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Evaluation of world castor (*Ricinus communis* L.) germplasm for resistance to Fusarium wilt (*Fusarium oxysporum* f. sp. *ricini*)

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Abstract Castor (*Ricinus communis* L.) is an important industrial oilseed crop grown worldwide. Wilt caused by *Fusarium oxysporum* f. sp. *ricini* is a devastating disease of this crop. The objective of this research was to identify stable sources of wilt resistance among the global castor germplasm collections available in India for use in cultivar improvement. The global collections comprising 1,779 Indian and 190 exotic accessions from 36 countries were screened against wilt in wilt sick plots at two sites in India in preliminary screening. None of the accessions showed high resistance to wilt, 133 accessions comprising 111 Indian and 22 exotic accessions representing 13 countries exhibited resistance. Thirteen of the 133 resistant accessions were tested further for multiple years in wilt sick plots and glasshouse under controlled artificial inoculation at two sites. All the 13 accessions consistently showed wilt resistance in both wilt sick plot and glasshouse at both sites in multiple years. Eleven of these 13 accessions were from India and two were from former USSR. Evaluation for agro-morphological traits identified high seed yielding and early maturing resistant accessions. Diversity analyses precisely revealed diversity among the resistant accessions. These 13 resistant accessions would be great value as donors of resistance.

Keywords Castor · Germplasm · Fusrium wilt · Resistance · Diversity

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Introduction

Castor (*Ricinus communis* L.) is an important non-edible industrial oilseed crop. Its oil contains more than 80 % ricinolic acid which confers distinctive industrial properties to the oil. Castor grows as an indeterminate annual or perennial crop depending on climate and soil types in tropical, sub-tropical and warm temperate regions in the world. It can be grown productively on underutilized marginal uplands. Castor is cultivated on commercial scale in 30 countries; India, China, Brazil, USSR are the major castor growing countries in the world (Damodaram and Hegde 2010). Global demand for castor oil is rising constantly at 3 to 5 % per annum (http://www.castoroil.in/). Castor is an ideal candidate for bio-oil production with 500 to 1,000 l ac^{-1} (Auld et al. 2009). Wilt caused by *Fusarium oxysporum* f. sp. *ricini* Nanda & Prasad is the most devastating soil and seed borne disease of castor reported in several castor growing countries (Nanda and Prasad 1974). Fusarium wilt attacks castor plant at any time throughout growing period. The extent of seed yield loss ranges from 39 to 77 % depending upon the stage of the crop (Raoof and Nageshwar Rao 1999). Chemical control of castor wilt is not effective and economical as the pathogen is soil and seed-borne in nature and difficult to eradicate. The use of wilt resistant cultivars is the best and cost effective method. Dependable source of resistance are needed for developing resistant cultivars. Castor germplasm is a valuable repository of desirable genes and gene combinations. The Directorate of Oilseeds Research, Hyderabad (DOR), India maintains 3,331 castor germplasm accessions of Indian and exotic origin. Screening of the vast germplasm collections against wilt is important to recognize resistant sources to utilize in castor breeding programmes. Therefore, a multiyear-multipronged programme to screen castor germplasm accessions against Fusarium wilt at different locations in wilt sick plots and glasshouse was taken up in India under the All India Coordinated Research Project (AICRP) on Castor. Hitherto 1,969 accessions of the global castor collection were screened against Fusarium wilt at two locations in batches between 1997–98 and 2011–12, and the reaction of these accessions against Fusarium wilt was discussed in the present paper. The resistant accessions identified in the present investigation were evaluated for various agro-morphological traits as well for genetic diversity in order to recognize diverse agronomically superior resistant sources to use in breeding programmes.

Materials and methods

Plant material

A working collection of 1,969 accessions comprising 1,779 Indian accessions, collected from 18 provinces and Andaman & Nicobar Islands, and 190 exotic collections introduced from 36 countries was used in the present study. The exotic collections were introduced through National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Most of the Indian collections were collected through explorations across the country by the DOR, Hyderabad, India in collaboration with National Bureau of Plant Genetic Resources, New Delhi, India. Prior to initiation of the present investigation, genetic uniformity was maintained in each accession through inbreeding for 7 years at DOR in order to maintain the working collection of global germplasm. The segregants were separated in each accession. The inbreeding and maintenance was done in batches over years.

Screening against *F. oxyspourm* f. sp. *ricini*

Screening in wilt sick plots

In the preliminary screening, 1,969 accessions were screened in batches against Fusarium wilt in wilt sick plots between 1997–98 and 2011–12 at two sites in India namely, DOR, Hyderabad, Andhra Pradesh (17.366°N and 78.478°E) and S. K. Nagar, Gujarat (24.19°N and 72.19°E) under AICRP on Castor. Permanent wilt sick plots were developed at both locations by growing and in situ incorporation of infected plant debris of highly susceptible variety, Aruna/VP1/JI35/GAUCH-1, and are being maintained since 1990 under AICRP on Castor at both locations The inoculum was incorporated in wilt sick plots prior to sowing. Inoculum load of $2–3\times10^3$ CFU/g of soil was maintained and monitored in wilt sick plots at both locations. Isolate for inoculum was obtained by single spore isolation technique (microconidia) from infected castor roots. A virulent isolate among many isolates obtained from the local area has been used. The inoculum for inoculations has been obtained by growing the virulent isolate on semi-cooked sorghum grain for 15 days. Wilt sick plots were irrigated regularly. Local isolate was used in each location but not the same isoalte in both locations. Isolates used in both locations differed in pathogenicity and variability at molecular level. The isolate used for controlled experiments and field screening was the same at each location.

