International Journal of Economic Plants 2016, 3(3):107-110 Review Article

# Fusarium Wilt Resistance in Castor: An Overview of the Recent Advances and Future Strategies for Genetic Improvement

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**Article History**

Article ID: IJEP106
Received in 24th June, 2016
Received in revised form 29th July, 2016
Accepted in final form 10th August, 2016

## Abstract

Castor is one of the important non-edible oilseeds with immense industrial and commercial values. The major castor producing countries in the world are India, Brazil, USSR and China. India is the world leader with regard to castor area (13.20 lakh ha), production (22.0 lakh tons) and productivity (1653 kg ha$^{-1}$). In castor, to release any hybrid or variety at state or national level in India, the entry should be wilt-resistant, in addition to possessing excellent yield and yield-contributing characters. Wilt caused by *Fusarium oxysporum* f.sp. *ricini* is the most important soil and seed borne disease of castor causing significant yield losses. In India, the disease is observed in all the castor growing regions of the country. The extent of yield loss depends on the stage at which plant wilts with the losses ranging from 77% at flowering to 63% at 90 days and 39% in later stages on secondary branches. To our knowledge, only few studies were carried out on inheritance of fusarium wilt resistance in castor and were inconclusive. To thoroughly characterize wilt resistance and to provide a better understanding of the differences among the genotypes with varying levels of resistance, an integrated approach focusing on quantifying the levels of *F. o.* f.sp. *ricini* colonization through fungal DNA quantification coupled with microscopic examination of resistance expression within the root tissues and visual evaluation of symptoms is necessary. This information will be valuable to the castor breeders interested in improving castor wilt resistance, because they can prioritize their efforts according to the type and level of resistance expression desired.

***Keywords:*** Castor, Fusarium oxysporum, Wilt resistance

## 1. Introduction

Castor is one of the important non-edible oilseeds with immense industrial and commercial values. This crop is widely distributed throughout the tropics and sub-tropics and is well adapted to the temperate regions of the world. The major castor producing countries in the world are India, Brazil, USSR and China. India is the world leader with regard to castor area (13.20 lakh ha), production (22.0 lakh tons) and productivity (1653 kg ha$^{-1}$) (Annual Report, Castor, 2013-14).

Wilt caused by *Fusarium oxysporum* f.sp. *ricini* (Nanda and Prasad) is the most important soil and seed borne disease of castor causing significant yield losses. In India, the disease is observed in all the castor growing regions of the country. The extent of yield loss depends on the stage at which plant wilts with the losses ranging from 77% at flowering to 63% at 90 days and 39% in later stages on secondary branches (Pushpawathi et al., 1998). An attempt has been made in this paper to review the progress made in breeding for fusarium wilt resistance in castor while suggesting the future strategies for evolving durable resistant cultivars.

## 2. Etiology and Symptomatology

The wilt disease was characterized completely and the etiology was established as *F. o.* f. sp. *ricini* (Nanda and Prasad, 1974). The fungus produces abundant white mycelial growth on semi-synthetic medium which turns to pink on incubation under fluorescent light. It forms micro and macro conidia are formed. Wilting is preceded by production of leaf blight symptoms and formation of dark stripes on the entire stem up to the infected leaves. Drooping of plants with few top leaves after drying and dropping of all the affected lower leaves is a characteristic symptom and ultimately plants die (Fig. 1).

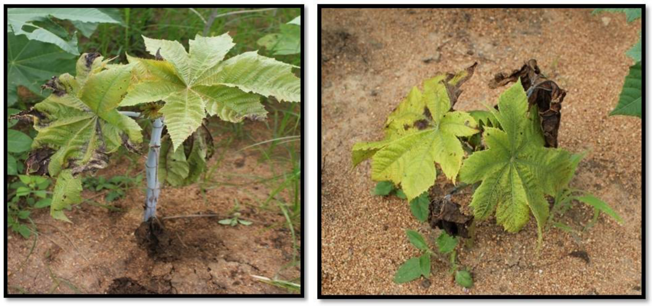

Figure 1: Fusarium wilt affected castor plants in the wilt sick plot, Palem, Telangana

## 3. Screening Techniques To Identify Wilt Resistance In Castor

### 3.1. Field screening

Wilt sick plot (WSP) method is the most common, effective and widely used method for screening of genotypes resistant to fusarium wilt. The main advantages of the method are that it clearly differentiates between susceptible and resistant lines thereby allowing for screening a large amount of genetic material. A WSP is developed by repeated incorporation of diseased plant debris, inoculum and cultivation of wilt susceptible cultivar and the soil inoculum load is maintained at 2.1 x $10^3$ colony forming units (CFU) per gram of soil. Wilt incidence is recorded as percentage at 30 days after sowing and there after at monthly intervals up to 180 DAS. Wilt reactions are categorized using the scale devised by Mayee and Datar (1986).

