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Research Article

Pattern of genetic diversity among *Fusarium* wilt resistant castor germplasm accessions (*Ricinus communis* L.)

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Abstract

Wilt caused by *Fusarium oxysporum* f.sp. *ricini* (Wr) Gordon is one of the major yield losing diseases in castor. Cultivating wilt resistant cultivars is an effective strategy to control the disease. Utilization of diverse sources of stable resistance is a prerequisite for durable resistance breeding. The experiment was conducted to identify genetically diverse resistant sources in castor germplasm. Genetic diversity among 20 identified wilt resistant germplasm was assessed using multivariate classificatory methods. Wide genetic diversity was demonstrated among these accessions. These accessions are valuable in wilt resistance breeding programme. They would serve as base diverse material for wilt resistance breeding, wilt resistant genepool construction and molecular tagging of resistant genes.

Keywords : Castor, diversity, wilt resistance

Introduction

Castor (*Ricinus communis* L.) is an important industrial non-edible oilseed crop. Its oil enjoys tremendous worldwide demand because of unlimited industrial applications. Castor is grown agriculturally worldwide and India is the world leader in castor production and export. Castor crop is particularly suitable for small-scale, resource poor farmers located in marginal areas due to its ability to thrive under low rainfall and marginal fertility conditions. Castor researchers worldwide are concentrating on genetic improvement of castor for high productivity coupled with resistance to major diseases and insect pests in order to make it competitive with other vegetable oils in energy market.

Continuous cultivation of castor in the same areas has led to several fold increase in the occurrence of soil-born wilt disease caused by *Fusarium oxysporum* f.sp. *ricini* (Wr) Gordon. It is one of the major yield reducing factors in castor and can cause 39-77% yield loss depending on the stage at which the plants wilt (Pushpavati, 1995). Chemical treatments are not efficient in controlling the disease (Siddaramaiah *et al*., 1980). Cultivation of wilt resistant cultivars was proved to be an effective strategy to minimize the losses. There is a need for identification of diverse sources of resistance to wilt to sustain wilt resistance in castor since the existing wilt resistant cultivars are no more effective in controlling the disease as they showed moderate to high susceptibility (40-100% wilt incidence). This was because of utilization of the same source of resistance repeatedly in wilt resistance breeding programme.

The experiment was taken up to assess the genetic diversity among 20 castor germplasm accessions identified as stable sources of resistance to wilt. The pattern of genetic diversity among wilt resistant accessions for nine quantitative traits was studied. The diverse resistant accessions would serve as base

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material to develop a wilt resistant genepool with broad genetic base for desirable quantitative traits and also as parental lines in breeding programmes aimed at development of wilt resistant high yielding cultivars and base material for molecular tagging of wilt resistant genes.

Material and methods

Twenty castor germplasm accession *viz.,* RG-45, RG-111, RG-224, RG-297, RG-398, RG-718, RG-737, RG-1354, RG-1608, RG-1624, RG-1631, RG-1633, RG-1922, RG-1925, RG-1941, RG-1958, RG-2019, RG-2661, RG-2818 and RG-2819, identified as stable sources of resistance to *Fusarium* wilt over years of contiguous screening in wilt sick plots at the Directorate of Oilseeds Research and All India Coordinated Research Project centre at S.K. Nagar, Gujarat (Anjani and Reddy, 2002; Anjani *et al.,* 2004) were used in the present study. The average wilt incidence among these accessions was between zero and 9.8%. As per the scale proposed by Mayee and Datar (1986), the castor genotypes possessing less than 20% wilt incidence are designated wilt resistant. These accessions were collected by the Directorate through explorations in Andhra Pradesh, Tamil Nadu, Bihar, Maharashtra, Assam, Meghalaya and Gujarat states. The 20 resistant accessions were planted in 2006, 2007 and 2008 in a randomized block design with three replications at the research farm of Directorate of Oilseeds Research, Hyderabad, India (17.36^{o}N and 78.47^{o}E). Each accession was planted in a three-row plot of 5 m length in each replication at recommended spacing of 90 x 45 cm plants. Data were collected on 10 plants in each entry in each replication for plant height up to primary raceme (cm), number of nodes up to primary raceme on main stem, length of primary raceme (cm), number of productive racemes/plant, days to 50% flowering of primary raceme, days to maturity of primary raceme, 100-seed weight (g) and seed yield/plant (g). Seed oil content (%) was estimated by an NMR instrument (Oxford-7005). Three years mean value of each quantitative trait recoded in each accession was used for diversity studies. Genetic divergence was assessed using established multivariate algorithms such as principal component analysis (Jolliffe, 1986), D^2-analysis (Mahalanobis, 1936) and cluster analysis using Ward's minimum variance method (Ward, 1963). INDOSTAT statistical software was used for data analysis.