About 150 accessions were screened in each year at both sites. Soil texture in wilt sick plots is red sandy loam at DOR, Hyderabad and sandy loam at S.K. Nagar. Each accession was planted in two rows of 5 m length and spacing followed was 60 cm between rows and 30 cm between plants. Either one of the susceptible checks viz., Aruna, VP-1, GAUCH-1, Kiran and JI-35, and one of the resistant checks namely, 48–1 and DCS-9, were planted after every 10 rows of experimental material in wilt sick plots in each year to determine the uniform spread of inoculum across the sick plot. Recommended dose of fertilizers and irrigations were provided and pests (other than wilt) control measures were taken as and when required.

Thirteen accessions which expressed wilt resistance reaction in the preliminary screening were further tested for 2 years in wilt sick plots at both sites. In addition, six of these 13 resistant accessions were tested further for 1–3 years more at both sites in wilt sick plots as a part of screening against multiple disease resistance under AICRP on Castor. The details of multiple disease resistance screening experiments were not given here, only the reactions of the accessions against wilt were presented. Wilt incidence (%) was recorded in wilt sick plots at 30 day intervals from 30 to 180 days after planting. Percent wilt incidence was derived from the ratio of the number of wilted plants to the total number of plants multiplied by 100. Cumulative number of wilted plants was considered to calculate percent wilt incidence in each accession. Reaction of experimental material against wilt (*F. oxysporum* f.sp. *ricini*) was categorized as per the scale given by Mayee and Datar (1986) as described in Table 1. The accessions showing 0 % wilt incidence were rated as highly resistant and those showing 0.1 to 20 % incidence were rated as resistant.

Screening in glasshouse using root-dip inoculation technique

The resistance reaction of 13 accessions, which consistently exhibited resistance reaction in the first 2 years in wilt sick plots at DOR, Hyderabad and S.K. Nagar, was confirmed by testing further for 2 to 4 years under controlled artificial inoculation conditions in glasshouse using root-dip inoculation technique (Raoof and Nageshwar Rao 1996) at both the locations. This method has been standardized over years with several test materials. As per this technique, plants were grown in pots filled with sterilized river bed sand. Ten day old seedlings were uprooted; one-third of root system of seedlings was clipped from distal end and dipped for 1 min in *F. o. ricini* spore suspension (1×10^6 spores/ml) from clipped end. Inoculated seedlings were transferred to earthen pots (25 cm diameter) filled with autoclaved soil. Twenty plants in each entry were tested for reaction against wilt along with one of the susceptible checks and resistant checks that were used in wilt sick plots. Development of wilt symptoms was observed periodically and the final wilt incidence was recorded 1 month after transplantation to pots. Since only resistant accessions were screened in glasshouse for confirmation of their resistant reaction, the accessions were not actually classified into any groups. Any accession which had less than 20 % wilt incidence in glasshouse screening was taken as a finally confirmed resistant accession.

Evaluation for agro-morphological traits and genetic divergence study

The 13 identified resistant accessions were evaluated for agro-morphological traits in a non-wilt sick plot at DOR, Hyderabad in a randomized block design with three replications during 2010–11 and 2011–12. Each accession was planted in a three-row plot of 5 m length at the recommended spacing of 90 cm between rows and 45 cm between plants in a row. Data were collected on 15 plants in each replication of an entry for 18 morphological traits viz., plant height up to primary raceme (cm), number of nodes on main stem, days to 50 % flowering of primary raceme, days to maturity of primary raceme, total length of primary raceme (cm), length of primary raceme covered by male flowers (cm), length of primary raceme covered by capsules (cm), number of productive racemes/plant, 100-seed weight (g), seed yield (g/plant) at different pickings at 120, 150, 180 and 210 days after planting and total seed (g/plant). Seed oil content (%) was estimated by an NMR instrument (Oxford-7005). Recommended doses of N-P-K (60-40-30 kg/ha) fertilizers were applied, and plant protection measures were taken as and when required. The recommended dose of phosphorus as well as of potassium and 50 % of nitrogen were applied as basal doses before sowing and the remaining nitrogen was applied at 40 days after planting. In both years, the accessions along with check, 48–1 were planted in June and grown

Table 1 Categorization of 1,425 castor accessions of Indian origin showing coherent reactions against Fusarium wilt in wilt sick plots at two locations

Score	Disease incidence (%)	Reaction against wilt	Number of accessions
0	0	High resistant	0
1	0.1–20	Resistant	111
2	20.1–40	Moderately resistant	212
3	40.1–50	Moderately susceptible	132
4	50.1–75	Susceptible	216
5	>75	Highly susceptible	754

under rainfed conditions. Mean value of 2 years was considered for each trait for diversity analyses. Genetic diversity was assessed using established multivariate algorithms such as principal component analysis (Jolliffe 1986) and cluster analysis using Ward's Minimum Variance method (Ward 1963). Squared Euclidean distance was employed in Ward's minimum variance method. INDOSTAT statistical software, Indostat services, India was used for statistical analysis of the data.