### 3.2. Screening under controlled conditions

### 3.2.1. Root dip inoculation technique

Root dip inoculation (RDI) technique is a simple and reliable technique to screen for wilt resistance under controlled conditions in the green house. The method involves germination of castor seeds in sterile sand followed by trimming of seedling roots, dipping in spore suspension of *F. o.* f.sp. *ricini* and transplanting in pots. Observations on wilt incidence are recorded periodically up to 30 days after transplanting (Desai and Dange, 2003; Santha Lakshmi Prasad et al., 2008).

## 4. Genetics of Wilt Resistance in Castor

To our knowledge, only 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; Singh et al., 2011) and recessive gene control (Sviridov 1988, Lavanya et al., 2011), and also two complementary genes (Hanumantharao et al., 2005; Gouri Shankar et al., 2010) and polygenic control (Desai et al., 2001). Inheritance analysis carried out for wilt resistance in three crosses revealed that in the cross VP-1 x 48-1, the resistance to fusarium wilt was recessive while in the other two crosses, DPC 13 x RG 297 and DPC 9 x RG 297, resistance was incompletely dominant (Lavanya et al., 2011). Patel and Pathak (2011) studied the inheritance of the resistance to wilt and found that both the parents should be wilt resistant for developing wilt resistant hybrids. Anjani et al. (2014) reported that the mode of inheritance of $F_1$, $F_2$ and subsequent generations of a cross is dependent on the inheritance mode of the parents.

## 5. Breeding for Fusarium Wilt Resistance

Identification of durable resistance is imperative to evolve reliable genotypes. Over the years, global castor collections comprising 1,779 Indian and 190 exotic accessions from 36 countries were screened against wilt, among which only 13 accessions (11 from India; 2 from the former USSR) consistently showed wilt resistance in both WSP and greenhouse conditions during multiple years (Anjani et al., 2014). These 13 resistant accessions (RG-43, RG-111, RG-109, RG-297, RG-1608, RG-1624, RG-2758, RG-2787, RG-2800, RG-2818, RG-2822, RG-3016 and RG-3105) would be of great value as donors of resistance.

In castor, to release any hybrid or variety at state or national level in India, the entry should be wilt-resistant, in addition to possessing excellent yield and yield-contributing characters. To achieve this, the entry must show wilt resistance in the national wilt nursery (NWN) at the Directorate of Oilseeds Research (DOR), Hyderabad. The resistant entry is subsequently evaluated at the three permanent WSPs that are currently being maintained at the DOR, Hyderabad (rainfed system), S.K. Nagar, Gujarat (irrigated system) and Palem, Telangana (rainfed system). The entry, which exhibits resistance to wilt in atleast one WSP among the three and has a yield advantage over national checks, will be advanced to the subsequent stages of evaluation. So far, several wilt resistant hybrids and varieties were released in India (Table 1).

Table 1: Varieties And Hybrids Of Castor Resistant or tolerant To Fusarium Wilt Caused By *Fusarium oxysporum* f.sp. *ricini*

| Sl. No. | Variety or hybrid | Year of release | Resistant or Tolerant to wilt |
|---|---|---|---|
| 1. | Jyothi (DCS-9) | 1994 | Resistant |
| 2. | Haritha (PCS 124) | 2002 | Resistant |
| 3. | Jwala (48-1) | 2007 | Resistant |
| 4. | DCS-107 | 2010 | Resistant |
| 5. | GCH 4 (VP-1 x 48-1) | 1988 | Tolerant |
| 6. | DCH 177 (DPC 9 x DCS 9) | 2000 | Resistant |
| 7. | GCH 7 (SKP-84 x SKI-215) | 2006 | Resistant to nematode-wilt complex |
| 8. | DCH 519 (M 574 x DCS 78) | 2006 | Resistant |
| 9. | PCH 111 (DPC 9 x CS-1) | 2010 | Resistant |
| 10. | PCH 222 (DPC 9 x CS-18) | 2012 | Resistant |

## 6. Molecular Markers

To our knowledge, so far only two studies identifying molecular makers linked to wilt resistance in castor have been reported. Three RAPD markers viz., RKC $23_{1375}$, RKC $21_{1080}$ and OPBE $18_{900}$ flanking the wilt resistance gene at genetic distance of 5 cM, 10.7 cM and 7.6 cM respectively were identified using linkage analysis carried out using '48-1' and 'VP-1' (Singh et al., 2011). In another study, two RAPD markers, OPH-$12_{4973}$ and OPJ-$15_{4268}$ tightly linked to 'Haritha' and '48-1' at a distance of 5.0 cM and 7.0 cM respectively to wilt resistance were identified (Rajashekar Reddy et al., 2011). These markers provide a valuable basis for marker-assisted selection in castor breeding programs.

