, A. and Hayward, A.C. 2005. Bacterial diseases of ginger and their control. **In**: *Ginger. The Genus Zingiber*, P.N. Ravindran, and K.N. Babu (Eds.). CRC Press, London, pp. 341-66.

2. Liu, S., Wang, H., Zhang, J., Fitt, B.D.L., Xu, Z., Evans, N., Liu, W., Yang, W. and Guo, X. 2005. *In vitro* mutation and selection of double haploid *Brassica napus* lines with improved resistance to *Sclerotinia sclerotiarum*. *Plant Cell Rep.* **24**: 133-44.

3. Paul, R. and Shylaja, M.R. 2009. Production of toxic metabolites in cultures by *Pythium aphanidermatum* and *Ralstonia solanacearum* and bioassay of the metabolites. *J. Mycol. Pl. Pathol.* **39**: 422-26.

4. Paul, R., Shylaja M.R., Abraham, K. and Nybe, E.V. 2011. Screening somaclones of ginger for soft rot disease. *J. Mycopathol. Res.* **49**: 83-87.

5. Paul, R., Shylaja, M.R. and Nazeem, P.A. 2012. Molecular characterization of somaclones in ginger. *Indian J. Hort.* **69**: 221-25.

6. Shylaja, M.R., Nair, G.S. and Matew, J. 1996. Screening of black pepper (*Piper nigrum* L.) calliclones for *Phytophthora* foot rot resistance/ tolerance. *J. Trop. Agri.* **34**: 115-20.

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