Results and discussion

Analysis of variance established significant differences for various traits among the accessions. The three multivariate methods employed in this study were widely used in genetic diversity analysis as they simultaneously analyse multiple measurements on each individual under investigation (Franco *et al*., 1998). These three methods using quantitative data clearly demonstrated distinct pattern of genetic diversity among resistant accessions. Genetic divergence studies using quantitative traits and one of the multivariate methods were reported in castor (Sevugaperumal *et al*., 2000; Anjani and Reddy, 2002; Figueiredo Neto *et al.,* 2004; Costa *et al*., 2006; Bahia *et al*., 2008). However, there were no studies reporting diversity among wilt resistant accessions in castor. Anjani and Reddy (2002) studied diversity among castor accessions collected from northeastern India which included some of the wilt resistant accessions reported in the present study.

Principal component analysis

The principal component analysis grouped the 20 accessions into four clusters on the first two principal components (Fig. 1). The analysis showed that the first component alone accounted for 72% of the variation (latent root =743.9) among the resistant accessions. The geometrical distances among accessions in the principal component plot reflected the genetic distances among them with minimal distortion. This allowed us to visualize the differences among the accessions and identify possible distinct groups. Since the 82% of total variation was explained by the first two principal components the relationships between close neighbours were not distorted. Cluster I comprised of eight accessions *viz*., RG-45, RG-111, RG-398, RG-718, RG-737, RG-1631, RG-1925 and RG-1958. Cluster II included RG-224, RG-297, RG-1354, RG-2019, RG-1922, RG-1941, RG-2818 and RG-2819. Three accessions namely, RG-1608, RG-1633 and RG-2661 formed cluster III and a lone accession RG-1624 formed cluster IV. Clusters I, III and IV were plotted apart from each other indicating their diverse nature, whereas cluster II was stretched in between cluster I and III showing the relative closeness of some of the accessions in this cluster with those in Cluster I and III. Thus, this method allowed us to detect individuals showing intermediary between groups. The distant position of Cluster IV from the remaining clusters indicates that the lone accession RG 1624 included in this cluster is very much diverse from the rest of the resistant accessions.

D^2-analysis

D^2-analysis grouped the 20 accessions into three clusters. Here, the accessions that were included in I and II clusters of principal component analysis were placed together in cluster I. And the accessions that formed II and III clusters were correspondingly the same as those formed III and IV clusters in principal component analysis. The groups were distantly apart from each other. The distance of cluster I from clusters II and III was 54.3 and 87.9, respectively while the distance between clusters II and III was 53.8. The large distances, among clusters indicated that the wilt resistant accessions were significantly diverse for different quantitative traits studied. Considerable variation also existed within cluster I (30.8) and cluster II (33.5). Accessions in cluster I are of early duration type with short plant height and low number of nodes up to primary raceme whereas those in Cluster II are of medium duration type with medium plant height and node number (Table 1). The lone accession (RG 1624) in cluster III is a very late type possessing tall plant height and high node number. The number of nodes up to primary raceme on main stem and plant height are considered to be indicators for detecting earliness in castor bean as they show strong positive relation with days to 50% flowering and days to maturity (Mehata and Vashil 1998). The clustering pattern of resistant accessions also followed the same relation.

Cluster analysis

Cluster analysis grouped the resistant accessions into different groups and subgroups at different phenon levels. The two groups (A and B) were further divided into four distinct sub-groups (sub-group I to IV) as the phenon levels decreased (Fig. 2). The composition of accessions in each of these four clusters was exactly the same as those in four clusters formed by principal component analysis. Further, cluster analysis provided additional precise grouping of sub-groups at still reduced phenon levels thus facilitating identification of moderately diverse accessions.