## 7. Mutation Breeding

Irradiation of VP-1, a wilt susceptible pistillate line, and selections made in subsequent generations for wilt resistance led to the development of will resistant pistillate lines M 619, M 571, M 568, M 574 and M 584. One of the derivatives, M 574 is the female parent of high yielding wilt resistant hybrid DCH-519, evolved from DOR, Hyderabad.

## 8. Pathogenic Variability

In India, studies performed at four locations (DOR, Hyderabad; Palem, Telangana; S.K. Nagar, Gujarat and Yethapur, Tamil Nadu) over the years under AICRP on castor revealed pathogenic variability of *F. o.* f.sp. *ricini*. Differential reactions of four castor cultivars to 29 isolates of *F. o.* f.sp. *ricini* revealed 5 pathotypes among the isolates and genetic diversity using RAPDs grouped the isolates into 5 clusters (Santha Lakshmi Prasad et al., 2008). Interestingly, the grouping of isolates based on pathogenic variation had limited or no correlation with grouping based on RAPD analysis with most of the test isolates. Desai et al. (2003) reported considerable variability in 15 different isolates of *F. o.* f.sp. *ricini* with respect to morphological, cultural characters and pathogenicity. Madhusudhana Reddy et al. (2013) observed 28-54% variability among the 146 isolates of *F. o.* f.sp. *ricini* using RAPD markers.

## 9. Molecular Host-pathogen Interaction

To our knowledge, there are only two studies on the resistance expression to fusarium wilt in castor at the molecular level. mRNA expression analysis of the lipoxygenase (LOX) gene, viz., LOX5 (Rc9-LOX) revealed higher expression level in resistant genotypes (48-1 and SKP-84) compared to the susceptible genotypes (VP-1 and VI-9) suggesting its involvement in defence response in an incompatible host-pathogen interaction. Thus RcLOX5 gene can be used for identification of wilt resistant genotypes (Somnath et al., 2013). Expression analysis of key genes of the phenylpropanoid pathway identified the role of phenylalanine ammonia lyase (PAL) and cinnamate 4-hydoxylase 2 (C4H2) genes as evident by their increased expression in resistant genoytpes (Pritham et al., 2013). Further, though phenolic profiling revealed that caffeic acid could be used as a marker for large scale screening of the castor germplasm.

## 10. Future Strategies

Research on identification of race specific resistant genes and pyramiding them in a single genotype is required for longer perpetuation of the varieties. Screening the progenies of every segregating generation under high inoculum load might lead to the development of durable resistant genotypes. Similarly, already released varieties and parental lines of hybrids may be regularly screened under the wilt sick conditions to select source seed from wilt resistant plants.

For the development of a hybrid, it is desirable for both the parents to be wilt resistant as there would be likelihood that the parents have evolved different mechanisms of resistance. Hence, while proceeding with a breeding programme, an appropriate method might be to perform a genome-wide QTL interval mapping based on anonymous genetic markers in the $F_2$ and/or backcrosses between the parents. An informed decision on suitable genetic improvement programme can then be taken depending on the size of the QTL detected and the performance of the parents and the $F_2$ and/or backcrosses. Marker assisted breeding for fusarium wilt resistance in castor is very limited and confined only to the development of RAPD markers. Hence, identifying more reliable markers for tagging wilt resistance genes followed by marker assisted selection will reduce the time lag in evolving wilt resistant cultivars.

To thoroughly characterize wilt resistance and to provide a better understanding of the differences among the genotypes with varying levels of resistance, an integrated approach focusing on quantifying the levels of *F. o.* f.sp. *ricini* colonization through fungal DNA quantification coupled with microscopic examination of resistance expression within the root tissues and visual evaluation of symptoms is necessary. This information will be valuable to the castor breeders interested in improving castor wilt resistance, because they can prioritize their efforts according to the type and level of resistance expression desired.

A strategy utilizing molecular markers to detect and exploit genetic variation in wilt resistance integrated with an understanding of the ultrastructural host-pathogen interaction could play a major role in understanding the genetic control of resistance to fusarium wilt in castor.

## 11. Conclusion

To thoroughly characterize wilt resistance and to provide a better understanding of the differences among the genotypes with varying levels of resistance, an integrated approach focusing on quantifying the levels of *F. o.* f.sp. *ricini* colonization through fungal DNA quantification coupled with microscopic examination of resistance expression within the root tissues and visual evaluation of symptoms is necessary. A strategy utilizing molecular markers to detect and exploit genetic variation in wilt resistance integrated with an understanding of the ultrastructural host-pathogen interaction could play a major role in understanding the genetic control of resistance to fusarium wilt in castor.

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