Results

Screening in wilt sick plots

Initial high plant stand (28–32 plants/accession) in all experimental materials was maintained in both wilt sick plots in all the years under study. There were no accessions exhibiting only early wilting or late wilting. Wilt infestation did not stop at a particular stage in any accession, it was observed right from germination stage to 150 to 180 days after planting in wilt sick plots. In seedling (2–3 leaves) stage, discolouration of hypocotyl and loss of turgidity of top leaves were observed in wilt infested seedlings. In some seedlings, spot or blight symptoms appeared along the edge of cotyledons and the first true leaves. The grown up susceptible plants exhibited typical wilt symptoms like stunted growth, gradual yellowing, shrivelling with marginal necrosis and complete drying of leaves and branches, vascular discolouration and death of entire plant. These plants showed blackish lesions above the collar region of stem which further covered the entire stem. Pith of the infected stem became black and covered with white cottony Fusarium fungus. Roots of wilted plants showed blackening and necrosis.

Among 1,779 accessions of Indian origin, 1,425 showed consistent reactions against wilt and 354 showed inconsistent reactions at both sites in wilt sick plots in preliminary screening. Among the 1,425 consistent Indian accessions, 1,102 showed moderate to high susceptible reactions against wilt, 111 exhibited resistant reactions, 212 accessions showed moderate resistant reactions and none showed a high resistance reaction at both locations (Table 1). Among the 190 exotic accessions, the accession, RG3322 from South Africa was found highly resistant, 21 were wilt resistant (Table 2), 31 were moderately resistant, five were moderately susceptible, 11 were susceptible and 82 were highly susceptible while 39 showed inconsistent reactions in the preliminary screening in wilt sick plots at both sites.

Five accessions namely, RG13, RG172, RG507, RG851 and RG2549 were highly resistant (0 %) to wilt at DOR, Hyderabad but had 100 % wilt incidence at S.K. Nagar. In contrast, six accessions viz., RG28, RG246, RG267, RG298, RG517 and RG620 were highly resistant to wilt at S.K. Nagar but were highly susceptible at DOR, Hyderabad. In addition, 46 other accessions also had altered reactions from resistance to susceptible or vice-versa between two sites in wilt sick plots.

Thirteen accessions viz., RG43, RG111, RG109, RG297, RG1608, RG1624, RG2758, RG2787, RG2800, RG2818, RG2822, RG3016 and RG3105 consistently showed resistance reaction at both locations in wilt sick plots over years of testing (Table 3). Both susceptible and resistant checks also confirmed their respective reactions against wilt in wilt sick plots in all years under study. Wilt incidence had ranged from zero to 18 % among the 13 resistant accessions while it was 92 to 100 % in various susceptible checks and zero to 17 % in resistant checks in wilt sick plots at both sites over years. Eleven of the 13 resistant accessions were of Indian origin whereas two viz., RG109 and RG111, were from the former USSR. These 11 Indian accessions have come from different provinces.

Screening in glasshouse using root-dip inoculation technique

The 13 resistant accessions consistently confirmed resistance reaction over years under high artificial inoculation condition in glasshouse at both the sites (Table 4). Wilt incidence among 13 resistant accessions ranged from zero to 18.3 % over years in glasshouse experiment at two locations. The susceptible and resistant checks had also confirmed their respective reactions; wilt incidence was 93–100 % in susceptible checks and zero to 8.3 % in resistant checks.

Evaluation for agro-morphological traits

ANOVA indicated that the variation due to accessions was significant. F-test was significant among 13 accessions for all the 15 traits. The F values were between 3.5 and 162 (*P*: 0.01–0.15). Two-year mean values of 15

Table 2 Passport details and wilt incidence in 22 resistant exotic accessions at two locations in the preliminary screening

