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Pathogenic Response of Three Cowpea Varieties to Infection with *Fusarium Oxysporum* F. Sp. Tracheiphylum in the Screen House

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Abstract

Cowpea is an important legume with immense benefits to humans, animals, and the soil. Its production is threatened by many diseases among which is vascular wilt disease caused by *Fusarium oxysporum* f. sp. tracheiphilum (Fot). This study was carried out to evaluate three cowpea varieties commonly grown in the study area for their response to Fot infection with a view to determining their degree of tolerance/susceptibility to the disease. A 3x4 factorial experiment involving three cowpea varieties (TVU-16877, TVU-16891 and TVU-17088) and four levels of inoculation (spore/mycelia suspension, inoculum meal, spore suspension + inoculum meal and control) was conducted in the screen house. It was fitted to a completely randomized design (CRD). Data on height, number of leaves, stem girth and disease severity rating (using the CIAT DSR scale) were recorded. Analysis of variance was carried out and mean values were separated using the Tukey's HSD at 5% level of significance. The results showed significant difference ($p<0.05$) in the growth parameters for the different cowpea varieties. Variety TVU-16891 was the most severely affected having the lowest mean number of leaves (at 8 and 10 WAP) and the smallest mean stem girth (at 4, 6, 8 and 10 WAP). Disease severity rating was significantly affected by the inoculation methods and the interaction between inoculation methods and varieties. This study also revealed that variety TVU-17088 was tolerant/resistant to spore/mycelia suspension + inoculum meal inoculation (to which the other cowpea varieties were susceptible) and showed intermediate response to the other two inoculation methods.

Keywords: *Fusarium oxysporum*, Cowpea, Vascular wilt, *Fusarium oxysporum,* root discolouration

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a food and feed crop grown in the semi-arid tropic of Africa and particularly in the savannah region of West Africa where Nigeria is reported to be the largest producer and consumer accounting for 61% of the production (IITA, 2009; Mouneke et al, 2012). The crop is known as "vegetable meat" in some parts of the world where it serves as a major source of protein to many people due to the high amount of protein in its grain with better biological value of dry weight basis, which contains more than 26.61% protein, 3.99% lipid, 56.24% carbohydrate, 8.60% moisture, 3.84% ash, 1.38% crude fibre, 1.51% gross energy and 54.85% nitrogen free extract (Owolabi et al., 2012).

Cowpea is cultivated on about 14.5 million hectares of land worldwide with annual global production of about 6.9 million metric tons in 2016 (FAOSTAT, 2016). Africa is responsible for 94% of this total production. Top producers are the West and Central African sub regions which contribute about 64% of the global production. Nigeria, the largest producer and consumer, accounts for over 64% of production in Africa and 60% worldwide (FAOSTAT, 2016).

There are many biotic and abiotic constraints that limit growth and yield of cowpea (Singh, 2005; Timko, Ehlers, & Roberts, 2007). Fusarium wilt disease caused by the fungal pathogen, *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot), is one of the diseases that pose a major threat to cowpea production worldwide. The disease causes

substantial annual yield losses ranging from 30% to 100% (Reddy et al., 1990; Mohammed and Sajo, 2018; Kusi et al, 2019). In the United States, high plant mortality with severe overall yield loss has also been

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reported (Pottorff et al, 2014). Fusarium wilt disease was first reported in Nigeria in the year 1975 (Armstrong and Armstrong, 1980). Further research has documented more isolates, from Ife Brown, TVu 4557 and Prima with disease incidence of 21, 15 and 55 per cent, respectively (Aigbe & Fawole, 2009).

Fusarium oxysporum is well represented among the communities of soil borne fungi, in every type of soil all over the world (Burges, 1981).

The fungus has been documented to be referred to as normal constituent of the fungi communities in the rhizosphere of plants (Gordon and Martyn, 1997). This has made it pathogenic to different plants species. They are however host specific, attacking only one or a few species of plants and at times only certain cultivars of the plant. This characteristics earn them the designate *formae spciale* and race of pathogen.

They penetrate into the roots where they invades the vascular system. Many other strains can penetrate roots but cannot invade vascular system or causes disease (Olivian and Alabouvette, 1997). There are also non-pathogenic form of *Fusarium* sp. Both pathogenic and non-pathogenic forms are difficult to distinguish by morphological differences.

