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Screening somaclones of ginger (*Zingiber officinale* Rose.) for Soft Rot disease

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Ginger somaclones of the two cultivars viz., Maran and Rio-de-Janeiro regenerated through bud culture were screened against soft rot disease caused by *Pythium aphanidermatum* at College of Horticulture, Kerala Agricultural University, Thrissur, Kerala during 2003 to 2005. Three screening methods viz., planting in sick field, inducing electrolyte leakage using toxic metabolite(s) of the pathogen and artificial inoculation of clones with soft rot pathogen inoculum were adopted. In natural screening in sick field, thirteen per cent of somaclones were not affected by soft rot disease and survived in sick field. In screening by electrolyte leakage method using toxic metabolite(s) of *Pythium aphanidermatum,* 60 per cent of somaclones exhibited low leakage of electrolytes as compared to conventionally propagated plants. Somaclones of Maran were found more tolerant to the disease as compared to clones of Rio-de-Janeiro. In artificial inoculation of the soft rot pathogen, all the clones took infection, but subsequent germination of rhizomes was observed in three somaclones viz., M VI, 364 R and R XI. Based on field evaluation and reaction of ginger somaclones in different screening methods, three somaclones viz., M VI, 364 R and R XI with high yield showing tolerance to soft rot disease could be located which could be used for further field evaluation / production programmes.

Key words: Artificial screening, electrolyte leakage, natural screening

INTRODUCTION

The major constraints in production of ginger *(Zingiber officinale* Rose.) are soft rot disease caused by *Pythium* spp. and bacterial wilt disease caused by *Ralstonia solanacearum.* Attempts to isolate resistant clones using conventional breeding techniques are not successful in ginger as the genetic variability available for disease resistance / tolerance is low and all the available cultivars / varieties are susceptible to the diseases. Studies are conducted to manage soft rot disease in ginger using cultural, chemical and biological methods by several workers and none of the methods exhibits a satisfactory control of the disease (Kurucheve, 1980; Shanmugham, 1996; Vilasini, 1996; Joseph, 1997; George, 1999). Broadening the genetic base through *in vitro* culture induces variability and exploitation of somaclonal variation for isolation of desirable plant types are of great significance in crop improvement programmes of ginger. Hence, investigations have been undertaken at College of Horticulture, Kerala Agricultural University, Thrissur, Kerala to screen somaclones of ginger regenerated through bud culture against soft rot disease.

MATERIALS AND METHODS

Somaclones of two ginger cultivars viz., Maran and Rio-de-Janeiro derived through *in vitro* adventitious bud regeneration after passing through 10 to 12 subculture cycles and planted out during 1999-2000 for rhizome development, formed the base material for the study. Somaclones were screened for resistance / tolerance to soft rot disease by three methods viz., screening in sick field, inducing electrolyte leakage from leaves using toxic metabolite(s) of pathogen and artificially inoculating

clones with pathogen inoculum. Conventionally propagated (CP) plants of cultivars Maran and Rio-de-Janeiro served as control.

Screening of somaclones in sick field

Seventy three somaclones of cultivar Maran and 78 somaclones of cultivar Rio-de-Janeiro along with conventionally propagated plants of the two cultivars were screened for field reaction to *Pythium* incited soft rot disease. Somaclones were screened in a sick field where ginger grown in the previous season was severely infected by soft rot disease. Inoculum level in the field was made uniform by ploughing the area and rhizome bits of somaclones (15-20 g) and control plants were planted in mounds of size 50 cm height and 30 cm base diameter. Three replications of each clone were maintained. Incidence of the disease was recorded at fortnightly intervals during the growth period of April to December 2003. The percentage infection was worked out noting the number of plants infected, out of the total number planted.

Screening by electrolyte leakage method

Seventy three somaclones of cv. Maran and 78 somaclones of cv. Rio-de-Janeiro were screened by electrolyte leakage method using toxic metabolite(s) of *P. aphanidermatum.* Liquid cultures of pathogen were initiated in liquid Asparagine or synthetic Mucor medium (Hesseltine, 1954) by inoculating five mm culture discs of seven day old cultures of the fungus grown in potato dextrose agar medium. The cultures were incubated for a period of 15 days under shaking condition. The culture filtrate was collected after filtering successively through a muslin cloth and Whatman No.I filter paper. The filtrate was concentrated to one tenth of its volume by keeping the filtrate on a hot plate maintained at 100° C to produce concentrated culture filtrate (CCF). The CCF was then diluted to 10 per cent with distilled water.

Medium mature leaves of somaclones used for electrolyte leakage studies were prepared as described by Vidyasekharan *et al.* (1986) and Shylaja *et al.* (1997). The leaves were cut into small pieces (1 cm long and 5 mm wide) and random samples (200 mg) were enclosed in cheese cloth and placed in test tubes. Three milliliters of diluted toxin preparation (10% v/v) was infiltered into the leaf sections in vacuo for 10 minutes. 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 Systronics 20 conductivity meter. Experiments were repeated thrice.

