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Proportional dominance of *Alternaria linicola* and *Alternaria lini* in infected tissues and evaluation of linseed (*Linum usitatissimum* L.) germplasm against *Alternaria* blight

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Abstract
Alternaria blight of is an important disease of Linseed (*Linum usitatissimum* L.) that hampers its productivity and oil content. During severe infections, spots coalesced and covered the large area of the leaves. Field experiments were conducted during 2015-16 and 2016-17 to evaluate the performance of promising lines/varieties of linseed against Alternaria blight disease. Microscopic examination of pathogens in diseased tissue reveled that *Alternaria linicola* Groves & Skolko and *Alternaria lini* Dey were two fungi associated with the spots on leaves and capsules. Studies of relative dominance of pathogens associated with the diseased tissue revealed the dominance of *Alternaria linicola* (95.86-98.63%) in comparison to *Alternaria lini* (1.31-4.14%) during different growth period of linseed crop. Sixty-three genotypes were evaluated against this disease under artificial epiphytotic condition to known the level of resistance and susceptibility in different genotypes under artificial inoculated conditions. Out of Sixty-three genotypes screened, 2 genotypes namely –LCK-152, LCK-7002, rated as resistant, under both the years. While 4 genotypes namely- BUAPUR LOCAL, CR-M-6X22-9, EC-9832, OLC-48 in 2015-16 and 3 genotypes in 2016-17 rated under highly susceptible lines against this disease.

Keywords: Alternaria, disease, fungicide, linseed

Introduction
Linseed (*Linum usitatissimum* L.) commonly known as flax is an important *rabi* oilseed crop and a major source of oil and fibre. There are different varieties of linseed meant for dual purposes. Oilseed-type plants are generally shorter, have more branches and produce more seeds, while fiber flax types are usually taller, have few branches, and have been selected for fiber (Gill, 1966) [6]. It is one of the most important oilseed crops of temperate and subtropical region of the world. It has nutritional, medicinal, industrial and agricultural uses. The major causes behind low production of linseed are that the crop is cultivated mostly in rainfed marginal/sub-marginal lands and also due to the biotic and abiotic stresses. Alternaria blight caused by *Alternaria lini* Dey and *A. linicola* Grooves and Skolko, is a major biotic stress limiting crop yield in hot and humid environment (Groves and Skolko, 1944) [7]. In the last few years, *A. linicola* infection has been the main reason for the failure of the linseed seed. The disease causes up to 60 per cent annual yield loss depending upon the severity of the disease (Chauhan and Srivastava, 1975) [3]. 18.0 to 43.88 per cent losses have also been reported in different cultivars due to Alternaria leaf blight (Singh *et al.*, 2003, Singh and Singh 2004[a]) [11, 13]. Only few resistant genotypes are available at the National level against Alternaria blight (Singh *et al*, 2006; Singh *et al*, 2009) [15, 16]. Various fungicides have been used in the past to control the Alternaria blight disease with dissimilar cost-benefit ratio (Das, 2015) [5], but the ideal and most economical means of managing the blight disease of linseed would be the use of resistant varieties. Under these circumstances there is a need to exploit genetically host resistance in existing lines/varieties for the identification of resistant sources. The main aim of the present investigation was to study the proportional dominance of the *Alternaria lini* and *Alternaria linicola* during growth periods of the linseed crop and evaluation of resistance sources of the linseed for identification of resistant parental stocks.

Materials and Methods

Collection of diseased samples and isolation of pathogens:
Infected leaf and pods of linseed having the characteristics symptoms was collected for the isolation of *Alternaria* spp. The infected leaves were washed with sterilized water and cut into small sections containing both the disease and healthy looking tissue by sterilized scalpel. The sections were surface sterilized by dipping into 0.25% sodium hypochloride solution for 15-20 seconds and washed by 5 changes of sterilized distilled water. Small sections of infected leaves were then demoisturized by placing those folds of sterilized blotting paper and transferred aseptically to Petri dishes containing the water agar medium. The Petri dishes were incubated for 25^0C±1 for 5 days for sparse growth and sporulation of each *Alternaria* spp. associated with the diseased tissue. In each Petri dish, 5 pieces of each infected tissues were inoculated. After incubation, the growths were observed under the microscope for production of conidia of different species of *Alternaria*.

