Establishing a high throughput screening method for large scale phenotyping of castor genotypes for resistance to *Fusarium* wilt disease

Ranjan K. Shaw[1,2], Mobeen Shaik[1], Zubair Ahmed Mir[1,3], M. Santha Lakshmi Prasad[1], R. D. Prasad[1], S. Senthilvel[1]*

[1]ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad – 500030, India

[2]Department of Genetics, Osmania University, Hyderabad – 500007, India

[3]Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Hyderabad – 500030, India

*Corresponding author

S. Senthilvel

e mail: senthilvel.senapathy@icar.gov.in

Telephone: +91 40 24598116

Fax: +91 40 24017969

The final publication is available at www.springerlink.com
https://link.springer.com/article/10.1007/s12600-016-0535-0

Abstract

Castor (*Ricinus communis* L.) is an important oilseed crop providing raw materials for many industries. Its cultivation is seriously affected by the wilt disease caused by *Fusarium oxysporum* f. sp. *ricini*. In India, no cultivar could be released for cultivation without wilt resistance. Breeding for wilt resistance is hampered due to the limitation on number of genotypes that can be screened in a traditional 'sick plot (field) method'. In the pursuit of establishing a high throughput screening method, we evaluated four different methods of artificial inoculation namely seed soaking, soil drenching, root dip and sick pot in glasshouse condition for their efficiency using a panel of eight genotypes with known disease reaction. The results showed that 'sick pot method' was the most ideal for accurate identification of resistance or susceptibility in plants in a short time with relative ease. In order to further validate the results, a large set of 132 castor inbred lines were evaluated in sick pot. Based on days-to-death data of the inbred lines, a scoring system was developed to suitably characterize the degree of resistance. Screening of the same set of inbred lines in the sick field produced similar results but moderate and highly resistant genotypes could not be differentiated suggesting the advantage of sick pot method over field screening. The screening method established and the set of resistant or susceptible inbred lines identified in this study could be of immense use in basic research concerning host-pathogen interactions, molecular genetics and breeding applications in castor.

Keywords

Fusarium oxysporum f. sp. *ricini*, *Ricinus communis* L., resistant lines, glasshouse screening, inoculation methods

Introduction

Castor (*Ricinus communis* L.) is the sole member of the genus *Ricinus* under Euphorbiaceae family. East Africa was considered as the probable origin of castor based on the prevalence of diversity (Vavilov 1951); but it is now widely distributed across the world. It is an important industrial oilseed crop grown especially in arid and semi-arid regions. The importance of castor lies in its unique seed oil that contains more than 80 per cent of ricinoleic acid (an unusual, monounsaturated, 18-carbon fatty acid), having many desirable industrial properties. Castor oil and its derivatives have widespread applications in various industries including paint, lubricant, cosmetics, nylon, pharmaceutical, plastics and textiles (Ogunniyi 2006). Castor is assuming even greater significance now because of its biodiesel potential (Berman et al. 2011).

Castor production is seriously affected by several pests and diseases of which, wilt caused by *Fusarium oxysporum* f. sp. *ricini* is the most important soil-borne disease (Nanda and Prasad 1974). The *Fusarium* wilt occurs in all castor growing areas across seasons and causes yield loss of up to 77 per cent depending on the stage at which the plants wilt (Pushpawathi et al. 1998). Symptoms of *Fusarium* wilt in castor include root degeneration, necrotic streaks at the base of stem, chlorosis of leaves and necrosis of affected tissues, finally leading to death of the plant. Though the pathogen is primarily a soil borne fungus, its seed born nature has also been confirmed (Naik 1994).

As the wilt is a vascular disease caused by the soil-borne fungus, chemical and physical control methods are not much effective and economically viable (Dange et al. 2006). Due to the systemic nature of the pathogen and the difficulties in controlling the pathogen after beginning of the infection process, development of castor cultivars with inherent resistance to wilt is the only viable option to manage the disease problem. The fungus survives for several years in the field

after its introduction. Growing wilt resistant cultivars would reduce the pathogen spread in the field and therefore contribute to effective integrated disease management.

Development of wilt resistant cultivars requires identification of dependable sources of resistance by screening large diverse germplasm collections and knowledge on mode of inheritance of resistance. Several wilt resistant genotypes both in germplasm collections as well as improved lines have been identified in castor (Prasad and Bhatnagar 1981; Raoof and Rao 1996; Sudhakar et al. 2010; Anjani et al. 2014). Classical genetic studies hitherto conducted on wilt resistance in castor indicated the involvement of recessive genes (Sviridov 1988; Podkuichenko 1989; Lavanya et al. 2011), dominant genes (Reddy et al. 2010; Reddy et al. 2011), duplicate genes (Sviridov 1988; Anjani and Raoof 2014), complimentary genes (Rao et al. 2005; Gourishankar et al. 2010) and polygenes (Desai et al. 2001; Lavanya et al., 2011; Patel and Pathak, 2011). These observations suggest the possibility of Mendelian and/or quantitative genetic basis of wilt resistance in castor.

Historically, screening for wilt resistance is done based on disease incidence in wilt sick plots under field conditions, which had been very effective but with limitations. A major limitation associated with field screening is the restriction on the number of entries that can be screened in a fixed sick plot. The field variability including uneven distribution of the pathogen load may influence the disease development and severity, which would lead to ‘escapes’ or ‘false positives’. Moreover, the field screening is time consuming and labour intensive. Hence, a rapid, reliable, reproducible and high throughput glasshouse based artificial screening method is needed for evaluating large number of genotypes for reaction to *Fusarium* wilt in castor.

In castor, a few glasshouse based wilt screening methods namely seed soaking, root dip inoculation, soil drenching and sick pot have been used (Raoof and Rao 1996; Desai and Dange

2003; Prasad et al. 2008). However, suitability of these methods for large scale evaluation has not yet been established. Therefore, this study was undertaken to identify a rapid and reliable screening method for *Fusarium* wilt resistance in castor and assess its efficacy by screening a large set of castor genotypes and comparing the results with the field screening.

