

Analysis of mode of inheritance of *Fusarium* wilt resistance in castor (*Ricinus communis* L.)

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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 in castor. The inheritance mode of wilt resistance was investigated. The F₁, F₂ and backcross generations of four crosses involving four resistant and three susceptible parents were developed. The role of digenic (R^1 and R^2) epistatic interactions on wilt resistance was confirmed. The 15 : 1, 9 : 7 and 13 : 3 ratios indicated duplicate dominant, duplicate recessive and dominant and recessive epistatic interactions, respectively. Castor parents used in the crosses exhibited varied inheritance modes. All generations of a cross exhibited similar inheritance mode when parents were comparable. However, generations varied in inheritance mode when parents were not comparable in inheritance mode. These results would have practical interest when decisions are required regarding the choice of parents and methodology in resistance and hybrid breeding. The results also provided a basis for investigating molecular genetics of wilt resistance mechanisms.

Key words: castor — digenic — epistasis — *Fusarium* wilt — inheritance — resistance

Castor (*Ricinus communis* L.) is cultivated on a commercial scale with 1 525 000 ha in 30 countries producing 1 581 000 metric tons (MT) of seed yield. India, China, Brazil and the USSR are the major castor growing countries in the world (Damodaram and Hegde 2010). Castor oil has multifarious applications for the production of a wide range of industrial products including medicines, lower molecular weight aviation fuels, fuel additives, biopolymers and biodiesel (Caupin 1997, Comar et al. 2004, Ogunniyi 2006). The demand for castor oil worldwide is rising constantly at 3–5% per annum (<http://www.castoroil.in>). Leading countries from practically every continent are showing renewed interest in the castor crop. The adaptability and productivity of castor is limited by major diseases. Wilt caused by *Fusarium oxysporum* f.sp. *ricini* is the most important soil- and seedborne disease of castor reported in several castor growing countries (Nanda and Prasad 1974, Moshkin 1986). *Fusarium* wilt attacks castor plants at any time throughout the growing season. The extent of seed yield loss ranges from 39 to 77% depending upon the stage of crop growth (Raof and Nageshwar Rao 1999). Several sources of resistance to *Fusarium* wilt were reported (Podukuichenko 1991, Anjani et al. 2004, Anjani 2012, Dange et al. 2006). Breeding for wilt resistance is the most cost-efficient and eco-friendly disease management method. An understanding of wilt resistance inheritance in castor would enable development of effective and durable-resistant parental lines and cultivars. Some accessions from the global

castor germplasm repository (Directorate of Oilseeds Research, Hyderabad, India) consistently exhibited less than 20% wilt incidence in multiyear and multilocation screening evaluations in field plots with a high incidence of *F. oxysporum* spores and in a glasshouse where artificial inoculations were conducted. Consistent occurrence of less than 20% wilt incidence in these sources is indicative of more than one gene controlling wilt resistance. Information on inheritance of *Fusarium* wilt resistance in castor is limited and inconclusive. Therefore, we attempted to determine the wilt resistant mechanism in different resistant sources.

Material and Methods

Plant material: Four wilt resistant parents *viz.*, RG2758, RG2822, RG109 and RG2529 and three wilt susceptible parents *viz.*, RG2368, RG2876 and RG2836 were used in this study.

Crosses and generations: This study was conducted at the research farm (Directorate of Oilseeds Research, Hyderabad) from 2008 to 2011. Parental plants were planted in soil blocks containing spores of *F. oxysporum* during July, 2008. A single plant from each parent was selected for use in the crossing programme. The same plant for each parent was self-pollinated to derive selfed-generations of parents. The castor raceme is monoclinal and monoecious resulting in the production of pistillate flowers near the apex and staminate flowers proximally. As castor has an indeterminate growth habit, several racemes are being produced on a single plant throughout the growing season. Staminate and pistillate flowers can be identified at the bud stage because they differ in shape. Staminate flowers in some racemes of a female parent plant were removed at the bud stage, and pollen grains collected from a male parent plant were brushed onto pistillate flowers of the female parent plant to produce F₁ seeds. Crosses were made between four resistant and three susceptible parents during 2008. Three crosses *viz.*, RG2368 × RG2758, RG2876 × RG2822, and RG2822 × RG2836 were between wilt resistant and susceptible parents, and one *viz.*, RG2529 × RG109 was between wilt resistant parents. The remaining racemes in the same parental plants were self-pollinated by covering with Butter paper bags at the bud stage until maturation of self-generated seeds. For backcrossing, the staminate flowers in the selected F₁ plants were removed at the bud stage and the pistillate flowers were pollinated with pollen collected from selected resistant and susceptible plants of both parents within a cross. All of the pollinations were made under controlled conditions to avoid pollen contamination.