Purification of *Altenaria linicola* and *A. lini*
The conidia of *Alternaria* spp. produced in the water agar plate were flood harvested by using fine jet of water and conidia were collected in a sterilized eppendarf tube (2ml.) and washed three times by distilled water. 0.1. ml of diluted spore suspension obtained from the culture was produced on plain agar in Petri dishes to form a very thin layer on it and the spore were allowed to settle down on the agar surface. Settled spores were separated from each other by using a L-shape glass rod several times and streaked across the water agar plate. A glass marking pen was used to draw 50-100 circles of 5.0 mm diameter on the bottom of each water agar medium (2% Bacto agar) Petri plates (90 mm diam.) After incubation of Petri dishes at 25 ^{0}C for 8-12 hours, each circle was observed under a microscope at 100× magnification and those circles containing a single germinating conidia of *Alternaria* spp. were marked and spores in those circles were individually transferred into Petri dishes containing potato dextrose agar medium. The Petri dishes were incubated at 25^0 C ±1 for 10 days for growth and sporulation. After sporulation, the fungi were identified by using the relevant literature for identification of *Alternaria* spp. and detail morphological characteristics like size of conidia, conidiophore and mycelial conidial station were recorded. For measurement a calibrated ocular by using ocular and stages micrometer was used. Regular sub culturing was done by sub culturing of *Alternaria lini* and *A. linicola* on potato dextrose agar medium at 15 days intervals. Pathogenicity test of *Alternaria* spp. Pathogenicity test of *Alternaria lini and A. linicola* was tested on the susceptible variety Chambal. The spore suspension of conidia (10^4 spore/ml.) was incubated on the adult plant grown on the student's instructional farm of ANDUA&T Ayodhya, and symptom expression was observed.

Proportional dominance of *Alternaria linicola* and *A. lini* species in infected tissues of linseed
Relative frequency of *Alternaria* spp. in leaves of linseed collected from different locations of Uttar Pradesh was studied by observing the proportion of conidia of each species in infected tissue. Conidia of *Alternaria* spp. were harvested from infected leaves of linseed in a small drop of water on glass slide and their proportional frequency were observed at an interval of 15 days.

Evaluation of linseed genotypes against Alternaria blight
Screening of genotypes was done at Student Instructional farm of ANDUA&T Kumarganj, Ayodhya. The 63 genotypes used in this study were 68IC-32676, BR-14, BS-2, BENGAL-23, BENGAL-63, BEHAMPUR, BUAPUR LOCAL, CI-765, RL-975, CI-1972, CR-M-6X22-9, EC-1410, EC-9832, EC-99080, EC-12077-B, EC-22850, EC-22592, EC-EC-41627, EC-41656, EC99009, EC-99025, GS-121, Gunawal Local, GANGROOCHI, ILS-153, Kanpur Local, L-43, L-48, LCK-152, NP-59, NPHY-6-2P, RR-454, T-397, SJKO-5, SJKO-49, SJKO-63, RSJ-24, OLC-48, OLC-56, RKY-16, RUK-9, LS-1692, UDN-3 UDN-7, UDN-15, UDN-17, UDN-31, UDN-32, UDN-42, UDN-58, UDNA-3, LCK-7002, Meera, Gaurav, Polf-17, Polf-19, ES-44, H-43, LCK-9436, Polf-5, PCL-39, Parwati and RCL-125. These linseed germplasms were received from the Project Coordinating Unit, Linseed, ICAR, Kanpur and a number of advanced line developed at this station. These lines were sown during *rabi* 2015-16 and 2016-17 in augmented block design with one check Shekher repeated after every 10 lines of the test entries. Each entry and check were grown in single rows of 3 m length spaced 10 cm apart. Besides this, test entries were inoculated with spore suspension of *Alternaria linicola* twice one at seedling and other at flowering stage to create the epiphytotic.
Artificial Inoculation of *Alternaria linicola and A.lini* for disease development
A pure culture of local isolate of *A. linicola and A. lini* known to be aggressive was used for inoculation during evaluation of resistance in genotypes against Alternaria blight. *A. linicola* and *A. lini* was multiplied on sorghum grains and spores were harvested in water. The spores were inoculated on all the genotypes and spreader row (susceptible Chambal variety check) during evening hours with a spore water suspension of fixed concentration (10^4 ml.) at three growth stages of the linseed crop (complete flowering, capsule formation and before the maturity). The fields were frequently irrigated to maintain high humidity and promote disease development.