Materials and Methods

To identify a suitable screening method, four widely used methods: (i) seed soaking, (ii) soil drenching, (iii) root dip and (iv) sick pot were evaluated for speed, reliability, consistency and ease of use using a panel of eight castor genotypes with known disease reaction. The genotype panel consisted of two standard checks: JI35 (susceptible) and 48-1 (resistant), three resistant lines: AP77, AP130, AP171 and three susceptible lines: AP45, AP56 and AP60. A large set of 132 castor inbred lines was used for validation of the selected inoculation method. These inbred lines were derived from the accessions of core germplasm (Sarada and Anjani 2013), which represent the spectrum of diversity in the germplasm collection of castor. The details of inbred lines are given in Online Resource 1. The details of the experimental procedures followed are described below.

Assessment of inoculation methods

Experimental setup

All experiments were conducted in plastic pots (30 × 15 × 13cm) filled with 4 kg of sterilized potting mixture (red soil, black soil and farmyard manure in the proportion of 5:3:1). Two test genotypes (one susceptible and one resistant) were sown in two halves of the same pot and 10 seedlings per genotype were maintained. The pots were kept in the glasshouse at 25 to 30°C and

watered whenever required. The same setup was replicated twice. The seedlings were observed regularly for development of disease symptoms.

Preparation of initial inoculum

The initial inoculum of *Fusarium oxysporium* f. sp. *ricini* was prepared by isolating the pathogen from the infected root of a susceptible castor genotype, JI35 (grown at the research farm of Indian Institute of Oilseeds Research [IIOR], Hyderabad) and by culturing it on potato dextrose agar (PDA) medium. The fungal culture was purified by single spore isolation technique and maintained in paraffin oil at -20°C (Nakasone et al. 2004).

Seed soaking method

The fungal culture was grown on PDA for seven days at 28 ± 2°C at BOD incubator. Five discs of the culture medium (3 mm size) containing conidia were removed from the petri plate and added to the Erlenmeyer flasks (250 ml) containing 100 ml of potato dextrose broth (PDB). The flasks were incubated at 25 ± 2°C on an orbital shaker at 100 rpm for 3 days and kept in the culture room at 28 ± 2°C for 4 days. The PDB culture was filtered through double layered muslin cloth. The spore concentration was estimated using a hemocytometer and adjusted to 1×10^6 spores / ml. The seeds were surface sterilized and soaked in 200 ml of spore suspension for two hours. The treated seeds were sown in plastic pots. Seeds soaked in sterile water were used as control.

Soil drenching method

In this method, each pot with 10 day-old seedlings was poured with 10 ml of spore suspension prepared as described in seed soaking method. The sterile PDB was used for the uninoculated control pots.

Root dip method

The seeds were surface sterilized and sown in plastic trays containing sterilized sand. The trays were kept in the glasshouse at ambient temperature. Ten day-old seedlings were uprooted and cleaned under running tap water. The tip of the roots were trimmed for about 2 cm and dipped for 3 min in the spore suspension prepared as described in seed soaking method. The inoculated seedlings were then transplanted in the pots. Controls pots were maintained by dipping the trimmed seedlings in sterile distilled water.

Sick pot method

The pathogen was mass-multiplied on sorghum (*Sorghum bicolor*) grains as substrate. Semi-cooked sorghum grains (100 gm in 250 ml of conical flask) were sterilized by autoclaving at 15 psi for 20 min at 121°C. The flasks were inoculated by actively growing fungal mycelial culture grown on PDA and incubated at 28 ± 2°C for 15 days. The flasks were hand shaken daily to ensure complete fungal colonization on the sorghum grains. The 15-day old fungal culture was added to the potting mixture at the rate of 3g/kg and thoroughly mixed. The pots were watered and kept for incubation for 24 hours before sowing. Control pots were maintained with only sterile soil.

Large scale evaluation of inbred lines in pot

The preparation of pots and inoculation was done as described above (sick pot method). The inbred lines were sown along with standard resistant (48-1) and susceptible (JI35) checks. Three test entries (5 plants / entry) were sown in a single pot and the same setup was replicated twice. The plants were observed for disease reaction up to 75 days after sowing (DAS).

Large scale evaluation of inbred lines in field

The wilt sick plot was prepared by repeated incorporation of wilt affected plant debris and continuous cultivation of wilt susceptible cultivar. The inoculum load in the soil before start of the experiment was 2×10^3 colony forming units (CFU) per gram of soil. Each inbred line was sown in 4.5 m long rows with the spacing of 90 cm (between rows) × 45 cm (between plants) during November-2014. The standard susceptible (JI35) and resistant checks (48-1) were sown after every five test entries. All test entries were replicated twice. The plants were observed for disease reaction up to 150 DAS.

Results and Discussion

Assessment of inoculation methods

The different inoculation methods were compared based on speed, reliability, consistency and ease of use using a panel of eight castor genotypes with known disease reaction. All the genotypes germinated within 8-10 days and the germination rate was more than 99 per cent. The plants were observed for wilt symptoms regularly. The symptoms of Fusarium infection started with necrotic spots in the leaves, chlorosis and discoloration of leaves, drooping of the plant and

extended to formation of black streak from collar to growing point and ultimately seedling death. The various disease symptoms are depicted in Fig 1.

Seed soaking method

The disease progress was slow and severity was less in seed soaking method compared with all other methods (Fig 2). The plants of susceptible genotypes showed disease symptoms 30 to 35 DAS and died between 35 and 40 DAS. All the plants in resistant genotypes: AP56, AP77 and AP171 and 16 out of 20 plants of resistant check, 48-1 survived, as expected. However, four to six plants of the susceptible genotypes did not die till the end of the experiment (Table 1). These plants could have escaped from the pathogen infection because of the non-availability of live spores at the time of root initiation and growth. Okiror (1998) also reported delay in disease occurrence and inconsistency in disease reaction in seed soaking method, while optimizing the screening methods for *Fusarium* wilt in pigeon pea. Even though seed soaking method is easy to perform, the reliability of results is a concern because of the possibility of escapes.

Soil drenching method

In soil drenching, the onset of the disease was little earlier than seed soaking method, comparable with root dip and later than sick pot method (Fig 2). The plants of susceptible lines died between 30 and 35 DAS. The disease severity was comparable with seed soaking but lesser than root dip and sick pot methods. The disease reactions of resistant and susceptible genotypes were as expected except for 48-1 in which eight out of 20 plants died after 58 DAS. Lee et al. (2015) observed very less disease severity in susceptible cultivars, when soil drenching method was used to screen melon genotypes for *Fusarium* wilt.