The use of chemical and cultural control by crop rotation have been shown to be inadequate for managing the disease problem (Nelson, 1981) as chemicals leave harmful residues in the environment while the thick resting spores (chlamydospores) can survive in the soil for years making crop rotation inadequate control measure. The most cost effective and environmentally safe control according to Fravel et al, (2003) is the use of resistant cultivars when they are available. This study was therefore carried out to determine the effect of the vascular wilt disease pathogen on three varieties of cowpea commonly grown by farmers in the study area with the view to identifying the variety with tolerance/resistance to the disease.

2. Material and Methods

Source of seeds and inoculum

Three of the commonly distributed cowpea varieties to farmers in the study area (TVU-16877, TVU-I6891 and TVU-17088) were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds were kept in the refrigerator until needed. The inoculum (*Fusarium oxysporum* f. sp. tracheiphilum (Fot) was obtained from the pathology laboratory of the IITA Ibadan, Nigeria. The inoculum was maintained in acidified Potato Dextrose Agar until needed.

Preparation of Inoculum

Spore/mycelia suspension was prepared by transferring ten 5mm diameter punch from 5-day-old culture of Fot into 100ml of sterile water using sterile cork borer. The mixture was comminuted in a warring blender for 5 minutes. The mixture was then stored in corked Erlenmeyer flask. Inoculum meal was prepared by pouring 5ml of the spore suspension prepared earlier into 100g of previously sterilized wheat powder and incubating the mixture for 2 weeks.

Soil Pasteurization

Sandy loam top soil was collected from the farm and steam pasteurized in a drum at 65 – 80^{o}C for at least 10 hours. Three kilogram of the pasteurized soil was then measured into perforated plastic pots (PPP) for the potted experiment.

Inoculation

There was four levels of inoculation involved in this study namely; Spore/mycelia suspension, Inoculum meal, inoculum meal + spore suspension and control (no inoculation).

Spore/mycelia suspension (Inoculation 1) consisted pouring 10ml of spore/mycelia suspension in each 3kg of pasteurized soil 3 days before planting in pots with treatment combination having Inoculation 1.

Inoculum meal (Inoculation 2) involved wounding the stem of the plant with sterile carborundum close to the soil surface and wrapping about 1g of the prepared inoculum meal around the wounded stem. This was done at the 4th week after planting in pots with treatment combination having Inoculation 2. Inoculation 3 was a combination of Inoculation 1 and 2 while Inoculation 4 consisted of control (without inoculation).

Disease Severity Rating

Disease severity rating was done beginning at 8 weeks of planting on sampled plants using the CIAT scale of 1 – 9. The plant is rated 1 if there are no visible symptom and 9 if the plant is dead or severely infected with 100% of the foliage showing wilting, chlorosis or necrosis. Rating of 3 shows 1 – 3 leaves representing about 10% of the total foliage are wilted/chlorotic while rating of 5 and 7 indicate that about 25% and 50% of the leaves respectively are wilted/chlorotic. Cowpea varieties with mean value for disease severity rating of 1 - 3, 3.1 - 6.0 and 6.1 - 9.0 were considered Resistant/Tolerant, Intermediate and Susceptible respectively (Pastor-Corrales and Abawi, 1987).

The sampled plants were uprooted at 10 weeks of planting and washed under running tap water. The tap root and stem were split open and rated for vascular discoloration. Vascular discoloration was rated as light (L), severe (S), very severe (VS) and none (ND).

Experimental layout

The experiment was a 3 x 4 factorial laid out in a completely randomized design. There were three varieties of cowpea (mentioned earlier) and four inoculation methods namely; Inoculation 1 (Spore/mycelia suspension), Inoculation 2 (Inoculum meal), Inoculation 3 (inoculum meal + spore suspension) and control (no inoculation).

Data collection and Analysis

Data collected on weeks 2, 4, 6, 8 and 10 after planting include; plant height, number of leaves per plant, stem girth and disease severity rating. All data were subjected to analysis of variance (ANOVA) and the mean values were separated where necessary by the use of Tukey HSD at $p = 0.05$.