Observations recorded
The observations on the progress of disease severity in each genotypes were recorded by selected five plants randomly from each genotypes. The disease severity was recorded at 10 days intervals to till maximum disease severity by using 0-5 rating given below:
Disease rating Disease severity description
0. No symptoms on leaf.
1. Small light brown spots scattered covering ≤5% leaf area.
2. Spots small, brown, with concentric rings, covering 5.1 to 10% leaf area.
3. Spots large, brown, irregular with concentric rings, covering 10.1 to 25% leaf area.
4. Spots large, brown, irregular lesions with typical blight symptoms, covering 25.1 to 50% leaf area.
5. Spots large, brown, irregular lesions with typical blight symptoms, covering > 50% leaf area.

The per cent disease intensity (PDI) was calculated by employing formula (Wheeler, 1969) [19] mentioned below:

$$\text{PDI} = \frac{Sum\ of\ total\ numerical\ ratings}{Total\ number\ of\ leaves\ observed}\ X\ \frac{100}{\text{Highest grade}}$$

The area under Disease Progress Curve (AUDPC) was calculated by the formula as under (Shaner and Finney, 1977) [10]:

$$AUDPC = \sum_{i=1}^{n} \left(\frac{yi+yi+1}{2}\right)(ti+1-i)$$

Where
Yi = Alternaria blight severity (%) at the 1st observation
$t_{(i+1)} - t$ = Time (days) between two disease scores
n = Total number of observation

The apparent infection rate of disease development (r) is measured of the speed at which an epidemic develops. Despite the presence of virulent pathogen and favorable environment, differences were observed in the rate of disease development on different date of sowing. The period from the initial appearance of symptoms and the final disease severity calculated according to the following formula (Vender plank, 1963) [18].

$$\text{Infection rate (r)} = \frac{2.3}{t_2 - t_1} \log_e \frac{X_2\,(1-X_1)}{X_1(1-1-X_2)}$$

Where
(e) = apparent infection rate
t_1 = time during first observation
t_2 = time (days) during second observations
$t_2 - t_1$ = time interval between two observations
x_1 = per cent disease intensity value in decimal at corresponding t_1 time
x_2 = per cent disease intensity value in decimal at corresponding t_2 time
Log_e = natural log

Result and Discussion

Proportional dominance of *Alternaria* spp. species in infected tissues of linseed

The relative frequency of *Alternaria* spp. in the diseased leaves of linseed collected from different locations is presented in table-1. The data indicates the higher population of *A. linicola* in all diseased samples collected from 5 locations of U.P. in the month of Feb. and March during crop season 2015-16 and 2016-17. The conidial population of *A. linicola* obtained from diseased leaf samples collected during Feb. to March in both the years ranged between 95.86 to 98.63% whereas population of *Alternaria lini* ranged from 1.31-4.14% (Table-1). Results indicate that *A. linicola* is most virulent and dominant pathogen of linseed in Uttar Pradesh. Kumar *et al.* (2016) [8] also worked on the relative dominance of Alternari leaf blight of mustard pathogens and reported that conidia of *A. brassicae* are most predominant in diseased tissue of rapeseed- mustard in comparison to *A. brassicicola* and *A. raphani.*

Evaluation of linseed genotypes against Alternaria blight:

The progress of the disease on the 63 genotypes of linseed was studied during Rabi 2015-2016 and 2016-2017 to evaluate the level of resistance and susceptibility in various germplasm collected from PC unit of Linseed, Kanpur, and U.P. Inoculation of spores of an aggressive isolate of *A. lonicola* and *A. lini* @ 10^4 spore per ml. induce the typical symptoms of Alternaria blight leaves of linseed in second week of Dec. in 2015 -2016 and 2016-17 with appearance of black points, which later enlarge to develop into spot of various sizes. The tissue damage of linseed leaves was lower in resistant genotypes in comparison to susceptible genotypes. The highest disease severity was found 66.5% in 2015-16 and 62.5% in 2016-17. Disease severity on 63 genotypes varied from 9.47 to 65.0 in 2015-16 and 7.21% to 62.54% in 2016-17 which indicated that artificial inoculation caused the disease in both the years. Lower disease severity on leaves (less than 10%) was noted on genotypes LCK-7002 and LCK-152 during 2015-16 and 2016-17 which indicates the components of resistance in these two genotypes. Out of 63 genotypes, 34 genotypes showed disease severity between 10.1-25%, 4 genotypes showed the disease severity between 25-40. 19 genotypes showed disease severity between 40-60 and only four genotypes namely Buapur local, CR-N-6X22-9, EC-9832 and OLC 48 showed severity ranged between 60.1-65.01 in the year 2015-16. More or less similar results were observed in the year 2016-17. In the year 2016-17, 33 genotypes showed disease severity between 10.1-25%, 9 genotypes showed the disease severity between 25-40, 11 genotypes showed disease severity between 40-60 and only 3 genotypes namely RKY16, OLC 48 and CR-N-6X22-9, showed severity ranged between 60.1-62.5% in the year 2016-17. Severity in susceptible check Chambal was noted more than 60% in both the years (Figure-1).

Area under disease progress curve (AUDPC – A value):

The Area Under Disease Progress was observed on leaves of all the 63 genotypes which were found variable depending on the susceptibility of genotypes against Alternaria blight pathogens. Area under disease progress curve (AUDPC) on different genotypes varied from 131.354 - 1272.80 in year 2015-16 and 117.5-1195.14 in 2016-17 (Table-2). The AUDPC below 200 was observed in genotype Gaurav, LCK-152, LCK-7002 and NPHY-6-2P during 2016-17. However only two genotypes namely LCK-152, LCK-7002 showed AUDPC below 200 in year 2015-16. Thirty-two genotypes showed 201-400 AUDPC in both the years. AUDPC of the rest of genotypes ranged between 400-1272.84 during 2015-16 and 2016-2017. RKY 16, Berhampur, EC-9832, OLC 56, Buapur local, CR-M-6X22-9, OLC-48 showed higher AUDPC in 2015-16. Higher ADUPC was recorded in genotypes RKY-16, OLC-56, OLC-48, CR-M-6X22-9, EC-9832 in 2016-17 (Fig. 2). Observations of AUDPC indicates that LCK 152 and LCK 7002 showed lower disease progress during both the years and these lines could be used as potential donor for breeding programs.

Apparent Infection Rate (r value):

The apparent infection rate of *Alternaria linicola* on linseed genotypes raged from 0.0185 to 0.1086 and in 2016-17 ranges 0.040 to 0.1044. The lowest infection rate was found in genotype Behrampur (0.0185) in 2015-16 and Ec-99089 (0.040) in 2016-17 while the maximum infection rate was noticed in genotype Parvati (0.108) in 2015-16 and NPHY-6-2P (0.104) in 2016-17. The details about r-value is different genotypes in given in Fig.3. However, Pant *et al.* (2001) [17] screened 30 linseed cultivars for the resistant to *Alternaria alternata* and reported 9 cultivars as resistant (1-1.5% infection) and 9 moderately resistant (5.1-10% infection).

Host reaction of Linseed genotypes against Alternaria blight

Based on lower disease intensity and lower AUDPC, only two genotypes LCK 152 and LCK 700 were found resistant in the year 2015-16 and 2016-17 (Fig.1 and Fig.2). Based on the severity of genotypes, 33-34 genotypes were found