In soil drenching, the conidial suspension is poured onto the surface of the soil after establishment of seedlings. The success of this method depends on the proportion of the roots exposed to the spores. The spore movement in the soil could be uneven, which could make the roots inaccessible to spores. Gracia-Garza and Fravel (1998) studied the effect of water percolation on dispersal of conidia through soil and found that nearly all propagules were retained within the upper 2 cm of the soil and CFU count was 10-fold lesser in the 8 to 10 cm depth compared with 0 to 2 cm depth. The dispersal of fungal propagules in soil water is greatly affected by the size, shape and electrical charge of the spore as well as the physical properties of soil. There is a possibility of spores getting filtered out as water percolates through the soil (Burke 1965). In general, the pathogen does not move more than a few centimeters by percolating water (Wallace 1978) and hydrophobic nature of the spore may lead to slow rate of passive transport within the soil (Ruddick et al. 1972). Hence, there is chance for uneven dispersion of conidial suspension poured on the soil surface. Most of the spores may remain in the upper layer of the soil and the roots of the plants may not be uniformly exposed to the fungal spores.

Root dip method

In root dip inoculation method, 2 to 3 transplanted plants in a few genotypes wilted quickly within a day or two, which might be due to transplantation shock. The wilt reaction in the susceptible genotypes started 20 days after transplantation. Gradually, all the susceptible lines started showing symptoms and collapsed before 25 days after transplantation. The time taken from sowing to disease scoring in root dip method was comparable to soil drenching method but more than sick pot method. Early fungal invasion and severe wilting symptom were expected in

root dip method due to pruning of roots. But, we did not find such a trend. Okiror (1998) has also reported late wilting and slow disease progress in pigeon pea, when root dip inoculation method was used. The root injury did not give any major advantage over intact root. Instead, wounding of the root system may reduce plant growth or cause early death as observed in this study.

A few plants (up to 7 out of 20) of resistant genotypes also collapsed in root dip experiment (Table 1; Fig 2). It could be due to high disease pressure caused by inoculation of broken or mechanically damaged roots. It is to be noted that infection in root dip process is in contrary to the natural process, wherein the fungus penetrates the intact roots. Hence, the disease reaction of a genotype in root dip inoculation method could be due to either its inherent susceptibility or high load of spores entered through the damaged roots, which otherwise could have not entered (Lopez-Lavalle et al. 2012). The root tip excision *a priori* may exclude the screening for resistance mechanisms that might be associated at the level of root penetration (Eynck et al. 2009). The other drawback is that plants inoculated by root dipping are exposed to the inoculum for only a few minutes (1 – 5 min) reducing the chance of equal fungal load intake resulting in plant to plant variation for disease reaction within a genotype (Wang et al. 1999; Lopez-Lavalle et al. 2012).

Although root dip inoculation is the commonly used method in castor for *Fusarium* wilt screening in glasshouse (Raoof and Rao 1996; Desai and Dange 2003; Prasad et al. 2008; Reddy et al. 2011), it has not been used in large scale screening. The root dip and transplanting are tedious, when large numbers of plants are involved because of the need for delicate handling (Hillocks 1984).

Sick pot method

In sick pot method, wilt symptoms developed early and also progressed fast (Fig 2). The plants of susceptible lines showed wilt symptoms from 10 days after sowing and died before 18 days (Table 1). In contrast to other methods, the standard resistant check, 48-1 also showed disease symptoms, but after 40 days. The plants of 48-1 survived up to 50 days whereas all the susceptible lines died within 18 days clearly discriminating 48-1 from susceptible lines. Other resistant inbred lines namely AP171, AP56 and AP77 survived till the end of experiment without any wilt symptoms. This result showed that the resistance level of 48-1 was only intermediary between susceptible and resistant groups in sick pot experiment. Using root tip inoculation method, Desai and Dange (2003) reported that 48-1 was resistant to wilt even at high inoculum concentration (1×10^7 spores/ml) with different root dipping time.

All the plants within each genotype showed uniform disease reaction in sick pot method compared to all other methods. As the inoculum is applied in dried form, thorough mixing with soil is possible and the plants are uniformly and continuously exposed to the fungal propagules which reduce the probability of escapes from the infection (Lopez-Lavalle et al. 2012). Sick pot method was found to give highly comparable results with field screening because the process of infection is similar to the natural process (Hillocks 1984). This method has been used extensively for screening against *Fusarium* in many crops such as pigeon pea (Okiror 1998; Nene and Kannaiyan 1982), cotton (Hillocks 1984; Lopez-Lavalle et al. 2012; Abd-Elsalam et al. 2014), chickpea (Nene and Haware 1980; Trapero-Casas and Jimenez-Diaz 1985; Ahmad et al. 2010), banana (Purwati et al. 2008), soybean (Leath and Carroll 1982) and safflower (Sastry and Chattopadhyay 2003).

Large scale evaluation of inbred lines in pots

Due to several advantages over other methods of inoculation, we selected sick pot as ideal method for evaluating *Fusarium* wilt resistance in castor. The efficacy of the 'sick pot method' for large scale evaluation was tested by screening a set 132 inbred lines. The susceptible check, JI35 died within 20 days after sowing, whereas the resistant check, 48-1 survived up to 50 days after sowing. The inbred lines showed differential reaction to fungal infection: some were similar to checks, some were in between the checks and some showed higher level of resistance than the resistant check (Fig 3).

Based on these observations, genotypes were categorized on the basis of 'days to death'. For each inbred line, the days to death was recorded when 80 per cent of the plants of the inbred line died. The observations on the disease reaction were continued till three weeks after the resistant check died (i.e. up to 75 days). The genotypes were scored on 1 (susceptible) to 4 (highly resistant) scale as given in Table 1. This system of scoring allowed us to categorize the inbred lines into different resistance levels.

Conventionally, wilt reaction is scored based on 'per cent wilt incidence', which is calculated as the proportion of died plants to the total number of plants screened in each genotype and the genotypes are categorized as: 0% - highly resistant, 0.1 to 20% - resistant, 20.1 to 40% - moderately resistant, 40.1 to 50% - moderately susceptible, 50.1 to 75% - susceptible and >75% - highly susceptible (Mayee and Datar 1986; Anjani et al. 2014). However, theoretically the disease incidence should be close to either 0% (resistant) or 100% (susceptible) because all the plants of an accession or inbred (having same genetic makeup) are expected to show uniform disease reaction. In practice, survival of a few plants in susceptible line or death of few plants in resistant line might be due to the genetic heterogeneity, uneven inoculum in the

field or escape from the infection. Therefore, the variation among genotypes with respect to disease incidence cannot be linked to quantitative expression of resistance. In our study, we found that more than 80 per cent of the plants either died (susceptible lines) or survived (resistant lines). We did not find the disease incidence varying from 0 to 100 per cent except in 12 lines: AP7, AP16, AP41, AP47, AP51, AP59, AP66, AP68, AP84, AP101, AP104 and AP120. There is a possibility that these lines are not genetically uniform (heterozygous/heterogeneous). The inbred lines used in this study were derived from germplasm accessions through selfing for four generations (Senthilvel et al. 2016). However, these 12 lines might perhaps still be segregating at loci responsible for wilt resistance.