3. Results and Discussion

Effect of treatments on height of cowpea varieties

The results in Table 1 shows the effect of treatments on height of cowpea plants. There was significant difference ($p<0.05$) in the mean height of the different cowpea varieties during the first four weeks after planting. Varieties TVU-16877 was the most affected having the lowest mean height of 15.457cm and 22.728cm in week 2 and week 4 after planting respectively. There was no significant difference ($p>0.05$) in the plant height from week 6 to week 10 of growth.

At 2 weeks after planting, when inoculation 2 (inoculum meal) and 3 (inoculum meal + spore/mycelia suspension) have not been applied, inoculation 1(spore/mycelia suspension) significantly reduced plant height in varieties TVU-16877 and TVU-17088 compared to the control. TVU-16877 was 16.943cm compared to the control (20.217cm) and TVU-17088 23.110cm compared to the control (26.417cm). There was no significant difference in the plant height at weeks 4 and 6 after planting but at weeks 8 and 10, the height of cowpea variety TVU-16891 was significantly affected by inoculation 2 and 3 compared to the control. At week 8 after planting, the height was 15.000cm and 22.333cm for inoculation 2 and 3 compared to 43.5000cm (control) while at week 10 after planting it was 0.000cmm and 23.667cm for inoculation 2 and 3 compared to 47.857cm (control).

Effect of treatments on number of leaves of cowpea varieties

Table 2 summarizes the effect of the treatments on the number of leaves of cowpea plants. The mean number of leaves was significantly affected by varietal difference only at weeks 8 and 10 after planting. Cowpea variety TVU-16891 had the lowest mean number of leaves, 9.569 and 8.006 for weeks 8 and 10 after planting respectively.

Generally the mean number of leaves differed significantly ($p<0.05$) for the different varieties and inoculation methods but with no specific pattern throughout the entire period of the study. Varieties TVU-16877 however showed the most frequently occurring highest mean number of leaves at weeks 2, 4 and 10 after planting. The corresponding mean number of leaves at weeks 2, 4 and 10 respectively were 8.000, 14.657 and 18.167.

Effect of treatment's on stem girth of cowpea varieties

The results in Table 3shows that there was significant difference ($p<0.05$) in the mean stem girth of the different cowpea varieties beginning from week 4 till the 10^{th} week after planting. TVU-16891 was the most affected, with the smallest mean stem girth for weeks 4 (0.638), 6 (1.154), 8 (1.208) and 10 (1.184). With respect to the combined effect of the treatments, there was no significant difference ($p>0.05$) in the stem girth at week 2 and week 6 after planting. At week 4 after planting, all the inoculation methods significantly reduced stem girth compared to the control in all the varieties. The same trend was repeated in week 8. At week 10 however, only variety TVX-16891 was the most seriously affected by the inoculation methods compared to the control with the lowest mean stem girth (0.633) observed for inoculum meal method followed (0.950) observed with inoculum meal + spore/mycelium suspension and also followed by (1.550) observed for spore/mycelia suspension.

Disease Severity Rating

The effect of inoculation methods on the disease severity rating (DSR) is summarized in Table 4. The DSR was found to be significantly different ($p<0.05$) for the different inoculation methods. The response of cowpea plants to spore/mycelia suspension inoculation with the DSR value of <3 was classified as Tolerant/resistant, those of Inoculum meal inoculation with the DSR of >3 was classified as Intermediate while those of spore/mycelia suspension + inoculum meal with the DSR of 6.2 was classified as Susceptible.

The DSR was significantly different ($p<0.05$) for the interaction between inoculation methods and cowpea varieties (Table 5). Only cowpea variety TVU17088 showed resistance/tolerance to even the most devastating inoculation method (spore/mycelia suspension + inoculum meal) to which the other two varieties (TVU16877 & TVU16891) were susceptible. The three cowpea varieties were classified as intermediate for spore/mycelia suspension inoculation methods. TVU17088 was intermediate while TVU16877 & TVU16891 were susceptible to Inoculum meal inoculation. With respect to vascular discoloration, only cowpea variety TVU17088 showed no discoloration of the vascular tissue.