Screening by artificial inoculation of P. aphanidermatum in selected superior somaclones

Artificial screening against soft rot disease was conducted during June to September 2005 in ten somaclones of ginger selected based on yield and disease resistance reaction assessed in sick plot and electrolyte leakage studies. Artificial inoculation and scoring were done as reported by Vilasini (1996) and Shanmugham (1996) with minor modifications in scoring. Ginger sprouts emerged from 15 g seed bits, one month after sprouting were used for artificial inoculation. These sprouts were planted in polybags (22 x 15 cm) filled with potting mixture prepared with sand, cow dung and sick soil (soil heavily infected with soft rot disease along with diseased rhizome bits) in the proportion 1:1:1. Three replications were maintained for each clone. The symptoms were recorded daily for a period of eight weeks. The symptoms were recorded using the following score : 0 - No disease; 1 - 1 - 25 % leaves showing yellowing; 2 - 2 -26 - 50 % leaves showing yellowing; 3 - 51-75 % leaves showing yellowing; and 4 - More than 75 % leaves showing yellowing and subsequent death

Field evaluation of somaclones for yield

Somaclones were evaluated for yield for three consecutive seasons (2002, 2003 and 2004). The field was prepared by ploughing and mounds of size 50 cm height and 30 cm base diameter were taken at a spacing of 40 cm. Raised beds of size l x l m were prepared with an interspace of 40 cm between beds. Rhizome bits of 15 to 20 g weight were used as seed material. In first year, planting was done in mounds. In second and third year, planting was done in beds or mounds as per the availability of seed rhizomes. The crop was managed as per the Package of Practices recommendations of Kerala Agricultural University (KAU, 2002). The rhizomes were harvested eight months after planting by uprooting individual clumps.

RESULTS AND DISCUSSION

Screening of somaclones in sick field

In natural screening of somaclones in sick field, the plants took infection 45 days after planting. The incidence of disease was less in somaclones as compared to CP plants of both cultivars. Soft rot incidence was noticed in 86 per cent clones of cv. Maran and 88 per cent clones of cv. Rio-de-Janeiro (Table 1). Pre emergence rotting was observed in 27.39 per cent clones of cv. Maran and 25.64 per cent clones of cv. Rio-de-Janeiro. Post emergence rotting was observed in 58.91 per cent clones of cv. Maran and 62.82 per cent clones of cv. Rio-de-Janeiro. Soft rot incidence was more in clones of Rio-de-Janeiro than in clones of Maran. The tolerance reaction of cv. Maran to soft rot disease was reported by Nair (1969), Nair (1975) and Nybe (1978). Fourteen per cent somaclones of cv. Maran and 12 per cent somaclones of cv. Rio-de-Janeiro were not affected by soft rot disease in the sick field. The clones that survived in the sick field showed no incidence of the disease in the clonal evaluation plot which was done simultaneously, showing the effectiveness of natural screening method in the selection of plant types tolerant to diseases.

Table 1 : Natural screening of ginger somaclones in sick field against soft rot disease

Cultivar	Total No. of somaclones planted	No. of somaclones affected by soft rot disease	Soft rot incidence (%)
Maran	73	63	86.30
Rio-de-Janeiro	78	69	88.46

Screening by electrolyte leakage method

Electrolyte leakage induced by toxic metabolite(s) from leaves of somaclones, 10 minutes after infiltration was used for comparison. Somaclones exhibited low leakage of electrolytes as compared to CP plants. Leakage of electrolytes induced by toxic metabolite(s) of *P. aphanidermatum* ranged from 14.75 to 59.85 µS in clones of Maran and from 16.10 to 58.35 µS in clones of Rio-de-Janeiro (Table 2). Somaclones that came in the first two classes of frequency table, exhibiting leakage value of less than 25 µS were designated as clones of low leakage group, next four classes as clones of medium leakage group (25.00 - 48.99 µS) and last two classes (> 49 µS) as clones of high leakage group (Table 3). The leakage of electrolytes was comparatively low in somaclones of Maran. The number of clones of Maran were higher in the low and medium leakage group and lower in the high leakage group. Only three per cent clones of Maran exhibited high leakage values as compared to eight per cent clones of Rio-de-Janeiro. Somaclones in general recorded lower electrolyte leakage values as compared to CP plants. Clones of Maran exhibited superiority over clones of Rio - de -Janeiro in resistance / tolerance to soft rot. Seventy one per

Table 2 : Screening of ginger somaclones by electrolyte leakage method against soft rot disease

Electrolyte leakage (µs)				Somaclones (Cvs.) superior to CP plants (%)	
Somaclones (Cvs.) (Range of electrolyte leakage)		Conventionally propagated plants (cvs.)			
Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro	Maran	Rio-de-Janeiro
14.75–59.85	16.10–58.35	37.95	35.00	71.43	56.00

Table 3 : Frequency distribution of electrolyte leakage induced by toxic metabolitie(s) of *P. aphanidermatum* in somaclones of ginger.