The disease reactions of the genotypes tested are given in Online Resource 1. Out of 120 inbred lines showing consistent disease reaction, 73 were scored as susceptible, 18 were moderate, 21 were resistant and 8 were highly resistant. The highly resistant lines did not show any disease symptom till the end of the experiment. There were at least eight lines showing significantly higher resistance than the standard check, 48-1. There could be different mechanisms of resistance operating in lines that exhibited varying level of resistance, which needs to be studied.

Large scale evaluation of inbred lines in field

To verify the results of glasshouse based screening, the entire set of 132 inbred lines was evaluated in a well maintained sick plot at IIOR, Hyderabad. The susceptible check, JI35 (planted across the field) died within 30 days of sowing. The resistant check, 48-1 grew normally without any wilt symptoms till the end of experiment (150 DAS). This was in contrary to the observation in the sick pot screening, where 48-1 died after 50 days of sowing. The reactions of

test entries were highly varying. Some of the inbred lines exhibited wilt symptoms early and died within 40 days after sowing. A few inbred lines showed disease symptoms (chlorosis/necrosis of leaves) with stunted growth. A group of inbred lines exhibited normal growth and vigor without any disease symptoms. Accordingly, the inbred lines were grouped in to three categories 'susceptible' (plants died), 'moderate' (survived but with disease symptoms) and 'resistant' (no disease symptoms).

Out of 132 inbred lines evaluated, 66 were found to be susceptible, 14 were moderate and 40 were resistant. The remaining 12 inbred lines could not be categorized into any group due to mixed reaction as observed in sick pot screening. From these results, it was evident that the inbred set represented good range of variability for reaction to *Fusarium* wilt. High frequency of resistant lines indicates that genetic resistance for *Fusarium* is rampant in castor germplasm. The highly resistant inbred lines identified here are genetically very diverse (Senthilvel et al. 2016) and could be exploited as potential sources of resistance in castor breeding programme for development of wilt resistant cultivars.

Comparison of glasshouse screening with field evaluation

When the result of sick pot method was compared with field evaluation, 66 inbred lines showed susceptible reactions in both the pot and field. Seven inbred lines: AP1, AP21, AP34, AP35, AP86, AP102 and AP129, which were scored as susceptible in the sick pot, were scored as moderate in the field screening. This might be due to low pathogen load or early vigour of those inbred lines enabling the plants to survive more days and scored as moderate type in the field. Correl (1991) opined that the results of pathogen reaction are dependent on the environmental factors and age of the host, which could affect the plant's ability to resist the fungus.

Seven inbred lines (AP10, AP14, AP27, AP54, AP61, AP85 and AP121) showed moderate reactions in both sick pot and field screenings, whereas 12 inbred lines (AP20, AP25, AP26, AP29, AP37, AP66, AP78, AP100, AP107, AP112, AP116, and AP124) which were scored as moderate in sick pot screening were found to be resistant in the field. The standard resistant check, 48-1 also showed disease symptoms in the pot screening but was healthy, when grown in the field. Similar results were obtained by Mohammadi et al. (2012) while screening lentil lines (*Lens culinaris*) for resistance to *Fusarium oxysporum* f. sp. *lentis* under greenhouse and field conditions. They found three lentil lines, which were scored as resistant under field conditions, were susceptible or moderately susceptible under greenhouse condition. This type of variation in expression may be attributed to differences in the level of inoculum and space availability for plant growth in pot and field. In pots, plant growth is constrained by small area. Also, continuous availability of inoculum is assured in pots due to containment. In field, plants can grow faster and tolerate a certain level of infection (Eynck et al. 2009). The expression of resistance in the field could also depend on the concentration or rate of production of constitutive antifungal components by the root. Genotypes having moderate resistance could produce root exudates which would restrict the hyphal spread and spore germination of *Fusarium* leading to less disease progress in the field. Stevenson et al. (1995) found that the kind of plant root exudates (which have antifungal activity) and the rate of exudation differ between susceptible and resistant plants.

It is important to note that any artificial inoculation test cannot completely simulate the disease progress in the field. In artificial inoculations, plants are exposed to high inoculum concentration at the early stage of growth under congenial conditions for the pathogen, which do not generally appear in the field. Therefore, there is a gap between the resistance evaluation test

carried out under controlled conditions in the greenhouse and under field conditions. Hence, improvements in the artificial inoculation methods are required so as to match the results of field screening.

The inbred lines scored as resistant (22) and highly resistant (7) in pot screening were found to be resistant in the field screening also. However, it was difficult to discriminate the highly resistant and resistant categories of inbred lines in the field screening. Overall, the congruence between sick pot and sick field evaluation was very high. These results confirm that the screening performed on young seedlings is a good predictor of *Fusarium* wilt resistance in adult plant under field conditions in castor. The sick pot method can easily be applied for rapid and high throughput screening of castor for *Fusarium* wilt resistance with the advantage of scoring for different level of resistance.

Acknowledgment

This study was funded by Indian Council of Agricultural Research, New Delhi. The authors thank J. Ilesh and Shaik Shamshuddin for their technical support.

References

Abd-Elsalam, K.A., Omar, M.R., Asran-Amal, A., Mansour, M.T., & Aly, A.H. (2014) Evaluation of a cotton germplasm collection against *Fusarium* wilt race 3 isolates from Egypt. *Tropical Plant Pathology*, 39, 095-103.

Ahmad, A.A., Iqbal, S.M., Ayub, N., Ahmad, Y., & Akram, A. (2010). Identification of resistance sources in chickpea against *Fusarium* wilts. *Pakistan Journal of Botany*, 42, 417–426.

Anjani, K. & Raoof, M.A. (2014). Analysis of mode of inheritance of Fusarium wilt resistance in castor (Ricinus communis L.). *Plant Breeding*, 133, 101–107.