Accession	Source/origin country	Indian national identity number	Wilt incidence in wilt sick plot (%)	
			DOR, Hyderabad	S.K. Nagar
RG-15	Italy	EC-151810	15.4	8.3
RG 109	Former USSR	EC-97703	18.2	0
RG-111	Former USSR	EC-97703 (A)	14.2	16.7
RG-155	Unknown	EC-154818	16.7	9.1
RG-611	USA	–	11.1	20
RG-817	USA	–	16.7	0
RG-963	Egypt	–	15.4	0
RG-967	Sri Lanka	–	20	16.7
RG-968	South Africa	–	15.4	0
RG-990	Unknown	EC-153581	20	9.1
RG-1076	USA	EC-254823	20	7.1
RG-1146	Unknown	EC-21276	18.2	0
RG-1148	South America	EC-13338	20	0
RG-1151	Nigeria	EC-14806	15.3	0
RG-2673	USA	EC-410738	20	9.1
RG-3020	Namibia	EC-501587	10	14.3
RG-3309	Zaire	EC-625805	12.5	0
RG-3315	Iran	EC-625811	20	14.3
RG-3322	South Africa	EC-625818	0	0
RG-3340	Turkey	EC-633124	16.7	11.1
RG-3359	Egypt	EC-633143	10	0
RG-3361	Ecuador	EC-633145	16.7	9.1

agro-morphological traits were given in the Table 5. A wide range of diversity was observed for each trait among resistant accessions. Short plant height was observed in RG111 followed by RG43 and RG109; RG43 matured earlier (104 days) than other resistant accessions. The longest primary raceme (72 cm) and the longest length of primary raceme covered by capsules (61 cm) were observed in RG2800. Highest number of productive racemes per plant was observed in RG109 and the heaviest 100-seed weight (66 g) in RG3105. Most of the accessions possessed an oil content between 48 and 49 %.

Wide diversity was observed for seed yield at different days after planting (DAP) and total seed yield (g/plant) among the resistant accessions (Table 6). Four accessions viz., RG43, RG111, RG297 and RG2758 yielded significantly higher than the high yielding check, 48–1 at 120 DAP. RG297 yielded the highest at 120 DAP and 150 DAP and RG2818 yielded the highest at 180 DAP while RG1624 yielded the highest at 210 DAP. Significantly higher total seed yield over the check, 48–1 was realized from RG1624, which was the tallest and very late maturing accession.

Genetic divergence study

The principle component analysis (PCA) performed on 15 agro-morphological characters of 13 wilt resistant accessions showed that the first four components explained 80.95 % of total variation (Table 7). The first four components were retained as per the Kaiser criterion (Kaiser 1960). This criterion retains only the components that have eigenvalues greater than 1 for interpretation. The first component accounted for 43.27 % of the variance and was loaded with 13 characters. The second component accounted for 17.7 % of total variation while the third and fourth components accounted for 12.26 % and 7.7 % of total variation, respectively. The most effective traits in the first component were number of nodes on main stem, seed yield at 210 days after

Table 3 Wilt incidence in 13 resistant sources in wilt sick plots at two sites in different years

Accession[a]	Year	Wilt incidence (%)		Accession[a]	Year	Wilt incidence (%)	
		DOR, Hyderabad	S.K. Nagar			DOR, Hyderabad	S.K. Nagar
RG 43 (IC0584671)	2002–03	14.2	0.0	RG2818 (IC346622)	2002–03	5.0	0.0
	2004–05	8.3	7.7		2003–04	0.0	0.0
	2006–07	9.0	10.0		2004–05	8.3	0.0
RG111	2001–02	14.2	16.7		2006–07	0.0	16.7
(EC97703)	2004–05	10.0	7.1		2007–08	16.6	0.0
	2010–11	10.0	10.5		2010–11	0.0	0.0
RG109 (EC97703)	2002–03	18.2	0.0	RG2822 (IC346626)	2003–04	18.0	0.0
	2004–05	14.2	7.7		2004–05	9.0	0.0
	2009–10	10.0	0.0		2006–07	12.5	15.3
RG297 (IC373889)	2000–01	9.1	8.7	RG3016 (IC257812)	2004–05	0.0	0.0
	2001–02	18.0	0.0		2005–06	13.3	0.0
	2004–05	0.0	7.7		2006–07	0.0	0.0
RG1608 (IC373978)	1997–98	11.1	0.0		2007–08	0.0	8.3
	2002–03	18.0	0.0		2009–10	0.0	0.0
	2003–04	10.5	7.1	RG3105	2008–09	15.4	0.0
RG1624 (IC373981)	1997–98	11.1	0.0		2009–10	0.0	–
	2002–03	18.0	0.0		2010–11	0.0	0.0
	2003–04	15.0	0.0	Checks[b]			
	2006–07	5.5	0.0		1997–98	97(10)	100(0)
	2007–08	0.0	0.0		2000–01	97(15)	–
	2009–10	0.0	0.0		2001–02	90 (7.1)	98(0)
RG2758 (IC346591)	2002–03	5.0	0.0		2002–03	97(9.1)	96(0)
	2003–04	0.0	0.0		2003–04	91(17)	100(0)
	2006–07	5.5	0.0		2004–05	96(5.4)	100(0)
	2007–08	0.0	0.0		2005–06	94 (9.8)	100(0)
RG2787 (IC374338)	2002–03	15.0	0.0		2006–07	100 (5)	97(0)
	2003–04	14.3	0.0		2007–08	100(0)	100(0)
	2005–06	5.8	0.0		2008–09	100(0)	100(0)
	2006–07	17.8	0.0		2009–10	100(0)	100(0)
	2007–08	15.3	10.0		2010–11	92 (0)	100(0)
	2009–10	0.0	0.0				
RG2800 (IC346604)	2003–04	18.0	0.0				
	2004–05	15.3	7.7				
	2005–06	12.5	0.0				
	2006–07	0.0	0.0				
	2009–10	0.0	16.7				