The parental selfed-generations were planted along with F₁ generations of four crosses in the wilt containing blocks during June 2009. One selected F₁ plant in each cross was self-pollinated and harvested separately to derive F₂ populations. The same F₁ plant from each cross was

backcrossed to resistant and susceptible plants selected from selfed-generations of both parents in 2009. Pollen grains were collected from susceptible plants prior to wilting. The F₂ and backcross generations of each cross were planted in the blocks containing wilt spores during June 2010. The selected resistant and susceptible F₂ plants in each cross were self-pollinated to generate F₃ generations. F₃ generations of each cross were planted during June 2011 in the wilt-infested blocks.

Screening of experimental material and disease assessment: The experimental blocks including parents, F₁, F₂, F₃ and backcross generations of four crosses were planted in plots consisting of 2–3 × 10³ CFUg⁻¹ soil inoculum of *Fusarium oxysporum* f. sp. *ricini*. The highly susceptible check variety 'Aruna' was included in one row after every five rows of the experimental material. The plot and plant spacing in the experimental blocks was 60 cm between rows and 30 cm between plants in a 5-m long row. Wilt incidence (%) was recorded at 15-day intervals and continued for 30 to 210 days after sowing during both years. The wilt (*F. oxysporum* f. sp. *ricini*) reactions on the experimental plants were categorized (Table 1) using the same scale by Mayee and Datar (1986). Wilt susceptible plants exhibited stunted growth, gradual yellowing, shriveling with marginal necrosis, and complete drying of plants, vascular discoloration, and eventual death of the entire plant. The infected plants showed black lesions above the stem collar region covering the entire stem. The pith of the infected stem became black and was covered with white cottony mycelium of the *Fusarium* fungus. The roots of wilted plants were black and necrotic. Plants with partial wilting were not observed in the experimental material. Wilt incidence in the highly susceptible check variety 'Aruna' ranged from 80 to 100% in all the experimental blocks during both years.

The parents, F₁, F₂, F₃ and backcross generations showing up to 20% wilt incidence were rated as resistant. The data on percentage wilt incidence were derived from the ratio of the number of wilted plants to the total number of plants multiplied by 100. No other diseases were observed on plants in the experimental blocks. Castor plants were fertilized, irrigated and pesticides were applied when required.

Results

The four crosses *viz.*, RG2368 × RG2758, RG2529 × RG109, RG2876 × RG2822 and RG2822 × RG2836, differed in inheritance mode. Therefore, crossing results are presented and genotypes representing F₁, F₂, F₃ and backcross progenies from each cross were determined based on segregation ratios.

Mode of inheritance for wilt resistance in RG2368 × RG2758

The inheritance of wilt resistance was determined in a cross between a highly wilt susceptible parent, RG2368, and a wilt resistant parent, RG2758. Wilt incidence in selfed-generations of parents, F₁, F₂, F₃ and backcross generations from the cross, RG2368 × RG2758, is given in Table 2. The selfed-generation from the resistant parent exhibited a resistant reaction, and sus-

ceptible parent was confirmed to be highly susceptible as expected. Castor plants in the F₁ generation were moderately resistant to wilt. Three F₂ generations derived separately from self-pollinations of resistant F₁ plants representing nos. 4, 29 and 77 showed resistant reactions; however, two F₂ generations derived from self-pollinations of susceptible F₁ plants representing nos. 27 and 46 were killed by wilt. The F₃ generations derived from a single resistant F₂ plant exhibited 16% wilt incidence, while the F₃ generation derived from a single susceptible F₂ plant showed 100% wilt incidence. Among the three backcrosses (derived using resistant F₁ plants nos. 29 and 4, resistant plant of RG2758 and the susceptible plant of parent RG2368), two backcrosses (resistant F₁ plant no. 29 × resistant plant, RG2758) (resistant F₁ plant no. 4 × resistant plant, RG2758) showed resistant reactions; however, the third backcross (resistant F₁ plant no. 29 × susceptible plant, RG2368) exhibited moderate susceptibility to wilt.