Plant height up to primary raceme, days to 50% flowering of primary raceme, days to maturity of primary raceme, number of productive racemes/plant, 100-seed weight, seed yield/plant and seed oil content had significant influence on classification by all the three methods. Earlier reports also identified the quantitative traits *viz.,* days to flowering, number of racemes per plant, plant height, potential yield and seed oil content promising for diversity studies in castor (Anjani and Reddy, 2002; Figueiredo Neto *et al.,* 2004; Costa *et al*., 2006; Bahia *et al*., 2008). The multivariate methods provided realistic picture of the relationships between different clusters of wilt resistant accessions and were effective in revealing the genetic relationships among accessions. Pattern of diversity among the resistant accessions was more or less same in all the methods. Contribution of quantitative data was effective in differentiating the resistant accessions while diverse geographical origin of accessions played no role in their grouping.

Conclusions

Analysis of genetic diversity facilitated reliable classification of wilt resistant accessions and also revealed realistic picture of the relationships among accessions. Identification of diverse resistant accessions would be useful for stratified sampling of diverse genotypes for resistance breeding programme as well to construct a wilt resistant genepool. The knowledge accrued from these studies would be highly useful in planning crosses and assigning unexploited resistant accessions to specific heterotic groups.

The resistant accessions from widely diverse clusters may give varying combinations of genes as well as intermediate types. The diverse resistant accessions would have perpetual value in scientific advancement in host-plant resistance breeding in castor. They are also promising sources for molecular characterization, tagging and mapping of wilt resistance genes in castor to utilize in marker-assisted breeding.

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Table 1. Cluster means for various characters based on D^2-analysis

Character	Cluster I	Cluster II	Cluster III
Plant height (cm)	91	231	298
Number of nodes up to primary raceme	15	23	33
Length of primary raceme (cm)	29	31	40
Number of productive racemes/ plant	9	8	5
Days to 50% flowering of primary raceme	57	73	125
Days to maturity of primary raceme	126	145	193
100-seed weight (g)	24	48	46
Seed oil content (%)	48	45	51
Seed yield (g/plant)	97	150	231

Fig. 1. Principal component analysis of 20 *Fusarium* wilt resistant accessions based on nine quantitative traits. The ellipses enclose the wilt resistant accessions of the concerned clusters.

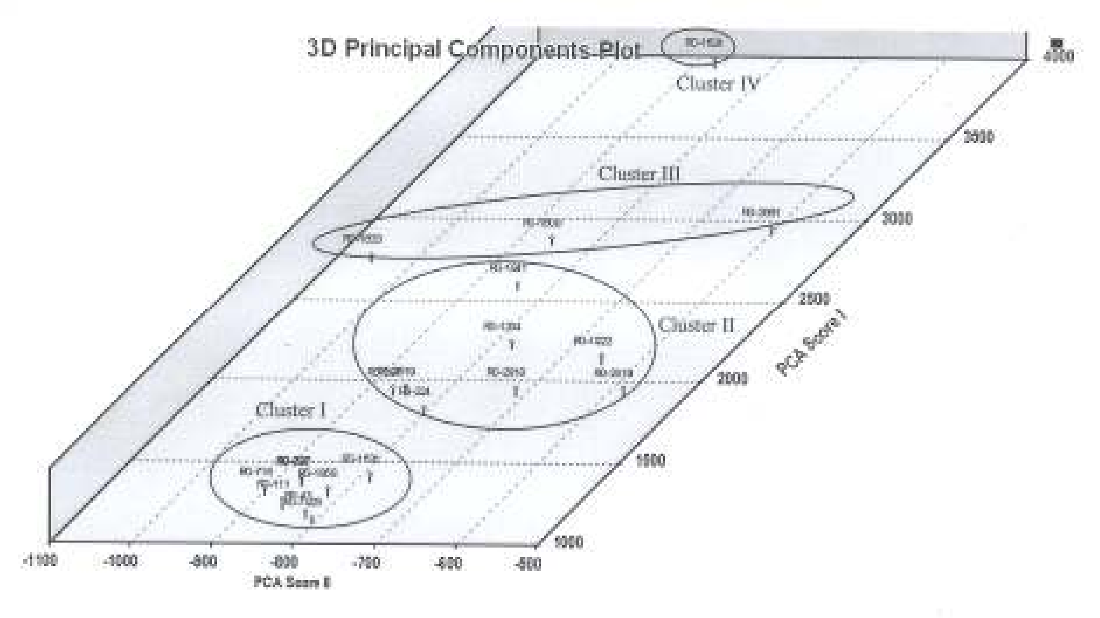

Fig. 2. Dendogram formed by cluster analysis based on Ward's minimum variance. Sub-clusters I, II, III and IV were selected for grouping the 20 wilt resistant accessions as these were precisely in agreement with those formed by principal component analysis.