Anjani, K., Raoof, M.A., Reddy, A.V., & Rao, C. H. (2003). Sources of resistance to major castor (*Ricinus communis L.*) diseases. *Plant Genetic Resources Newsletter*, 137, 46-48.

Anjani, K., Raoof, M.A., & Desai, A.G. (2014). Evaluation of world castor (*Ricinus communis* L.) germplasm for resistance to *Fusarium* wilt (*Fusarium oxysporum* f. sp. *ricini*). *European Journal of Plant Pathology*, 139, 567–578.

Berman, P., Nizri, S., & Wiesman, Z. (2011). Castor oil biodiesel and its blends as alternative fuel. *Biomass and Bioenergy,* 35, 2861-2866.

Burke, D.W. (1965). The near immobility of *Fusarium solani* f. sp. *phaseoli* in natural soils. *Phytopathology*, 55, 1188-1190.

Correl, J.C. (1991). The relationship between formae specialis, races, and vegetative compatibility group *in Fusarium oxysporum*. *Phytopathology*, 81, 1061–1064.

Dange, S.R.S., Desai, A.G., & Patel, S.I. (2006). Wilt of castor and its management – a review. *Agricultural Reviews*, 272, 147–151.

Desai, A.G., & Dange, S.R.S. (2003). Standardization of root dip inoculation technique for screening of resistance to wilt of castor. *Journal of Mycology and Plant Pathology,* 33, 73-75.

Desai, A.G., Dange, S.R.S. & Pathak, H.C. (2001). Genetics of resistance to wilt in castor caused by *Fusarium oxysporum* f.sp. *ricini*. Nanda and Prasad. *Journal of Mycology and Plant Pathology*, 31(3), 322-326.

Eynck, C., Koopmann, B., & Tiedemann, A.V. (2009). Identification of *Brassica* accessions with enhanced resistance to *Verticillium longisporum* under controlled and field conditions. *Journal of Plant Diseases and Protection*, 116, 63–72.

Gourishankar, V, Rao, P.V.R. & Reddy, A.V. (2010). Inheritance of certain morphological characters and Fusarium wilt resistance in Castor *Ricinus communis* L. *SABRAO Journal of Breeding and Genetics*, 42(2), 56-63.

Gracia-Garza, J.A., & Fravel, D.R. (1998). Effect of relative humidity on sporulation of *Fusarium oxysporum* in various formulations and effect of water on spore movement through soil. *Phytopathology*, 88, 544-549.

Hillocks, R.J. (1984). Production of cotton varieties with resistance to *Fusarium* wilt with special reference to Tanzania. *Tropical Pest Management*, 30, 234–246.

Lavanya, C., Raoof, M.A. & Prasad, M. S. L. (2011). Genetics of resistance to Fusarium wilt in castor by *Fusarium oxysporum* f.sp. *ricini*. *Indian Journal of Phytopathology*, 64, 151-153.

Leath, S., & Carroll, R.B. (1982). Screening for resistance to *Fusarium oxysporum* in soybean. *Plant Disease*, 66, 1140-1143.

Lee, W.J., Jang, K.S., Choi, Y.H., Kim, H.T., Kim, J-C., & Choi G.J. (2015). Development of an efficient simple mass-screening method for resistant melon to *Fusarium oxysporum* f. sp. *melonis*. *Research in Plant Diseases*, 21(3), 201-207.

Lopez-Lavallea, L.A.B., Potter, N., & Brubaker, C.L. (2012). Development of a rapid, accurate glasshouse bioassay for assessing fusarium wilt disease responses in cultivated *Gossypium* species. *Plant Pathology*, 61, 1112–1120.

Mayee, C.D., & Datar, V.V. (1986). *Phytopathometry*. Technical Bulletin 1, 218. Marathwada Agricultural University, Parbhani, India.

Mohammadi, N., Puralibaba, H., Mohammadi, E.G., Ahari, A.B., & Sardrood, B.P. (2012). Advanced lentil lines screened for resistance to *Fusarium oxysporum* f. sp. *lentis* under greenhouse and field conditions. *Phytoparasitica*, 40, 69–76.

Naik, M.K. (1994). Seed borne nature of *Fusarium* in castor. *Indian Journal of Mycology and Plant Pathology*, 24, 62-63.

Nakasone, K., Peterson, S. W., & Foster, M. S. (2004). Preservation and distribution of fungal cultures. In: G. M. Mueller, G. F. Bills, & M. S. Foster (Eds.) *Biodiversity of Fungi: Inventory and Monitoring Methods* (pp. 37-47). London: Elsevier.

Nanda, S., & Prasad, N. (1974). Wilt of castor a new record. *Indian Journal of Mycology and Plant Pathology*, 4, 103–105.

Nene, Y.L., & Haware, M.P. (1980). Screening chickpea for resistance to wilt. *Plant Disease*, 64, 379-380.

Nene, Y.L., & Kannaiyan, J. (1982). Screening pigeonpea for resistance to *Fusarium* wilt. *Plant Disease*, 66, 306-307.

Ogunniyi, D.S. (2006). Castor oil: a vital industrial raw material. *Bioresource Technology*, 97, 1086-1091.

Okiror, M. A. (1998). Screening techniques for *Fusarium* wilt of pigeonpea. *African Crop Science Journal*, 6, 345-350.

Patel, P.B., & Pathak, H.C. (2011). Genetics of resistance to wilt in castor caused by *Fusarium oxysporum* f. sp. *ricini* Nanda and Prasad. *Agricultural Science Digest*, 31, 30-34.

Podkuichenko, G.V. (1989). Donors of resistance to Fusarium in castor oil plant. *Instituta Maslichnykh Kullar*, 1, 24-26

Prasad, M.S.L., Sujatha, M., & Raoof, M.A. (2008). Morphological, pathogenic and genetic variability in castor wilt isolates. *Indian Phytopathology*, 61, 18-27.

Prasad, N., & Bhatnagar, A. (1981). Evaluation of resistant varieties of castor to wilt pathogen. *Journal of Mycology and Plant Pathology*, 11, 137-138.

Purwati, R.D., Hidayah, N., Sudjindro, and Sudarsono (2008). Inoculation methods and conidial densities of *Fusarium oxysporum* f.sp. *cubense* in Abaca. *Hayati Journal of Biosciences*, 15, 1-7.