Discussion

This study showed that all the inoculation methods successfully infected the cowpea varieties tested reducing the height, number of leaves and the stem girth. Some root discolorations were also observed as evidence of infection by the pathogen. The reduced values of the growth parameters can be linked to the competition between the pathogen and the host plant for nutrients needed to survive. The host plant is deprived of adequate nutrients for normal growth and development. This is in agreement with the report of (Smith, 2007) who observed that development of infectious disease on or within the host involves the processes of invasion and resource consumption, competition for growth-limiting resources potentially may occur between pathogens and cellular or sub-cellular component of the host ecosystem. The degree of infection however varied for the different cowpea varieties and the inoculation types.

The contrasting results obtained in the different growth parameters could be the result of the effect of the nature and concentration of inoculum as well as variation in pathogen. Rodriquez (1960) found that fungus in a 10-day-old inoculum caused higher percentage of wilt than when the fungus is grown for 2 to 5 days supposedly due to high amount of toxic metabolites that accumulated. This explained why inoculum meal prepared by incubating the fungus in wheat medium for two weeks produced more severe effect in the course of the study. The reduction of virulence or complete loss of pathogenicity of isolates in laboratory media has been noted (Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1969; Armstrong and Armstrong, 1975). Mutation in sterile culture has also been reported (Mc. Keen and Wensley, 1962). This might be the reason for the reduced virulence observed in cases where inoculum meal was stored for later inoculation. It was recommended to store cultures in sterilized soils to maintain the wild type and presumably the virulence of the cultures (Miller, 1945; Mc. Keen, 1951 & Mc. Keen and Wensley, 1961). The synergistic effect of spore/mycelia suspension + inoculum meal and the possible accumulation of toxic metabolites could be the reason for the high virulence of that treatment combination.

Table 1: Effects of variety and inoculation methods on the plant height (cm)

		Weeks after inoculation (WAP)				
Variety	Inoculation	2	4	6	8	10
TVU-16877	Ino 1	16.943± 2.574^{bcd}	22.900± 0.954^{a}	37.483± 21.242^{a}	36.767± 19.744^{ab}	43.617± 26.205^{ab}
	Ino 2	10.600± 0.563^{d}	20.767± 2.136^{a}	63.833± 10.664^{a}	63.583± 8.647^{a}	70.667± 3.055^{a}
	Ino 3	14.067± 7.305^{cd}	21.600± 9.441^{a}	43.720± 26.153^{a}	42.470± 23.175^{ab}	42.777± 23.497^{ab}
	Ino 4	20.217± 1.578^{abc}	25.643± 4.012^{a}	29.333± 3.686^{a}	32.320± 2.821^{ab}	34.000± 2.291^{ab}
TVU-17088	Ino 1	23.110± 2.100^{ab}	32.543± 2.435^{a}	40.723± 3.427^{a}	39.573± 4.943^{ab}	43.190± 1.053^{ab}
	Ino 2	19.667± 0.597^{abc}	29.017± 3.103^{a}	33.750± 1.323^{a}	36.167± 1.041^{ab}	40.780± 1.078^{ab}
	Ino 3	25.080± 3.858^{ab}	39.533± 13.771^{a}	48.450± 15.251^{a}	51.490± 14.289^{ab}	54.367± 16.460^{a}
	Ino 4	26.417± 3.449^{a}	40.167± 2.809^{a}	46.417± 3.301^{a}	47.847± 2.388^{ab}	49.533± 3.465^{a}

TVU-16891	Ino 1	23.273± 4.121^{ab}	35.443± 9.759^{a}	52.000± 20.694^{a}	57.417± 27.107^{ab}	63.160± 28.727^{a}
	Ino 2	23.833± 1.258^{ab}	36.250± 11.924^{a}	35.627± 12.761^{a}	15.000± 25.981^{b}	0.000± 0.000^{b}
	Ino 3	19.270± 3.159^{abc}	28.693± 4.601^{a}	21.333± 8.312^{a}	22.333± 9.504^{ab}	23.667± 10.599^{ab}
	Ino 4	18.667± 1.258^{abcd}	31.233± 3.803^{a}	45.800± 28.766^{a}	43.500± 21.219^{ab}	47.857± 26.538^{a}
			Total effects of the varieties			
TVU-16877		15.457± 5.016^{b}	22.728± 4.884^{b}	43.593± 20.172^{a}	43.785± 18.445^{a}	47.765± 20.835^{a}
TVU-17088		23.568± 3.575^{a}	35.315± 7.933^{a}	42.335± 9.071^{a}	43.769± 9.174^{a}	46.968± 9.106^{a}
TVU-16891		21.261± 3.362^{a}	32.905± 7.749^{a}	38.690± 20.428^{a}	34.563± 25.768^{a}	33.671± 30.448^{a}