moderately resistant in the year 2015-16 and 2016-17. Some common genotypes that were found moderately resistance in both the years were Polf-17, Gaurav, BS-2, EC-22592, NPHY-6-2P, RCL-125, UDN-31, EC-99025, GUNAWAL LOCAL, UDN-32, PCL-39, RR-454, ES-44, L-48, T-397, CI-765, Polf-19, EC-41627, GANGROOCHI, SJKO-49, H-43, SJKO-63, GS-121, LCK-9436, Meera, L-43, NP-59, EC-1410, 68IC-32676, BENGAL-23, EC-2285O, EC-41656 and EC-99009. Some of these genotypes showing low severity and lower AUDPC could also be used as a donor for breeding programme. Rest of the genotypes were found moderately susceptible to highly susceptible to Alternaria blight (Fig. 1). Similarly, Singh *et al.* (1995a) [14] screened 450 genotypes of linseed and reported eight genotypes *viz.*, Acc No. 2883, Acc. No. 2921, Ayogi, Cherapuram, ES-44, KL-31, and RI-50-3 as resistant to the disease during the year 1991-92 to 1993-94. Das R. *et al.* (2016) [4] conducted during 2009-10, 2010-11, 2011-12, 2012-13 and 2013-14 to evaluate the performance of promising 24 lines/varieties of linseed against Alternaria blight disease with one susceptible check variety (Chambal) and one resistant check variety (Sheela). They revealed that, Meera, Gaurav, EC-41590, A-202 and JRF- 3 showed lowest per cent disease intensity in leaf (<10%) as well as bud infection to the extent of 8.4, 8.0, 9.0, 7.5, 7.8% respectively. These lines/varieties can be used as good donor for evolving resistant varieties against Alternaria blight in linseed. Singh and Singh (2011) [12] evaluated 200 genotypes against alternaria blight under artificial inoculated condition and found none was free from the disease, during 2007-08 and 2008-09. Ten genotypes namely Polf-12, Polf-13, Polf-15, Polf-16, Polf-19, Polf-22, Polf-23, Polf-29, Polf-39 and RL-18-1 were recorded resistant 15 moderate resistant 29 moderately susceptible, 59 susceptible and 98 as highly susceptible.

Table 1: Relative dominance (%) of Alternaria blight pathogen on leaves of linseed in different location of U.P.

Location	Periods											
	13/02/16		28/02/16		15/03/16		13/02/17		28/02/17		15/03/17	
	A. linicola	*A. lini*	*A. linicola*	*A. lini*	*A. linicola*	*A. lini*	*A. linicola*	*A. lini*	*A. linicola*	*A. lini*	*A. linicola*	*A. lini*
Kumarganj NDUA&T, Faizabad	97.52	1.48	97.83	2.17	96.92	3.08	97.12	1.88	96.53	3.47	95.86	4.14
CSAUA&T, Kanpur	98.20	1.80	98.28	1.72	97.98	2.02	98.53	1.47	97.39	2.71	97.02	2.98
Masaudha, Faizabad	98.12	1.88	97.41	2.59	96.67	3.33	97.72	2.27	97.16	2.84	96.09	3.91
Haidargadh, Barabanki	97.86	2.14	97.66	2.44	96.33	3.77	98.69	1.31	97.82	2.18	98.11	1.89
Lakhimpur Khiri	97.92	2.08	97.10	2.90	96.24	3.76	97.25	2.75	96.40	3.60	96.93	3.07

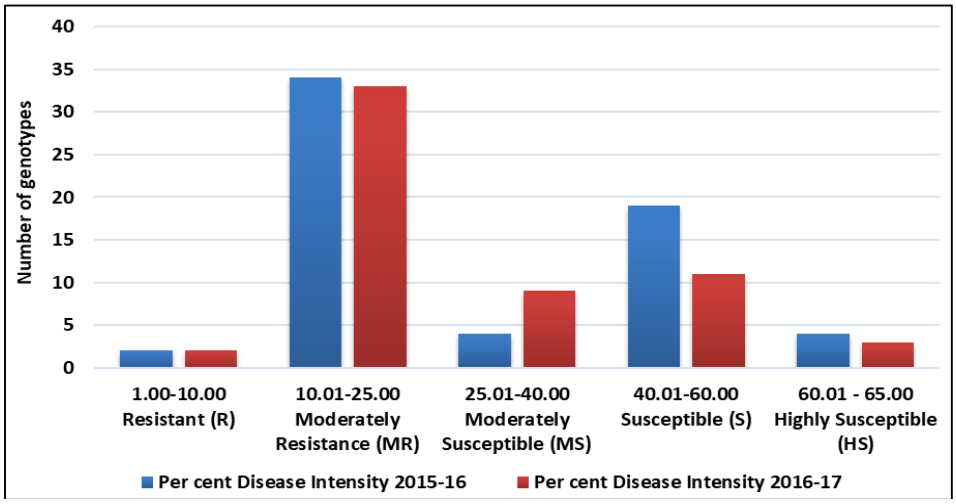

Fig 1: Per cent disease intensity and host reaction of different genotypes of linseed during 2015-16 and 2016-17.