[a] The number in parenthesis following the accession number is a national identification number allotted by National Bureau of Plant Genetic Resources, India

[b] Wilt incidence (%) in resistant check is given in parentheses

–not assessed

planting, plant height up to primary raceme, days to maturity of primary raceme, days to 50 % flowering of primary raceme, seed yield at 180 days after planting, total length of primary raceme, 100-seed weight, length

Table 4 Wilt incidence in resistance sources in castor germplasm in glasshouse under artificial inoculation conditions at two sites in different years

Accession[a]	Year	Wilt incidence (%)		Accession[a]	Year	Wilt incidence (%)	
		DOR, Hyderabad	S. K. Nagar			DOR, Hyderabad	S. K. Nagar
RG43	2002–03	0	–	RG2822	2003–04	14.3	0
(IC0584671)	2005–06	0	5	(IC346626)	2004–05	13.2	16.7
RG111	2002–03	18.2	–	RG3016	2005–06	8.3	0
(EC97703)	2005–06	9.1	8.3	(IC257812)	2011–12	12	5.3
RG109	2002–03	6.3	0.0	RG3105	2009–10	0	0
(EC97703)	2005–06	16.7	7.5		2011–12	13.3	0
RG297	2001–02	4.5	0		2010–11	6.7	0
(IC373889)	2002–03	0	0	Checks[b]			
RG1608	2002–03	0	0		2000–01	95 (0)	100(0)
(IC373978)	2003–04	20	0		2001–02	98 (8.3)	93 (0)
RG1624	2002–03	10	0		2002–03	93 (0)	95(0)
(IC373981)	2010–11	12.5	0		2003–04	97 (0)	97 (0)
RG2758	2004–05	8.3	0		2004–05	95 (0)	100(0)
(IC346591)	2005–06	0	0		2005–06	100 (0)	100 (0)
RG2787	2007–08	10.5	20		2006–07	95 (0)	90 (0)
(IC374338)	2010–11	5.3	0		2007–08	96(0)	100 (0)
RG2800	2004–05	0	0		2008–09	96(0)	100 (0)
(IC346604)	2005–06	0	0		2009–10	100 (0)	94 (0)
	2010–11	16.7	10.5		2010–11	95(0)	100 (0)
	2011–12	18.3	8.3		2011–12	−(1.6)	100 (0)
RG2818	2003–04	7.1	0				
(IC346622)	2004–05	0	0				

[a] The number in parenthesis following the accession number is a national identification number allotted by National Bureau of Plant Genetic Resources, India

[b] Wilt incidence (%) in resistant check is given in parentheses

–not assessed

of primary raceme covered by capsules and total seed yield. Oil content and length of primary raceme covered by male flowers were the effective traits for the third and fourth components, respectively. Seed yield at 150 days after planting was little discriminating while number of productive racemes/plant was the least discerning trait in the evaluated accessions.

Participation of 13 resistant accessions into two major groups could be visualized from principal component plot (Fig. 1). The geometrical distances among accessions in the principal component plot reflected no distortion of the genetic distances. Hence, the differences among the accessions could be visualized without ambiguity. The PCA had clustered six resistant accessions viz., RG43, RG111, RG109, RG297, RG2787 and RG3016 into one visualized major group while the remaining seven resistant accessions viz., RG1608, RG1624, RG2758, RG2800, RG2818, RG2822 and RG3105 along with check, 48–1 were clustered into another major group.

Wards' minimum variance method of cluster analysis also divided the 13 resistant accessions into two major clusters (A and B). Each major cluster further divided into two sub-clusters and these sub-clusters successively divided into their own sub-clusters (Fig. 2). This process continued until there were as many clusters as accessions. The clustering pattern and composition of clusters clearly indicated effectiveness of agro-morphological data set in discriminating the resistant accessions. Six resistant accessions viz., RG43, RG111, RG2787,

Table 5 Mean performance of 13 resistant accessions over 2 years for agronomic and yield traits under rainfed conditions