Segregation patterns of wilt resistance in selfed-generations of parents, F₁, F₂, F₃ and backcross generations from the cross are presented in Table 2. The selfed-generation of the resistant parent (RG2758) showed a 13 : 3 ratio for resistance and susceptibility, while the susceptible parent (RG2368) showed a 4 : 12 segregation ratio. The resistant and susceptible plants in the F₁ generation segregated in a 10 : 6 ratio. The F₂ plants derived from resistant F₁ plants (nos. 29, 4 and 75) segregated in a 13 : 3 ratio. The F₃ generation derived from a single resistant F₂ plant segregated in a 13 : 3 ratio, while all the F₃ plants derived from a single susceptible F₂ plant were susceptible to wilt.

Plants from two backcrosses (resistant F₁ plant no. 29 × resistant plant, RG2758) (resistant F₁ plant no. 4 × resistant plant, RG2758) segregated in a 13 : 3 ratio for resistance and susceptibility to wilt, while the third backcross (resistant F₁ plant no. 29 × susceptible plant, RG2368) segregated in 1 : 1 ratio. The segregation patterns for wilt resistance and susceptibility in these generations have indicated the role of two different genes (*R*¹ and *R*²) in dominant and recessive interaction mode for controlling *Fusarium* wilt resistance. In dominant and recessive interaction (13 : 3) model, the dominant alleles of one gene locus (*R*¹) in homozygous (*R*¹*R*¹) and heterozygous (*R*¹*r*¹) conditions, and the homozygous recessive alleles (*r*²*r*²) of another gene locus (*R*²) produce one phenotype and the dominant alleles of the second gene locus (*R*²) in homozygous (*R*²*R*²) and heterozygous (*R*²*r*²) conditions produce another phenotype. Therefore, genotypes, *R*¹*R*¹*R*²*R*², *R*¹*R*¹*R*²*r*², *R*¹*R*¹*r*²*r*², *R*¹*r*¹*R*²*R*², *R*¹*r*¹*R*²*r*², *R*¹*r*¹*r*²*r*², exhibit resistance reactions against wilt, and the genotypes, *r*¹*r*¹*R*²*R*² and *r*¹*r*¹*R*²*r*², show susceptible reactions.

The 4 : 12 segregation of resistant and susceptible progenies in selfed-generation of RG2368 established an *r*¹*r*¹*R*²*r*² genotype for the susceptible plant which was self-pollinated to derive selfed-generation of RG2368. The 13 : 3 segregation in the selfed-generation of RG2758, determined an *R*¹*r*¹*R*²*r*² genotype for the resistant plant from which selfed-generation of RG2758 was derived. As the same plants of both parents were used for deriving selfed-generations as well as in the initial crossing, genotypes of these parental plants could be confirmed by a 10 : 6 segregation ratio in the F₁ generation.

The 13 : 3 segregation in the three F₂ generations derived from the resistant F₁ plants, nos. 4, 29 and 75 confirmed the *R*¹*r*¹*R*²*r*² genotype of these resistant F₁ plants. The high susceptibility (100%) of F₂ generations derived from the susceptible F₁ plants nos. 27 and 46 confirmed the *r*¹*r*¹*R*²*R*² genotype of these susceptible F₁ plants. The 13 : 3 segregation in the F₃ generation derived from a single resistant F₂ plant confirmed the dominant and recessive

Table 1: The scale of evaluation used through the investigation

Score	Wilt incidence (%)	Category
0	0.00	Highly resistant (HR)
1	00.1–20	Resistant (R)
2	20.1–40	Moderately resistant (MR)
3	40.1–50	Moderately susceptible (MS)
4	50.1–75	Susceptible (S)
5	>75	Highly susceptible (HS)

Mayee and Datar (1986).