Note: Ino 1 = spore/mycelia suspension, Ino 2 = inoculum meal, Ino 3 = inoculum meal + spore/mycelia suspension, Ino 4 = control (sterile water). Post hoc used = Tukey HSD at p = 0.05, SPSS version 26. Values are means of three replicates and mean values followed by the same superscript are not significantly different at 5% level of significance.

Table 2: Effects of both variety and inoculation type on the number of leaves

		Weeks after inoculation (WAP)				
Variety	Inoculation	2	4	6	8	10
TVU-16877	Ino 1	7.333± 1.155^{ab}	13.167± 2.363^{ab}	13.557± 3.357^{a}	15.167± 1.258^{a}	18.167± 1.041^{a}
	Ino 2	8.000± 0.000^{a}	14.657± 0.890^{a}	13.167± 2.754^{a}	14.807± 2.253^{a}	16.083± 3.126^{ab}
	Ino 3	5.333± 1.528^{ab}	13.513± 1.066^{ab}	15.110± 3.167^{a}	14.610± 1.273^{a}	14.667± 1.443^{ab}
	Ino 4	3.277± 1.111^{b}	7.557± 2.696^{b}	7.000± 1.732^{a}	10.277± 2.812^{a}	15.000± 1.000^{ab}
TVU-17088	Ino 1	7.000± 1.000^{ab}	11.533± 2.501^{ab}	14.610± 1.990^{a}	13.277± 1.495^{a}	15.750± 2.537^{ab}
	Ino 2	7.033± 0.950^{ab}	9.100± 1.153^{ab}	11.833± 3.175^{a}	15.500± 4.330^{a}	15.333± 6.807^{ab}
	Ino 3	6.700± 1.127^{ab}	11.867± 1.501^{ab}	15.110± 4.728^{a}	16.277± 3.094^{a}	13.1667± 3.753^{ab}
	Ino 4	5.100± 2.551^{ab}	9.110± 2.714^{ab}	11.267± 0.751^{a}	14.417± 5.768^{a}	15.083± 4.304^{ab}
TVU-16891	Ino 1	6.700± 2.252^{ab}	14.333± 5.281^{ab}	16.667± 10.681^{a}	17.777± 5.467^{a}	12.500± 4.924^{ab}
	Ino 2	7.000± 1.732^{ab}	11.833± 2.254^{ab}	5.667± 5.508^{a}	0.000± 0.000^{b}	0.000± 0.000^{c}
	Ino 3	5.333± 0.577^{ab}	10.333± 0.577^{ab}	8.000± 4.359^{a}	7.667± 3.786^{ab}	6.190± 4.686^{bc}
	Ino 4	4.000± 1.732^{ab}	10.667± 0.577^{ab}	12.833± 8.519^{a}	12.833± 5.923^{a}	13.333± 3.786^{ab}
			Total effects of the varieties			
TVU-16877		5.986± 2.147^{a}	12.223± 3.307^{a}	12.208± 4.029^{a}	13.715± 2.699^{a}	15.979± 2.139^{a}
TVU-17088		6.458± 1.565^{a}	10.403± 2.231^{a}	13.205± 3.128^{a}	14.868± 3.606^{a}	14.833± 4.074^{a}
TVU-16891		5.758± 1.905^{a}	11.792± 2.973^{a}	10.792± 7.921^{a}	9.569± 7.852^{b}	8.006± 6.529^{b}

Note: Ino 1 = spore/mycelia suspension, Ino 2 = inoculum meal, Ino 3 = inoculum meal + spore/mycelia suspension, Ino 4 = control (sterile water). Post hoc used = Tukey HSD at p = 0.05, SPSS version 26. Values are means of three replicates and mean values followed by the same superscript are not significantly different at 5% level of significance.