Accession	PH (cm)	NN	DF	DM	LPM (cm)	LPC (cm)	TLP (cm)	NPR	100-SW (g)	OC (%)
RG-43	36	9	36	104	11.5	5	17	10	24	48
RG-109	58	14	56	109	13.0	23	36	22	30	48
RG-111	29	11	56	114	5	13	18	15	28	47
RG-297	71	16	51	117	8	27	35	12	28	48
RG-1608	211	21	70	152	10	21	32	8	45	47
RG-1624	243	32	119	204	11	34	45	4	45	49
RG-2758	129	17	53	115	13	28	41	16	36	40
RG-2787	101	14	54	116	12	32	44	12	32	48
RG-2800	87	18	54	118	11	61	72	13	24	45
RG-2818	117	18	75	122	7	26	33	9	15	48
RG-2822	150	21	73	120	9	29	34	7	14	39
RG-3016	92	17	74	118	11	24	35	13	20	48
RG-3105	213	31	81	140	7	37	44	11	66	48
48-1(Check)	118	21	75	132	6	35	41	16	30	48
Mean	118	18	66	127	10	28	37	12	31	46
Range	29-243	9-32	36-119	104-204	5-13	5-61	17-72	4-22	14-66	39-49
CV (%)	15.3	6.4	2.1	2.2	20.2	32.8	14	18	1.8	2.6
S.Em	12.8	0.86	1.0	1.98	1.4	6.57	3.7	1.56	0.41	0.87
CD(P=0.05)	39.3	2.6	3.9	6.0	4.2	20	11.5	4.7	1.2	2.66

PH plant height up to primary raceme; *NN* number of nodes on main stem; *DF* days to 50 % flowering; *DM* days to maturity of primary raceme; *LPM* length of primary raceme covered by male flowers; *LPC* length of primary raceme covered by capsules; *TLP* total length of primary raceme; *NPR* number of productive racemes/plant; 100-*SW* 100-seed weight; *OC* oil content (%); *CV* coefficient of variation; *S.Em* standard error of mean; *CD* critical difference; *P* Probabilit

RG3016, RG109 and RG297, were placed in the major cluster-A; while seven accessions viz., RG1608, RG3105, RG2818, RG2800, RG2758, RG2822 and RG1624 along with the check 48–1, were placed in cluster-B. The accessions, RG43 and RG111, were placed in one sub-cluster of cluster-A, and the accessions, RG2787, RG3016, RG109 and RG297, were placed in another sub-cluster of cluster-A. One sub-cluster of cluster-B comprised six accessions viz., RG 1608, RG3105, RG2818, RG2800, RG2758 and RG2822 and the check 48–1, while the other one had only one accession, RG1624. The composition of accessions in two major clusters was in agreement with the results of PCA. However, cluster analysis could discriminate precisely the resistant accessions by further dividing the major clusters at reduced distance levels. The two exotic accessions, RG109 and RG111, which had common parental origin (EC97703), were placed in different sub-clusters of cluster-A along with Indian accessions. The two high yielding accessions, RG1624 and RG297 were placed in two individual sub-clusters of clusters-A and cluster-B. These accessions were collected from diverse geographic regions in India. RG297 was collected from Tamil Nadu province of southern India and RG1624 was collected from Bihar province of eastern India.

The cluster means revealed that cluster-A had the lowest values for number of nodes on main stem (14), days to 50 % flowering (55) and days to maturity (113) and the highest values for oil content (48 %), seed yield in early pickings that were at 120 DAP (41 g/plant) and 150 DAP (43 g/plant). Cluster analysis placed the relatively very early maturing accessions, RG43 and RG109, precisely in one sub-cluster of cluster-A. Cluster-B had the highest mean values for yield contributing traits like length of primary raceme covered by capsules (34 cm) and 100-seed weight (34 g), it also had the highest mean values for seed yield at 180 DAP (54 g/plant) and 210 DAP (63 g/plant) as well as total seed yield (157 g/plant). The accessions included in this cluster were relatively late maturing type giving low

Table 6 Mean seed yield performance of 13 resistant accessions over two years under rainfed conditions

Accession	Seed yield (g/plant)				
	120 DAP	150 DAP	180 DAP	210 DAP	Total
RG-43	50	28	19	3	100
RG-109	36	64	46	18	165
RG-111	45	26	26	12.5	109
RG-297	83	72	36	53	244
RG-1608	12	18	49	62	142
RG-1624	20	55	62	113	250
RG-2758	40	10	38	38	126
RG-2787	27	37	44	24	132
RG-2800	27	18	51	59	157
RG-2818	5	10	77	49	142
RG-2822	6	6	31	44	88
RG-3016	3	28	13	24	69
RG-3105	1	10	60	59	131
48-1(Check)	27	51	60	81	220
Mean	27	31	44	46	148
CV (%)	17	21	15	14.4	8.5
S.Em	3.4	4.6	4.7	4.7	8.9
CD (P=0.05)	10.6	14.2	14.5	14.3	27.4

DAP days after planting; *CV* coefficient of variation; *S.Em* standard error of mean; *CD* critical difference; *P* probabilit

yields at early pickings but higher yields at late pickings that were at 180 DAP and 210 DAP. Cluster-B mean values for yield at these pickings (54 g and 63 g/plant, respectively) were higher than those estimated for

Table 7 Principal component analysis for 15 agro-morphological traits observed in 13 wilt resistant castor accessions