Table 2: Fusarium wilt incidence and segregation pattern of wilt resistance in F₁, F₂, F₃ and backcross generations of the cross, RG2368 × RG2758 and selfed-generations of resistant and susceptible parents

Generation	Number of plants			WI (%)	Expected ratio	χ^2	P (%)
	Total	R	S				
Selfed-generations							
Resistant parent RG2758	53	45	8	15	13 : 3	0.45	50
Susceptible parent RG2368	61	14	47	77	4 : 12	0.13	70–75
F ₁ generation	184	120	64	35	10 : 6	0.57	40–45
F ₂ generations							
F ₂ of R-F ₁ plant no. 29	288	238	50	17	13 : 3	0.35	55–65
F ₂ of R-F ₁ plant no. 4	278	232	46	16	13 : 3	0.84	35–40
F ₂ of R-F ₁ plant no. 75	276	227	49	18	13 : 3	0.17	65–70
F ₂ of S-F ₁ plant no. 27	124	0	124	100	–	–	–
F ₂ of S-F ₁ plant no. 46	157	0	157	100	–	–	–
F ₃ generations							
F ₃ of selected R-F ₂ plant ¹	161	134	27	16	13 : 3	0.4	50
F ₃ of selected S-F ₂ plant ²	78	0	78	100	–	–	–
Backcross generations							
(R-F ₁ plant no. 29 × R-plant-RG2758)	178	142	36	20	13 : 3	0.14	70–75
(R-F ₁ plant no. 4 × R-plant-RG2758)	165	138	27	16	13 : 3	0.62	40–45
(R-F ₁ plant no. 29 × S-plant-RG2368)	158	83	75	47	1 : 1	0.40	50–55

R, resistant; S, susceptible; WI, wilt incidence.

¹Resistant F₂ progeny of resistant F₁ plant no. 29.

²Susceptible F₂ progeny of resistant F₁ plant no. 29.

sive interaction mode of inheritance for wilt resistance in this cross. This also determined an R¹r¹R²r² genotype for the resistant F₂ plant from which the F₃ generation was derived. The 100% wilt incidence in the F₃ generation derived from a single susceptible F₂ plant determined the r¹r¹R²r² genotype for the susceptible F₂ plant from which the F₃ generation was derived. The susceptible and resistant F₂ plants from which the F₃ generations were derived were progenies of the resistant F₁ plant no. 29.

The 13 : 3 segregation ratio from two backcrosses (resistant F₁ plant no. 29 × resistant plant, RG2758) (resistant F₁ plant no. 4 × resistant plant, RG2758) confirmed the R¹r¹R²r² genotype of the resistant F₁ plants nos. 29 and 4 and resistant plant of RG2758 used in the backcross. The 1 : 1 ratio in the backcross (resistant F₁ plant no. 29 × susceptible plant, RG2368) confirmed again the R¹r¹R²r² genotype of resistant F₁ plant no. 29 and r¹r¹R²r² genotypes of the susceptible plant of the susceptible parent, RG2368 used in the backcross.

Mode of inheritance for wilt resistance in RG2529 × RG109

Wilt incidence in selfed-generations of parents, F₁, F₂, F₃ and backcross generations from the cross, RG2529 × RG109 is presented in Table 3. Selfed-generations of both parents were found to be wilt resistant. The F₁ and the F₂ generations derived separately through self-pollinations of resistant F₁ plants, nos. 56, 62 and 88 were resistant to Fusarium wilt. The F₃ generation derived from a single resistant F₂ plant was resistant to wilt, while the F₃ generation derived from a susceptible F₂ plant was highly susceptible (100%). One backcross (resistant F₁ plant no. 56 × resistant plant, RG109) was 100% resistant to wilt. However, two backcrosses (resistant F₁ plant no. 56 × susceptible plant, RG109) (resistant F₁ plant no. 56 × susceptible plant, RG2529) were moderately resistant.

Segregation patterns for wilt resistance in the selfed-generations of parents, F₁, F₂, F₃ and backcross generations of the cross are presented in Table 3. The selfed-generations of parents, F₁ generation and F₂ generations of the resistant F₁ plants representing nos. 56, 62 and 88 exhibited duplicate dominance (15 : 1) for wilt resistance. The duplicate dominance control of

wilt resistance was confirmed by a 15 : 1 segregation ratio in the F₃ generation. In this model, two independently segregating dominant genes (R¹ and R²) influence wilt resistance in the same way; therefore, the genotype having dominant alleles for any of these genes would exhibit resistance to wilt, and genotypes having homozygous recessive alleles at both genes (r¹r¹r²r²) would be wilt susceptible. The 3 : 1 ratio in the backcross generations (resistant F₁ plant no. 56 × susceptible plant, RG109) (resistant F₁ plant no. 56 × susceptible plant, RG2529) also confirmed the duplicate dominance mode of inheritance.