Table 3: Effects of both variety and inoculation type on the stem girth

	Weeks after inoculation (WAP)

Variety	Inoculation	2	4	6	8	10
TVU-16877	Ino 1	0.533± 0.058^{a}	0.640± 0.036^{b}	1.210± 0.101^{a}	1.377± 0.157b^{bcd}	1.607± 0.110^{bcd}
	Ino 2	0.617± 0.076^{a}	0.747± 0.021^{b}	1.197± 0.035^{a}	1.317± 0.144^{bcd}	1.780± 0.203^{bc}
	Ino 3	0.540± 0.069^{a}	0.807± 0.136^{ab}	1.210± 0.101^{a}	1.343± 0.150^{bcd}	1.567± 0.306^{bcd}
	Ino 4	0.507± 0.068^{a}	1.020± 0.017^{a}	1.190± 0.017^{a}	1.303± 0.025^{bcd}	1.357± 0.040^{cd}
TVU-17088	Ino 1	0.533± 0.058^{a}	0.650± 0.086^{b}	1.370± 0.320^{a}	1.840± 0.149^{ab}	2.167± 0.351^{b}
	Ino 2	0.533± 0.058^{a}	0.650± 0.000^{b}	1.583± 0.388^{a}	2.333± 0.577^{a}	2.833± 0.577^{a}
	Ino 3	0.607± 0.133^{a}	0.797± 0.191^{ab}	1.473± 0.494^{a}	1.900± 0.173^{ab}	2.083± 0.257^{b}
	Ino 4	0.527± 0.025^{a}	0.650± 0.050^{b}	1.153± 0.150^{a}	1.500± 0.070^{bc}	1.653± 0.040^{bc}
TVU-16891	Ino 1	0.517± 0.029^{a}	0.617± 0.029^{b}	1.150± 0.180^{a}	1.413± 0.180^{bc}	1.550± 0.180^{bcd}
	Ino 2	0.517± 0.029^{a}	0.633± 0.058^{b}	1.167± 0.153^{a}	0.800± 0.100^{d}	0.633± 0.115^{e}
	Ino 3	0.437± 0.100^{a}	0.567± 0.029^{b}	0.967± 0.152^{a}	1.100± 0.173^{cd}	0.950± 0.087^{de}
	Ino 4	0.467± 0.058^{a}	0.733± 0.189^{b}	1.333± 0.058^{a}	1.517± 0.076^{bc}	1.603± 0.045^{bcd}
		Total effects of the varieties				
TVU-16877		0.549± 0.072^{a}	0.803± 0.157^{a}	1.202± 0.064^{ab}	1.335± 0.116^{b}	1.578± 0.227^{b}
TVU-17088		0.550± 0.076^{a}	0.687± 0.113^{b}	1.395± 0.349^{a}	1.893± 0.408^{a}	2.184± 0.538^{a}
TVU-16891		0.484± 0.063^{a}	0.638± 0.107^{b}	1.154± 0.182^{b}	1.208± 0.317^{b}	1.184± 0.438^{c}

Note: Ino 1 = spore/mycelia suspension, Ino 2 = inoculum meal, Ino 3 = inoculum meal + spore/mycelia suspension, Ino 4 = control (sterile water). Post hoc used = Tukey HSD at p = 0.05, SPSS version 26. Values are means of three replicates and mean values followed by the same superscript are not significantly different at 5% level of significance.

Table 4: Effect of inoculation methods on disease severity rating

Inoculation Methods	Symptom scoring	DSR	Reaction Class
Spore/mycelia suspension	5	<3^{a}	Tolerant/Resistant
Inoculum meal	13	>3^{ab}	Intermediate
Spore/mycelia suspension +Inoculum meal	50	6.2^{b}	Susceptible

Values are mean of three replicates. Values followed by the same superscript(s) are not significantly different at p=0.05 by Tukey HSD.

4. Conclusions

It was established in this study that the three cowpea varieties showed varying degree of susceptibility to Fot applied in three different ways. Only cowpea variety TVU17088 showed appreciable tolerance/resistance to even the most devastating method of inoculation (spore/mycelia suspension + inoculum meal). Cowpea variety TVU17088 is therefore recommended for further genetic studies to understand the basis of the tolerance exhibited and take advantage of the source of resistance for breeding purpose.

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