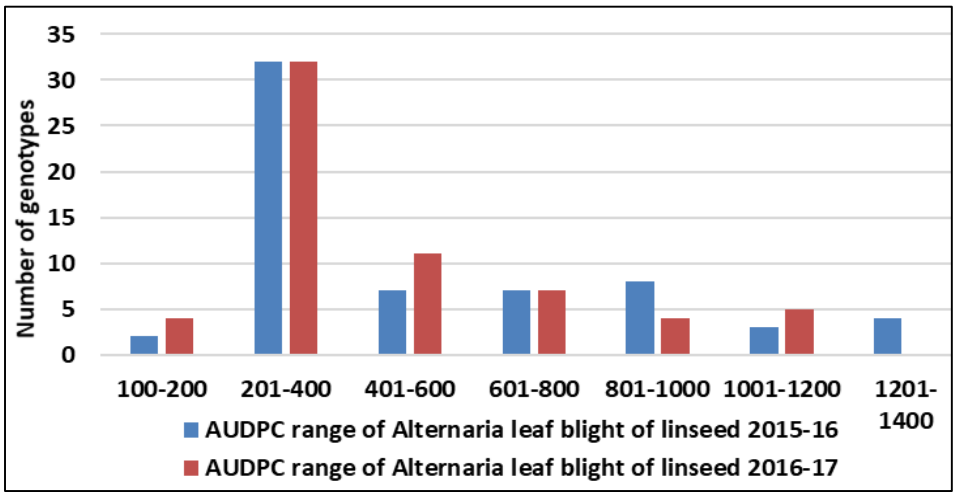

Fig 2: Distribution of 63 genotypes of linseed in different range of AUCPC (Area under disease progress curve) of Alternaria blight during 2015-16 and 2016-17.

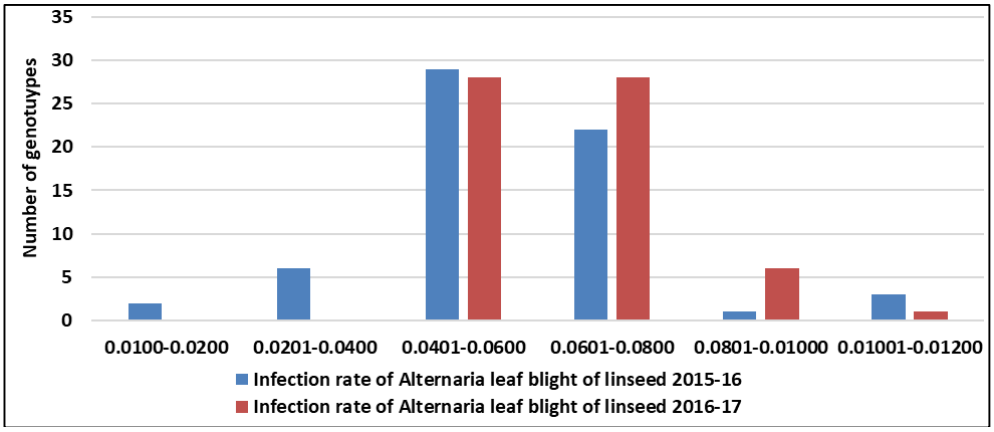

Fig 3: Distribution of 63 genotypes of linseed in different range of infection rate of Alternaria blight disease/ pathogens during 2015-16 and 2016-17.

Conclusion

The conidial proportion of A. *linicola* in the infected tissue ranged from 91.35 to 96.31% whereas frequencies of A. *lini* was only 1.31-4.14 during 03/02/2015 to 15/04/2017. This indicated that *A. linicola* is dominant and aggressive pathogen in comparison to *A. lini*. Sixty-three genotypes were evaluated against the Alternaria blight to find out the level of resistance / susceptibility. The disease severity in different genotypes ranged from 9.47 to 65.01% during the year 2015-16 and 7.21 to 62.54 during the year 2016-17. The minimum disease severity was recorded on genotypes LCK-7002, LCK-152 having in both years respectively. AUDPC and apparent infection rate on leaves were recorded on genotype LCK-7002 followed by genotypes LCK-152, NPHY-6-2P, and Gaurav in both years respectively. Significantly maximum disease severity (PDI), AUDPC and infection rate was observed in genotype OLC-48 followed by genotypes CR-M-6X22-9, and EC-9832 in 2015-16 and OLC-48, RKY-16, EC-9832 and CR-M-6X22-9 in 2016-17.

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