Trait	PC1	PC2	PC3	PC4
Plant height up to primary raceme	0.901	0.292	0.113	0.186
Number of nodes on main stem	0.948	0.135	0.033	0.055
Total length of primary raceme	0.659	-0.036	-0.718	0.051
Length of primary raceme covered by male flowers	−0.066	0.098	-0.400	0.832
Length of primary raceme covered by capsules	0.543	0.002	-0.738	-0.234
Number productive racemes/plant	−0.504	-0.339	-0.489	-0.005
Days to 50% flowering of primary raceme	0.876	0.088	0.254	0.010
Days to maturity of primary raceme	0.889	-0.089	0.307	0.211
100-seed weight	0.567	-0.064	0.100	0.328
Oil content	0.173	-0.594	0.409	0.002
Seed yield/plant at 120 DAP	-0.467	-0.716	-0.016	0.072
Seed yield/plant at 150 DAP	0.023	-0.922	0.023	0.209
Seed yield/plant at 180 DAP	0.710	-0.146	-0.152	-0.377
Seed yield/plant at 210 DAP	0.928	-0.183	-0.025	-0.129
Total seed yield/plant	0.540	-0.795	-0.060	-0.079
Eigenvalues	6.490	2.655	1.840	1.156
Variance (%)	43.271	17.704	12.268	7.706
Cumulative variance (%)	43.271	60.976	73.244	80.951

DAP days after planting: *PC* principal component

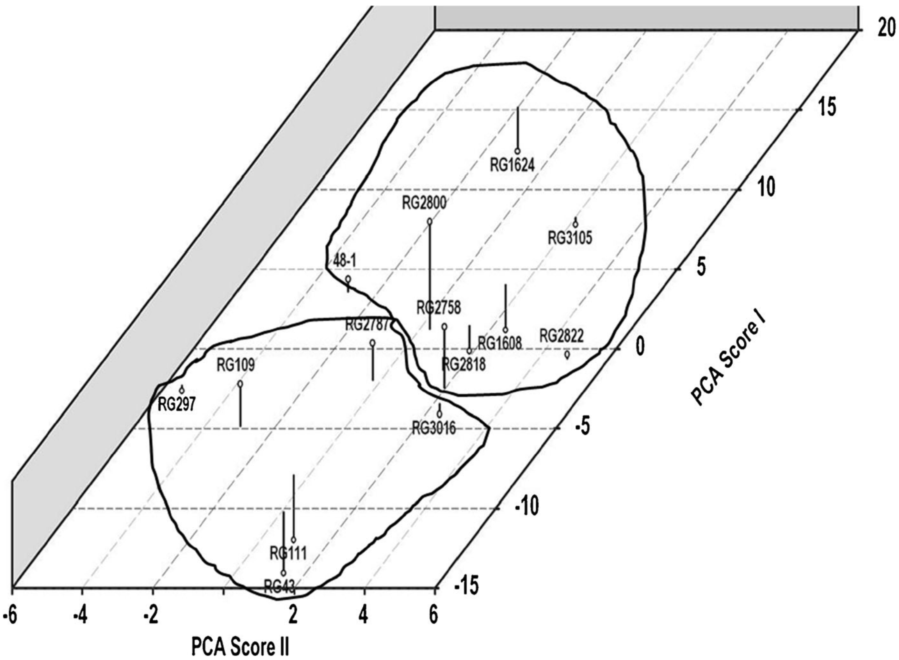


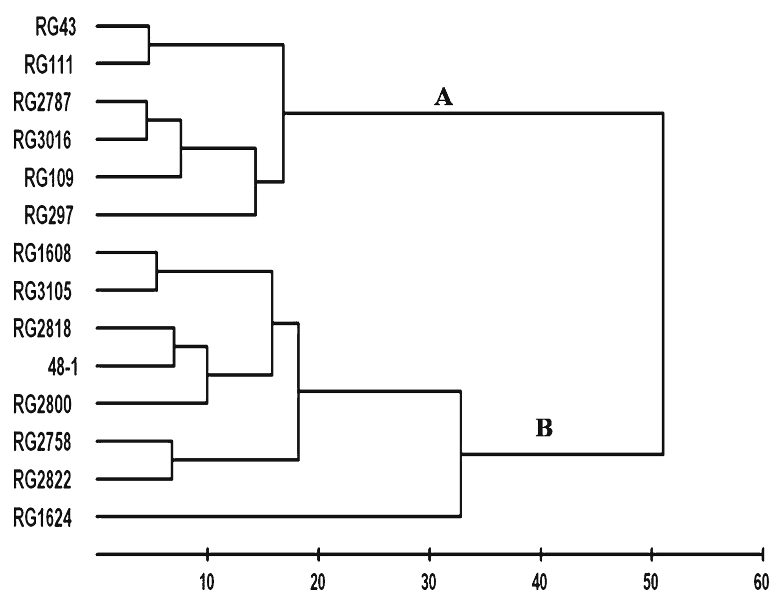
Fig. 1 Principal component analysis plot of 13 Fusarium wilt resistant accessions. The two visualized major groups in the plot were circled

cluster-A (31 g and 22 g/plant, respectively). Thereby mean value of cluster-B for total seed yield (157 g/plant) was higher than that estimated for cluster-A (137 g/plant). Cluster analysis precisely placed the highest yielding very late maturing accession, RG1624, separately in one sub-cluster of cluster-B.