Pushpawathi, B., Sarwar, H.A.K., Raoof, M.A., & Babu, R.R. (1998) Management of wilt disease in castor. Indian Journal of Plant Protection 26(2), 177–180.

Rao, C.H., Raoof, M.A. & Lavanya, C. (2005). Study on segregation patterns and linkages between morphological characters and wilt resistance in castor (*Ricinus communis*). *Journal of Oilseeds Research*, 22(1), 114-118.

Raoof, M.A., & Rao, N.T.G. (1996). A simple screening technique for early detection of resistance to castor wilt. *Journal of Indian Phytopathology*, 49, 389-392.

Reddy, A.V.V., Janila, P., Rao, P.V.R., Ahammed, S.K., Reddy,N.R., Shankar, V.G. & Singh, T.V.K. (2010). Molecular tagging of Fusarium wilt resistance gene(s) in castor, *Ricinus communis* L. *Journal of Oilseeds Research*, 27, 92-95.

Reddy, R.N., Sujatha, M., Reddy, A.V., & Reddy, A.P. (2011). Inheritance and molecular mapping of wilt resistance gene (s) in castor (*Ricinus communis* L.). *International Journal of Plant Breeding*, 5, 84-87.

Ruddick, S. M., & Williams, S.T. (1972). Studies on the ecology of actinomycetes in soil V. Some factors influencing the dispersal and adsorption of spores in soil. *Soil Biology and Biochemistry*, 4, 93-103.

Sarada, C., & Anjani, K. (2013). Establishment of castor core collection utilizing self-organizing mapping (SOM) networks. *Journal of Indian Society of Agricultural Statistics*, 67, 71-78.

Sastry, R.K., & Chattopadhyay, C. (2003). Development of *Fusarium* wilt resistant genotypes in safflower (*Carthamus tinctorius*). *European Journal of Plant Pathology,* 109, 147-151.

Senthilvel, S., Shaik, M., Anjani, K., Shaw, R.K., Kumari, P., Sarada, C., & Kiran, B.U. (2016). Genetic variability and population structure in a collection of inbred lines derived from a core germplasm of castor. *Journal of Plant Biochemistry and Biotechnology* doi:10.1007/s13562-016-0356-8.

Stevenson, P.C., Padgham, D.E., & Haware., M.P. (1995). Root exudates associated with the resistance of fourchickpea cultivars (*Cicer arietinum*) to two races of *Fusarium oxysporum* f. sp. *ciceri*. *Plant Pathology*, 44, 686–694.

Sudhakar, R., Ahammed, S. K., Srinivas, T. & Reddy, A. V. (2010). Evaluation of advanced breeding material of castor (*Ricinus communis* L.) for Fusarium wilt resistance. *Journal of Oilseeds Research*, 27, 290-292.

Sviridov, A.A. (1988) Inheritance of resistance to Fusarium in Castorbean. *Genetika*, 24, 908-913.

Trapero-Casas, A., & Jimenez-Diaz, R.M. (1985). Fungal wilt and root rot diseases of chickpea in Southern Spain. *Phytopathology,* 75, 1146-1151.

Vavilov, N.I. (1951). The origin, variation, immunity and breeding of cultivated plants. *Chronica Botanica*, 13, 1-364.

Wallace, H.R. (1978). Dispersal in time and space: Soil pathogens. In: J. G. Horsfall & E. B. Cowling. (Eds.) *Plant Disease: An Advanced Treatise Vol. II How Disease Develops in Populations* (pp. 181-202). New York, USA: Academic Press.

Wang, B., Dale, M.L., & Kochman, J.K. (1999). Studies on a pathogenicity assay for screening cotton germplasms for resistance to *Fusarium oxysporum* f.sp. *vasinfectum* in the glasshouse. *Australian Journal of Experimental Agriculture*, 39, 967-974.

Online Resource 1: **Details of 132 castor inbred lines evaluated for Fusarium wilt resistance**

S.No.	Inbred ID	Source germplasm ID	Morphological features			Disease response	
			Stem colour	Wax coating[1]	Spine on capsule	Days to death	Reaction[2]
1	AP1	RG43	Red	3	Yes	29	S
2	AP2	RG61	Red	1	Yes	28	S
3	AP3	RG72	Red	2	Yes	29	S
4	AP4	RG94	Red	2	Yes	22	S
5	AP5	RG111	Red	2	Yes	28	S
6	AP6	RG178	Green	2	Yes	30	S
7	AP7	RG193	Red	2	No	-	NC
8	AP8	RG220	Red	2	Yes	60	R
9	AP9	RG224	Red	2	Yes	25	S
10	AP10	RG249	Green	3	Yes	42	M
11	AP11	RG252	Red	1	Yes	28	S
12	AP12	RG260	Red	2	Yes	28	S
13	AP13	RG264	Green	2	Yes	26	S
14	AP14	RG289	Red	2	Yes	45	M
15	AP15	RG294	Red	2	Yes	28	S
16	AP16	RG297	Green	3	Yes	-	NC
17	AP17	RG408	Red	2	Yes	26	S
18	AP18	RG426	Green	1	Yes	26	S
19	AP19	RG430	Green	1	Yes	25	S
20	AP20	RG433	Red	2	No	40	M
21	AP21	RG489	Red	1	Yes	25	S
22	AP22	RG537	Green	3	Yes	26	S
23	AP23	RG551	Red	1	Yes	28	S
24	AP24	RG558	Red	0	No	61	R
25	AP25	RG565	Green	2	Yes	45	M
26	AP26	RG566	Red	2	No	46	M
27	AP27	RG589	Red	2	Yes	36	M
28	AP28	RG607	Green	2	Yes	60	R
29	AP29	RG673	Green	2	Yes	45	M
30	AP30	RG714	Red	3	Yes	29	S
31	AP31	RG732	Red	2	Yes	26	S
32	AP32	RG735	Red	2	Yes	27	S
33	AP33	RG784	Red	2	Yes	>65	HR
34	AP34	RG790	Red	1	Yes	28	S
35	AP35	RG829	Red	2	Yes	29	S
36	AP36	RG886	Green	2	Yes	24	S
37	AP37	RG892	Red	2	No	47	M
38	AP38	RG905	Mahogany	1	Yes	28	S
39	AP39	RG908	Mahogany	3	Yes	26	S