The 15 : 1 ratio in selfed-generations of both parents confirmed the R¹r¹R²r² genotype for the parental plants that were self-pollinated to derive selfed-generations as well as involved in the initial cross. The R¹r¹R²r² genotype of these parental plants was confirmed by a 15 : 1 segregation in the F₁ generation. The 15 : 1 segregation in F₂ generations confirmed the R¹r¹R²r² genotype of the resistant F₁ plants representing nos. 56, 62 and 88. The 3 : 1 segregation in the backcrosses (resistant F₁ plant no. 56 × susceptible plant, RG109) (resistant F₁ plant no. 56 × susceptible plant, RG2529) further confirmed the R¹r¹R²r² genotype of the resistant F₁ plant no. 56 and the r¹r¹r²r² genotype of the susceptible plants of RG109 and RG2529 involved in the backcross. Zero percentage wilt incidence in the backcross (resistant F₁ plant no. 56 × resistant plant, RG109) indicates that any of the resistant genotypes *viz.*, R¹R¹R²R², R¹R¹R²r², R¹R¹r²r², R¹r¹R²R², r¹r¹R²R² could be the genotype of the resistant parent plant, RG109, used in this backcross. The 15 : 1 ratio in the F₃ generation determined an R¹r¹R²r² genotype for the resistant F₂ plant from which the F₃ was derived.

Mode of inheritance for wilt resistance in RG2876 × RG2822

In this cross, the parent RG2876 is highly susceptible to wilt and the parent RG2822 is resistant. Wilt incidence in selfed-generations of parents, F₁, F₂, F₃ and backcross generations of the cross, RG2876 × RG2822 are given in Table 4. Selfed-generation of RG2822 exhibited resistance reaction, and the highly susceptible parent (RG2786) showed 100% susceptibility.

Table 3: Fusarium wilt incidence and segregation pattern of wilt resistance in F₁, F₂, F₃ and backcross generations of the cross, RG2529 x RG109 and selfed-generations of both resistant parents

Generation	Number of plants			WI (%)	Expected ratio	χ^2	P (%)
	Total	R	S				
Selfed-generations							
Resistant parent RG2529	50	47	3	6	15 : 1	0.01	90-95
Resistant parent RG109	54	52	2	4	15 : 1	0.59	45-50
F ₁ generation	158	147	11	7	15 : 1	0.13	70-75
F ₂ generations							
F ₂ of R-F ₁ plant no. 56	182	168	14	8	15 : 1	0.64	45-50
F ₂ of R-F ₁ plant no. 62	191	183	8	4	15 : 1	1.37	25-30
F ₂ of R-F ₁ plant no. 88	214	202	12	7	15 : 1	0.15	70
F ₃ generations							
F ₃ of selected R-F ₂ plant ¹	147	140	7	5	15 : 1	0.55	45-50
F ₃ of selected S-F ₂ plant ²	89	0	89	100	-	-	-
Backcross generations							
(R-F ₁ plant no. 56 x R-RG109)	189	189	0	0	-	-	-
(R-F ₁ plant no. 56 x S-RG109)	172	131	42	24	3 : 1	0.05	80-85
(R-F ₁ plant no. 56 x S-RG2529)	155	118	36	23	3 : 1	0.22	65-70

R, resistant; S, susceptible; WI, wilt incidence.

¹Resistant F₂ progeny of resistant F₁ plant no. 56.

²Susceptible F₂ progeny of resistant F₁ plant no. 56.

The F₁ generation was susceptible to wilt, while the F₂ generations derived separately through self-pollinations of resistant F₁ plants representing nos. 12, 39 and 25 exhibited resistant reactions. The two F₃ generations derived separately from two resistant F₂ plants showed resistance reactions, while the F₃ generations derived from two susceptible F₂ plants showed 100% wilt incidence. The backcross (resistant F₁ plant no. 25 x susceptible plant, RG2822) was resistant to wilt, and the backcross (resistant F₁ plant no. 25 x susceptible plant, RG2876) was susceptible; however, the backcross (resistant F₁ plant no. 25 x resistant plant, RG2822) was free from wilt.