Discussion

Successful screening for diverse disease resistance sources is based on the availability of large and diverse germplasm collections and the availability of reliable screening techniques. Screening wilt resistance under

Fig. 2 Dendrogram constructed by Ward's minimum variance method of cluster analysis using 15 agro-morphological traits of 13 wilt resistant castor accessions



natural conditions might give misleading information; therefore, the preliminary screening under wilt sick plot conditions followed by confirmation of resistance in glasshouse using root-dip inoculation technique was considered a dependable and economical method for identifying stable sources of resistance to Fusarium wilt. In the present investigation, noticeable differences were not found between the glasshouse and wilt sick plot data on reaction of resistant sources as well as of susceptible and resistant checks against wilt. Wilt incidence in the resistant accessions and checks was within the range of resistance category (<20 %) in both wilt sick plots and glasshouse experiments. In case of susceptible checks, wilt incidence was within the range of the high susceptibility category (>75 %). This indicates that both screening methods were reliable for discriminating resistant and susceptible genotypes. Wilt sick plots have been used in other crops also for identifying reliable sources of resistance to Fusarium wilt (Bayaa et al. 1997; Gwata et al. 2006; Iqbal et al. 2010).

A wide range of disease response was found in the castor germplasm collection. About 66 % of the total accessions were categorized into moderately susceptible, susceptible and highly susceptible categories. None of the accessions were highly resistant or immune to wilt. However, identification of 22 resistant exotic accessions in preliminary screening is indicative of the possibility of identifying more geographically diverse wilt resistant sources.

Inconsistent reaction observed in 22 % of the total accessions at two locations might be due to presence of different races of *F.o.ricini* in the two locations. The studies on *F. o. ricini* isolates in India envisaged the existence of different races but could not resolve the race pattern (Desai et al. 2003; Santha Lakshmi Prasad et al. 2008; Madhusudhana Reddy et al. 2012). Differentials to races are yet to be identified to determine races in castor wilt. However, 13 accessions including two from former USSR consistently exhibited resistant reaction against wilt at both locations over years in wilt sick plots as well as in glasshouse in the present investigation. These accessions are likely to withstand high inoculum levels as the inoculum load maintained under wilt sick plot and glasshouse conditions was very high. These 13 resistant accessions would be of great use in breeding programmes aimed at developing wilt resistant cultivars.

Analysis of genetic diversity in resistant accessions was done to facilitate reliable classification of accessions for stratified sampling of diverse resistant genotypes for breeding programmes. The two multivariate methods employed in this study were widely used in genetic divergence studies as they simultaneously analyse multiple measurements on each individual under investigation (Franco et al. 1998). The results of the two multivariate procedures showed that there was a certain consistence in classification of genotypes by different methodologies. Distinction of accessions based on agro-morphological traits was efficient. By combined application of PCA and cluster analysis, the traits contributing most to total variation in the evaluated accessions could be identified as well as comprehensive information about internal relatedness of evaluated accessions could be obtained.

In PCA, the contribution of a trait to the total variability was relative as its identification was based on the principle component. The traits having high discriminating power were retained and those having little discriminating power in the evaluated accessions were discarded. Thus PCA retained 12 agro-morphological traits that contributed high to total variability and discard three traits which contributed least to the genetic divergence. The traits retained were the major traits of interest for genetic improvement in castor.

The dendrogram constructed by Ward's minimum variance method indicated degree of divergence between accessions. From this analysis, it was understood that generation of additional variability would be possible by inter crossing the accessions from different clusters. The resistant accessions included in cluster-A were relatively early maturing type giving high seed yield at early pickings that can be used to breed early maturing high yielding wilt resistant cultivars in castor.

The information generated on genetic divergence in resistant accessions would be useful for selecting diverse wilt resistant genotypes to use in breeding programmes. Some of the wilt resistant accessions were identified earlier as sources of resistance to other biotic stresses as well. The wilt resistant accession, RG2787 was also identified as a source of resistance to root rot (*Macrophomina phaseolina*) and *Botrytis ricini* (Godfrey) (Anjani and Raoof 2009), and the early maturing wilt resistant accession, RG43 was also identified as a source of resistance to leafhopper, *Empoasca flavescens* Fabr. (Cicadellidae: Homoptera) (Lakshminarayana and Anjani 2009). Using these multiple resistant sources, it might be possible to stack the genes conferring resistance to wilt, root rot, Botrytis and leafhopper in one elite background in the future through marker-assisted selection as well through conventional breeding.

In conclusion, the use of well reliable tests for both field and glasshouse screening has allowed an understanding of the reaction of a wide germplasm collection of castor against Fusarium wilt and identification of stable sources of resistance to Fusarium wilt. The 13 resistant accessions identified would serve as donors of wilt resistance in castor resistance breeding programmes and for molecular mapping of diverse wilt resistance genes.

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