40	AP40	RG941	Green	3	Yes	23	S
41	AP41	RG969	Red	2	Yes	-	NC
42	AP42	RG999	Red	3	Yes	62	R
43	AP43	RG1068	Green	3	Yes	23	S
44	AP44	RG1114	Red	1	Yes	26	S
45	AP45	RG1125	Red	1	Yes	23	S
46	AP46	RG1142	Red	1	Yes	26	S
47	AP47	RG1146	Green	3	Yes	-	NC
48	AP48	RG1149	Green	3	Yes	63	R
49	AP49	RG1173	Red	3	Yes	26	S
50	AP50	RG1180	Green	3	No	27	S
51	AP51	RG1274	Red	1	Yes	-	NC
52	AP52	RG1289	Mahogany	3	Yes	29	S
53	AP53	RG1305	Red	2	Yes	60	R
54	AP54	RG1313	Red	2	Yes	42	M
55	AP55	RG1340	Red	2	No	62	R
56	AP56	RG1354	Green	2	Yes	>65	HR
57	AP57	RG1364	Red	1	Yes	27	S
58	AP58	RG1383	Green	3	Yes	26	S
59	AP59	RG1406	Red	2	Yes	-	NC
60	AP60	RG1507	Red	1	Yes	25	S
61	AP61	RG1523	Red	2	Yes	40	M
62	AP62	RG1545	Red	3	Yes	26	S
63	AP63	RG1579	Red	2	Yes	63	R
64	AP64	RG1627	Red	2	Yes	28	S
65	AP65	RG1647	Green	2	Yes	>65	HR
66	AP66	RG1654	Green	2	Yes	-	NC
67	AP67	RG1669	Red	2	Yes	65	R
68	AP68	RG1689	Mahogany	0	Yes	-	NC
69	AP69	RG1696	Green	3	No	25	S
70	AP70	RG1707	Green	2	Yes	64	R
71	AP71	RG1709	Red	2	Yes	22	S
72	AP72	RG1759	Green	1	Yes	22	S
73	AP73	RG1849	Green	2	Yes	29	S
74	AP74	RG1864	Green	3	Yes	29	S
75	AP75	RG1904	Green	2	Yes	65	R
76	AP76	RG1952	Red	2	No	27	S
77	AP77	RG1963	Red	0	Yes	>65	HR
78	AP78	RG1978	Mahogany	3	Yes	45	M
79	AP79	RG1981	Red	2	Yes	26	S
80	AP80	RG1999	Green	2	Yes	63	R
81	AP81	RG2014	Red	3	Yes	60	R
82	AP82	RG2022	Mahogany	0	Yes	62	R
83	AP83	RG2024	Red	3	Yes	58	R
84	AP84	RG2035	Red	2	Yes	-	NC

85	AP85	RG2184	Red	3	Yes	40	M
86	AP86	RG2195	Red	2	No	30	S
87	AP87	RG2266	Green	3	Yes	26	S
88	AP88	RG2269	Red	2	Yes	29	S
89	AP89	RG2288	Red	1	Yes	26	S
90	AP90	RG2320	Red	2	Yes	22	S
91	AP91	RG2326	Red	2	Yes	28	S
92	AP92	RG2375	Red	2	Yes	29	S
93	AP93	RG2377	Red	2	Yes	29	S
94	AP94	RG2378	Red	2	Yes	24	S
95	AP95	RG2390	Red	2	Yes	29	S
96	AP96	RG2430	Red	3	Yes	60	R
97	AP97	RG2451	Red	3	Yes	26	S
98	AP98	RG2454	Red	1	Yes	61	R
99	AP99	RG2457	Red	2	Yes	30	S
100	AP100	RG2465	Red	1	Yes	40	M
101	AP101	RG2473	Red	2	Yes	-	NC
102	AP102	RG2474	Red	2	Yes	26	S
103	AP103	RG2481	Green	2	No	30	S
104	AP104	RG2498	Mahogany	3	Yes	-	NC
105	AP105	RG2582	Red	2	Yes	29	S
106	AP106	RG2588	Red	2	Yes	21	S
107	AP107	RG2593	Red	1	Yes	42	M
108	AP109	RG2676	Green	1	Yes	29	S
109	AP110	RG2681	Mahogany	3	Yes	>65	HR
110	AP111	RG2685	Green	3	Yes	>65	HR
111	AP112	RG2705	Red	0	Yes	47	M
112	AP113	RG2717	Green	1	Yes	60	R
113	AP114	RG2719	Red	2	Yes	65	R
114	AP116	RG2719	Red	0	Yes	50	M
115	AP117	RG2725	Red	2	Yes	29	S
116	AP118	RG2789	Red	3	Yes	25	S
117	AP119	RG2810	Green	3	Yes	65	R
118	AP120	RG2818	Red	2	Yes	-	NC
119	AP121	RG2819	Green	3	Yes	46	M
120	AP122	RG2821	Green	0	Yes	29	S
121	AP123	RG2839	Red	2	Yes	30	S
122	AP124	RG2866	Red	2	No	50	M
123	AP125	RG2874	Red	3	No	>65	HR
124	AP126	RG2902	Red	2	Yes	28	S
125	AP127	RG2944	Red	1	Yes	>65	HR
126	AP128	RG2958	Red	3	Yes	26	S
127	AP129	RG2980	Red	2	No	27	S
128	AP130	RG2991	Red	1	Yes	25	S
129	AP131	RG3005	Red	2	Yes	29	S

130	AP132	RG3013	Red	2	Yes	65	R
131	AP133	RG3013	Green	0	Yes	27	S
132	AP134	RG3037	Green	2	Yes	25	S

[1]Waxi coating: 1 – Present only on stem, 2 – Present on stem and lower surface of the leaf, 3 – Present on stem, lower and upper surface of leaves

[2]Reaction: S-Susceptible, M – Moderate, R – Resistant, HR – Highly Resistant, NC – Not categorized due to heterogeneity

Table 1. Reactions of castor genotypes to Fusarium wilt under different methods of artificial inoculation in pot culture

Genotype	**Seed soaking method**			**Soil drenching method**			**Root dip method**			**Sick pot method**		
	% of died plants	**Days to death**	**Score***	**% of died plants**	**Days to death**	**Score***	**% of died plants**	**Days to death**	**Score***	**% of died plants**	**Days to death**	**Score***
JI-35	75	-	NC	90	32	M	100	35	M	100	18	S
AP130	100	38	M	100	32	M	90	32	M	100	15	S
AP45	80	40	M	90	35	M	100	35	M	100	18	S
AP60	70	-	NC	85	32	M	100	32	M	100	15	S
48-1	20	64	R	40	-	NC	40	-	NC	85	50	M
AP56	0	>65	HR	0	>65	HR	16	60	R	0	>65	HR
AP171	0	>65	HR	0	>65	HR	30	60	NC	0	>65	HR
AP77	0	>65	HR	5	>65	HR	35	61	NC	5	>65	HR