Segregation patterns for wilt resistance in selfed-generations of parents, F₁, F₂, F₃ and backcross generations from the cross are

presented in Table 4. The segregation ratio of resistant and susceptible plants in selfed-generation of RG2822 indicated duplicate dominant gene action (15 : 1) for wilt resistance. The genotype was R¹r¹R²r² for the resistant plant from which selfed-generation of RG2822 was derived. The mode of inheritance in selfed-generation of RG2876 could not be determined with certainty due to its 100% wilt susceptibility. However, a 4 : 12 ratio for resistance and susceptibility in the F₁ generation clearly points out that the mode of inheritance in RG2876 was not similar to that of RG2822. The 4 : 12 segregation pattern in the F₁ generation indicated a duplicate recessive inheritance mode, and the 15 : 1 ratio in F₂ generations derived from resistant F₁ plant nos. 39 and 25 indicated duplicate dominant interactions, thus confirming the

Table 4: Fusarium wilt incidence and segregation pattern of wilt resistance in F₁, F₂, F₃ and backcross generations of the cross, RG 2876 x RG 2822 and selfed-generations of resistant and susceptible parents

Generation	Number of plants			WI (%)	Expected ratio	χ^2	P (%)
	Total	R	S				
Selfed-generations							
Resistant parent RG2822	84	77	7	8	15 : 1	0.61	40-50
Susceptible parent RG2876	87	0	87	100	-	-	-
F ₁ generation	184	49	138	75	4 : 12	0.14	70-75
F ₂ generations							
F ₂ of R-F ₁ plant no. 12	238	198	40	17	-	-	-
F ₂ of R-F ₁ plant no. 39	248	219	29	12	15 : 1	1.57	20-25
F ₂ of R-F ₁ plant no. 25	246	234	12	9	15 : 1	0.78	40-45
F ₃ generations							
F ₃ of selected R-F ₂ plant ¹	263	250	13	5	15 : 1	0.75	40-45
F ₃ of selected S-F ₂ plant ²	56	0	56	100	-	-	-
F ₃ of selected R-F ₂ plant ³	177	148	29	16	3 : 1/12 : 4	7.00	<1
F ₃ of selected S-F ₂ plant ⁴	35	0	35	100	-	-	-
Backcross generations							
(R-F ₁ plant no. 25 x S-RG2822)	69	55	14	20	12 : 4	0.80	45-50
(R-F ₁ plant no. 25 x S-RG2876)	78	25	54	69	4 : 12	0.49	40-50
(R-F ₁ plant no. 25 x R-RG2822)	169	169	0	0	-	-	-

¹Resistant F₂ progeny of resistant F₁ plant no. 39.

²Susceptible F₂ progeny of resistant F₁ plant no. 39.

³Resistant F₂ progeny of resistant F₁ plant no. 12.

⁴Susceptible F₂ progeny of resistant F₁ plant no. 12.

R, resistant; S, susceptible; WI, wilt incidence.

$R^1r^1R^2r^2$ genotype of these resistant F_1 plants. However, no ratio could be fit into the F_2 generation derived from resistant F_1 plant no. 12. The 15 : 1 ratio in F_3 generation derived from a resistant F_2 progeny of resistant F_1 plant no. 39, and the 100% wilt susceptibility of F_3 generation derived from a susceptible F_2 progeny of resistant F_1 plant no. 39, indicated duplicate dominant interactions. Therefore, we determined that the $R^1r^1R^2r^2$ genotype was for the resistant F_2 plant, and the $r^1r^1r^2r^2$ genotype was for the susceptible F_2 plant. However, the segregation ratio in the F_3 generation derived from a resistant F_2 progeny of resistant F_1 plant no. 12 exhibited a 3 : 1 or 12 : 4 ratio.

The proportion of resistant to susceptible plants was 12 : 4 in the backcross (resistant F_1 plant no. 25 \times susceptible plant, RG2822). This proportion could only be possible if a duplicate dominance mode of inheritance was considered in this backcross, as the $r^1r^1r^2r^2$ genotype of susceptible plant from RG2822 and $R^1r^1R^2r^2$ genotype of resistant F_1 plant no. 25 have been determined. The absence of wilt in the backcross (resistant F_1 plant no. 25 \times resistant plant, RG2822) could be due to duplicate dominant gene action (15 : 1), as both resistant F_1 plant no. 25 and RG2822 showed a 15 : 1 ratio. The 4 : 12 ratio in the backcross (resistant F_1 plant no. 25 \times susceptible plant, RG2876) could only be possible when the two genes interact in a dominant recessive mode, and $r^1r^1r^2r^2$ is the genotype of the susceptible plant of RG2876.