Group No.	Mean electrolyte leakage (µS)	Frequency (%)	
		Maran	Rio-de-Javeiro
1	13.00—18.99	11.68	13.33
2	19.00—24.99	19.48	18.67
3	25.00—30.99	22.08	13.33
4	31.00—36.99	12.99	13.33
5	37.00—42.99	23.38	8.00
6	43.00—48.99	7.79	25.33
7	49.00—54.99	1.30	5.33
8	55.00—60.99	1.30	2.67

cent clones of Maran exhibited lower leakage values than CP plants compared to 56 per cent clones of Rio-de-Janeiro. Similar observations were made by Shylaja *et al.* (1996), 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.

Artificial screening of selected superior somaclones

In artificial screening done by soil inoculation method, symptoms of soft rot disease were noticed in all the somaclones inoculated with *P. aphanidermatum,* but the intensity and time taken for appearance of symptoms varied and it took 9 to 41 days for appearance of symptoms in different somaclones (Table 4). The clone 342 M (9.33 days) and the CP plants of cv. Maran (14 days) and Rio-de-Janeiro (12.67 days) were the first to take infection. Rotting and subsequent drying of tillers were observed in these clones three weeks after inoculation. In two clones viz., 364 R and M VI, there were considerable delay in the appearance of symptoms and rotting and subsequent drying of pseudostem occurred six to seven weeks after inoculation. In five somaclones viz. M VI, 292 R, 364 R, R V and R XI, after yellowing and drying of tillers, subsequent germination of rhizome was observed. The clones in which infection of soft rot was late and subsequent germination and growth occurred (M VI, 364 R and R XI) were designated as tolerant clones.

Table 4 : Incidence of soft rot in selected somaclones of ginger in artifical screening

Clone No.	Days taken for apperance of symptoms	Soft rot incidence scores 1 WAI*	2 WAI	3 WAI	4 WAI	5 WAI	6 WAI	7 WAI	Subsequent germination after rotting
99 M[a]	22.33	0	0	1	3	4	4	4	Not Germinated
342 M	9.33	0	2	4	4	4	4	4	Not Germinated
393 M	22.67	0	0	1	3	3	4	4	Not Germinated
970 M	20.67	0	0	2	3	4	4	4	Not Germinated
M VI	40.67	0	0	0	0	0	2	4	Germinated
Control M	14.00	0	2	4	4	4	4	4	Not Germinated
281 R[b]	16.67	0	0	1	3	3	4	4	Not Germinated
292 R	21.33	0	0	2	2	3	4	4	Germinated
364 R	34.00	0	0	0	0	2	3	4	Germinated
RV	16.33	0	0	2	4	4	4	4	Germinated
RXI	25.33	0	0	0	3	3	4	4	Germinated
Control R	12.67	0	3	4	4	4	4	4	Not Germinated

*WAI-Weeks after inoculation; a = Maran, cvs.; b = Rio-de-Janerio cvs.

Table 5 : Characters of selected superior somaclones and conventionally propagated plants in ginger.

Character		Clone No. M VI[a]	364 R[b]	R XI[b]	Conventionally propagated (CP) Maran	Rio-de-janeiro
Fresh yield of	1year (2002)	306.25	148.75	66.66	200.00	160.00
rhizomes/	II Year (2003)	333.33	176.67	140.00	333.33	116.67
plant (g)	III Year (2004)	406.67	426.67	333.33	413.34	336.67
	Mean	348.75	250.70	180.00	315.56	204.45
		Reaction to diseases				
Survival in sick field		Survived	Survived	Survived	Not survived	Not survived
Electrolyte leakage (µS) induced by *P. aphanidermatum*		22.75	22.30	21.75	37.95	35.00
Artificial screening		Resistant	Resistant	Resistant	Susceptible	Susceptible

a = cv. of Maran; b = cvs. of Rio-de-Janerio.

Fields evaluation of somaclones for yield

Yield and disease reaction of selected somaclones (M VI, 364 R and R XI) are presented in Table 5. The clone MVI was highest yielder with 348.75 g/ plant followed by 364 R (250.70 g/plant). These clones survived in natural screening, exhibited low leakage of electrolytes when screened with toxic metabolite(s) of *P. aphanidermatum* and also found resistant in artificial screening experiment.

Three different screening methods viz. natural screening in sick field, screening by electrolyte leakage method and artificial screening using *P. aphanidermatum* were compared for assessing the disease reaction of somaclones. In all the three screening methods, somaclones were found superior to CP plants in resistance reaction to soft rot disease. Somaclones of cv. Maran exhibited more tolerance to the disease as compared to clones of Rio-de-Janeiro. Three somaclones viz. M VI, 364 R and R XI, with high yield which were found tolerant in artificial screening experiment also survived in natural screening in sick field and exhibited low leakage of electrolytes when screened with toxic metabolite(s) of *P. aphanidermatum.* Based on results of three screening methods, three high yielding somaclones of ginger viz. M VI, 364 R and R XI could be located as tolerant to soft rot disease which could be used for further field evaluation / production programmes.

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