*'S'- Susceptible [days to death <30]; 'M' - Moderate [days to death = 31-50]; 'R' - Resistant [days to death = 51-65]; 'HR' - Highly resistant [days to death >65] and 'NC' - Not categorized [score was given only when >80% of the plants died (susceptible) or survived (resistant)

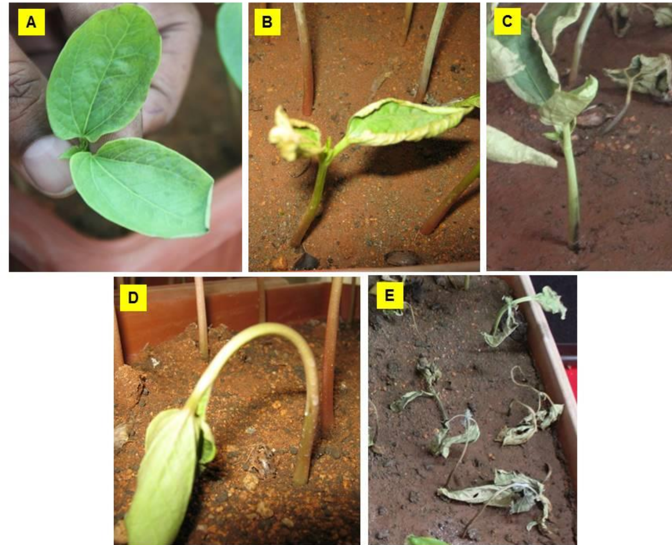

Fig. 1 Symptoms of *Fusarium oxysporum* f.sp. *ricini* infection in castor seedlings
[A: Necrosis, B: Discouloration and drying of leaf, C: Black streak from color region, D: Drooping of seedling, E: Death of seedling]

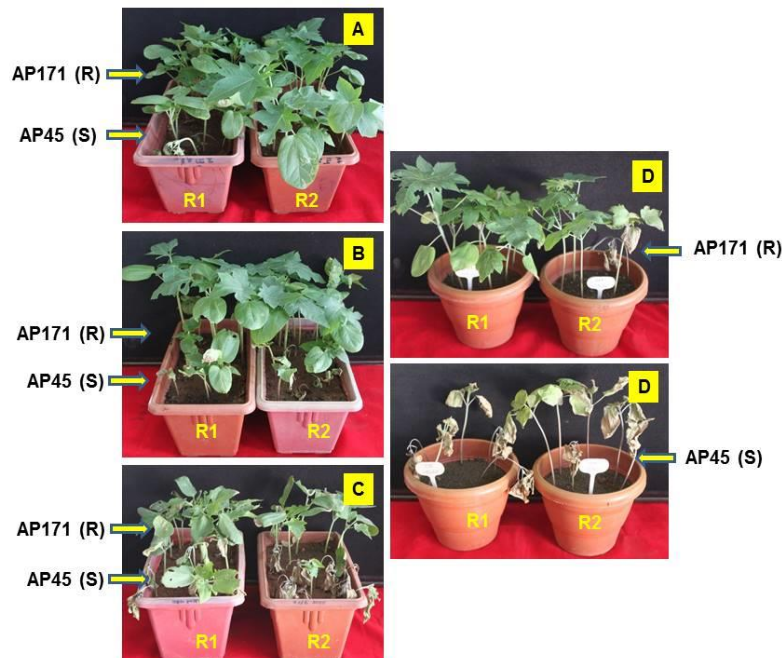

Fig. 2 Reaction of susceptible and resistant genotypes in different methods of inoculation at 30 days after sowing [‘R’ stands for ‘Replication’. A: Seed soaking, B: Soil drenching; C: Sick pot, D: Root dip].

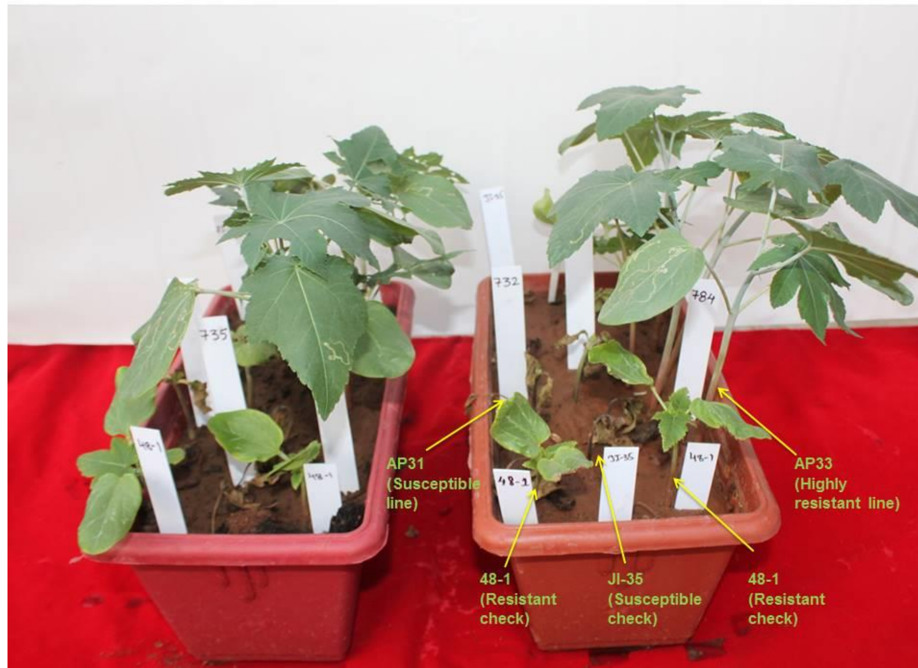

Fig. 3 Reaction of castor inbred lines to *Fusarium* wilt in sick pot at 45 days after sowing
[AP31 (left row) and AP32 (middle row) showed susceptible reaction and died along with susceptible check (JI35), whereas AP33 (right row) displaying higher resistance (no disease symptoms) compared with 48-1