Mode of inheritance for wilt resistance in RG2822 \times RG2836

The inheritance of wilt resistance was determined in a cross between resistant (RG2822) and highly susceptible (RG2836) parents. Wilt incidence in selfed-generations of parents, F_1 , F_2 , F_3 and backcross generations of the cross, RG2822 \times RG2836 is presented in Table 5. The F_1 generation exhibited moderate susceptibility to wilt, while the two F_2 generations derived sepa-

rately from resistant F_1 plants representing nos. 7 and 56 showed resistant reactions, and the third F_2 generation derived from resistant F_1 plant no. 28 exhibited moderate resistant reaction to wilt. The F_3 generations derived from two resistant F_2 plants exhibited resistance reactions, while the F_3 generations derived from susceptible F_2 plants were 100% susceptible to wilt. Three backcrosses (resistant F_1 plant no. 28 \times resistant plant, RG2822), (resistant F_1 plant no. 28 \times susceptible plant, RG2822) (resistant F_1 plant no. 28 \times resistant plant, RG2836) were free from wilt incidence, and the fourth backcross (resistant F_1 plant no. 28 \times susceptible plant, RG2836) showed a moderately susceptible reaction.

Segregation patterns of wilt resistance in selfed-generations of parents, F_1 , F_2 , F_3 and backcross generations of the cross are presented in Table 5. The selfed-generation of the resistant parent (RG2822) exhibited a duplicate dominant gene action (15 : 1), while all plants of susceptible parent (RG2836) died due to wilt. The significant 9 : 7 ratio in the F_1 signifies duplicate recessive gene action and is indicative of different inheritance modes between parents. In a duplicate recessive model, the presence of both dominant genes (R_1 and R_2) confers resistance and absence of either of the genes or both genes confers wilt susceptibility. Varying segregation ratios were observed in three F_2 generations derived from three different resistant F_1 plants. The F_2 generations derived from resistant F_1 plants representing nos. 7 and 56 segregated in a 15 : 1 ratio. This ratio reveals an $R^1r^1R^2r^2$ genotype for these resistant F_1 plants. The third F_2 generation derived from resistant F_1 plant no. 28 segregated in a 12 : 4 ratio for resistant and susceptible progenies. If a duplicate recessive model of inheritance was presumed in this F_2 , the genotype of the F_1 plant no. 28 would be $R^1R^1R^2r^2$ or $R^1r^1R^2R^2$. The 15 : 1 segregation in the F_3 generation derived from a resistant F_2 progeny of resistant F_1 plant no. 7 confirmed the duplicate dominant gene interaction. However, no significant

Table 5: Fusarium wilt incidence and segregation pattern of wilt resistance in F_1 , F_2 , F_3 and backcross generations of the cross, RG2822 \times RG2836 and selfed-generations of resistant and susceptible parents

Generation	Number of plants			WI (%)	Expected ratio	χ^2	P (%)
	Total	R	S				
Selfed-generations							
Resistant parent RG2822	153	145	8	5	15 : 1	0.27	65–70
Highly susceptible parent RG2836	117	0	100	100	–	–	–
F_1 generation	154	86	68	44	9 : 7	0.013	85–90
F_2 generations							
F_2 of R- F_1 plant no. 7	233	220	13	14	15 : 1	0.17	60–65
F_2 of R- F_1 plant no. 56	180	165	15	8	15 : 1	1.32	25
F_2 of R- F_1 plant no. 28	182	140	42	23	12 : 4	0.34	55–60
F_3 generations							
F_3 of selected R- F_2 plant ¹	149	142	7	4	15 : 1	0.6	40–45
F_3 of selected S- F_2 plant ²	41	0	41	100	–	–	–
F_3 of selected R- F_2 plant ³	134	112	22	16	12 : 4	5.25	<1
F_3 of selected S- F_2 plant ⁴	32	0	32	100	–	–	–
Backcross generations							
(R- F_1 - plant no. 28 \times R-RG2836)	91	91	0	0	–	–	–
(R- F_1 - plant no. 28 \times S-RG2822)	121	121	0	0	–	–	–
(R- F_1 - plant no. 28 \times R-RG2822)	147	147	0	0	–	–	–
(R- F_1 - plant no. 28 \times S-RG2836)	107	55	53	49	1 : 1	0.04	80–85

R, resistant; S, susceptible; WI, wilt incidence.

¹Resistant F_2 progeny of resistant F_1 plant no. 7.

²Susceptible F_2 progeny of resistant F_1 plant no. 7.

³Resistant F_2 progeny of resistant F_1 plant no. 28.

⁴Susceptible F_2 progeny of resistant F_1 plant no. 28.

digenic epistatic interaction was observed in the F_3 generation derived from a resistant F_2 progeny of resistant F_1 plant no. 28.

The backcross (resistant F_1 plant no. 28 \times susceptible plant, RG2836) exhibited a significant 1 : 1 segregation and the other backcross (resistant F_1 plant no. 28 \times resistant plant, RG2836) showed high resistance to wilt. When the resistant F_1 plant no. 28 was backcrossed to both resistant and susceptible plants of RG2822, progenies of both backcrosses were free from wilt; therefore, no ratios could be determined. However, the absence of wilt in these backcrosses could be due to duplicate dominant gene action; otherwise, there would have been a segregation pattern among backcrossed progeny. Inconsistency in inheritance patterns of wilt resistance observed in this cross was in accordance with that observed in the cross, RG2876 \times RG2822 because both parents showed different modes of inheritance. This inconsistency could be due to parental differences in the inheritance mode. However, the results could not explain how the gene interactions in the F_2 generation varied within itself and the F_1 generation.

Discussion

Considering the potential of *Fusarium* wilt in causing economic losses and its inevitable spread from field to field, resistant castor cultivars are needed for long-term disease management. Although resistance to wilt is an essential selection criterion in breeding of castor cultivars, the genetic basis for resistance has not been definitively characterized. Only a few studies were carried out on inheritance of *Fusarium* wilt resistance in castor and were inconclusive. Resistance was reported to be both monogenic dominant (Hanumantharao et al. 2005; Vishnuvardhan Reddy et al. 2010, Singh et al. 2011) and recessive gene control (Sviridov 1988, Lavanya et al. 2011), and also two complimentary genes (Gouri Shankar et al. 2010) and polygenic control (Desai et al. 2001). Singh et al. (2011) reported monogenic dominant control of wilt resistance in the cross VP-1 \times 48-1; however, Lavanya et al. (2011) found monogenic recessive control in the same cross where VP-1 and 48-1 are highly wilt susceptible and resistant parents, respectively. These differing inheritance patterns might be due to the use of a single location for the evaluations using unreliable suboptimal infection conditions in the field, different parental lines without predetermining the inheritance patterns of wilt resistance in them, and the presence of high levels of heterozygosity and genetic heterogeneity within parents and early generation (F_2 and F_3) families.

The consistent occurrence of less than 20% wilt incidence in the resistant inbred parental lines over years and locations observed prior to this investigation indicated the influence of more than one gene for wilt resistance. The present investigation conclusively confirmed the control of two genes (R^1 and R^2) on wilt resistance. Deviations from the expected 9 : 3 : 3 : 1 dihybrid ratio confirmed epistatic interactions between the two genes. The ratios 15 : 1, 9 : 7 and 13 : 3 indicated duplicate dominant, duplicate recessive and dominant and recessive modes of inheritance, respectively. In a 15 : 1 gene action, dominant alleles of both gene loci produced wilt resistance and the absence of both genes caused susceptibility. However, in a 9 : 7 gene action, the presence of dominant alleles for two genes together produced wilt resistance and the absence of either of them caused susceptibility. In a 13 : 3 gene action, the dominant alleles of one gene locus in homozygous and heterozygous conditions, and the homozygous recessive alleles of another gene locus produced

wilt resistance. Lavanya et al. (2011) suggested a role of oligo-genes with epistatic interactions in controlling wilt resistance in castor but not the inheritance mode or the epistatic interactions. The investigation showed that mode of inheritance and frequency of resistant progenies in F_1 , F_2 and backcross generations of a cross were dependent on the inheritance mode of the parents. When both parents of a cross were comparable in their inheritance mode, the inheritance mode observed in all generations studied were comparable with parents. On the contrary, varying inheritance modes were observed in different generations when parents of a cross differed in their inheritance modes. The results are important when making decisions regarding the choice of parents for developing new wilt resistant castor varieties. This study provides conclusive evidence on the mode for inheritance of wilt resistance and suggests that parental selection should be based on the inheritance mode for use in wilt resistant hybrid breeding programmes.

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