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# Study about Identification of Purple Blotch and Thrips Resistant Lines of Onion (*Allium cepa* L.)

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#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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#### Original Research Article

#### **ABSTRACT**

Thirty six elite genotypes were evaluated during the winter season (rabi) 2022-23 at three different environments in 3 replications. RO-1, RO-59, RO-1 × RO-59, RO-1 × Pusa Madhavi, RO-59 × Pusa Madhavi, RO-59 × Kashi No. 1 genotypes had showed thrips population less than 22.90 and highly resistant to thrips infestation. Purple blotch disease, caused by *Alternaria porri* is the most common devastating disease of onion under field conditions throughout the globe. RO-1, Pusa Shobha, RO-1 × RO-59 and Bhima Kiran × Bhima Shakti were found resistant with a grade scale of 1. The per cent leaf area infection ranged from 1-10 per cent.

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Keywords: Onion; Allium cepa L; germplasm; purple blotch disease; thrips.

#### 1. INTRODUCTION

Onion (Allium cepa L.) is a prime vegetable crop of the genus Allium belongs to the family alliaceae with chromosome number 2n (2x) = 16and it is confounded to be originated from Central Asia centre of origin and near East and Mediterranean regions are considered secondary centers of origin [1,2]. Onions are used as raw as salad, vegetable and spice all over the world [3]. The bulb and greens are rich in vitamin C, dietary fiber, mineral potassium, folic acid and it is richest source of mineral vanadium. It is also contains calcium, iron and high quality protein with low sodium without fat [4]. Onion bulb contains 86.8 per cent of moisture, 11 g of carbohydrates, 1.2 g of protein, 0.6 g of fiber, 0.08 mg of thiamine, 11 mg of vitamin- C, 180 mg of calcium, 50 mg of phosphorus, 0.7 mg of iron, 0.4 mg of nicotinic acid and 0.01 mg of riboflavin per 100 g of edible portion [5].

Onion is susceptible to foliar diseases that reduce bulb yield and quality [6], among foliar diseases purple blotch disease caused by Alternaria porri (Ellis). Cif. major threat for onion production, purple blotch infected leaves are reddish brown septate non-sporulating mycelium [7], air borne spores of A. porri were responsible for increased disease incidence of onion cultivar Creamish-golden coloured leaves requiredleaf wetness duration at 5 °C for 16 h and 8 h at 10-25°C. The numbers of lesions are increased with increase of leaf wetness duration and temperature reported by Suheri and Price [8]. It was first reported by Ajrekar [9] from Bombay state of India, and it is a major disease onion across the world [6] prevalent in warm humid climate [8]. infestation causes on leaves and flower stalks, reduces onion tops yield by 62-92 % [8] loss of bulb yield by 30 % and seed by 10 % under congenial environmental [10], causing heavy yield loss conditions ranging from 2.5 to 87.8 % during kharif season [11].

Among several insect pest onion thrips (*Thrips tabaci* Lindeman, Thysanoptera: Thripidae) pose severe problem (Gupta et al. 1994) and major limiting factor affecting yield, larvae and adults were rasping of leaves and sucking of sap from plant parts could lead to the chlorotic spot of leaves, deformities of foliage and finally it led to

reduced size and yield of bulb [12]. The highest infestation recorded from 35 to 100% thrips incidence and 3.5-30.3 nymphs per plant, both nymphs and adults of thrips by rasping of leaves, suck the exuding sap. T. tabaci Lindeman densities increased matured plant and low on young plants on which higher numbers of larvae gathered than adults at the plants as hiding place [13], as cryptic life style stay under the leaf surface and places far from reach control problematic, and consequently difficult to control by means of cultural and chemicals and cause yield losses estimated due to the incidence of thrips in onion has to the tune of 40 to 50% (Gupta et al. 1994).

#### 2. MATERIALS AND METHODS

The experiment was conducted at Horticulture farm, SKN College of Agriculture, Jobner (Jaipur) (Rajasthan), during rabi season of 2022-23. Eight genetically diverse parents namely, RO-1, RO-59, Bhima Kiran, Bhima Shakti, Pusa Shobha, Pusa Madhavi. Kashi No. 1 and Pusa Red were crossed in diallel fashion excluding reciprocals. All the 28 F<sub>1</sub>s were evaluated in a randomized block design with three replications under 3 different date of sowing. The seedlings were planted in row 15 cm apart by hand dibbling method with a row to row spacing of 10 cm. The standard cultural practices were followed to raise the crop. Number of thrips (both nymphs and adults) was recorded from 5 randomly selected plants in each plot by keeping a white paper below the plant and then shaking the plants with finger. The tested genotypes were also grouped into four categories of resistance viz., highly resistant, resistant, susceptible and highly susceptible based on number of thrips per plant. For purple blotch disease incidence was recorded by using the disease scale given by Bhangale and Joi [14] Disease incidence recorded after 75 transplanting on each cultivar by using following formula.

Disease incidence % = Total infected plants observed X 100 / Total plants observed

The genotypes were placed in different categories of resistance and susceptibility as follows on the basis of score method.

Scale: 0 = (I) Immune

1 = (R) Resistant (1- 10%)

2 = (MR) moderately resistant (11-20%)

3 = (MS) moderately susceptible (21-40%)

4 = (S) Susceptible (41-60%)

5 = (HS) highly susceptible (61% and above)

#### 3. RESULTS AND DISCUSSION

## 3.1 Screening of Different Genotypes of Onion against Purple Blotch Disease (Alternaria porri)

A total of 36 onion genotypes were screened in field under natural condition. The genotypes were grouped as per the scale of Bhangale and Joi [14] and data is presented in Table 2.

The results revealed that at 75 DAP in E<sub>1</sub>, among the genotypes none of them were found immune, while four genotypes viz., RO-1, Pusa Shobha, RO-1 x RO-59 and Bhima Kiran x Bhima Shakti were found resistant with grade scale of 1. The per cent leaf area infection ranged from 1-10 per cent. Thirteen genotypes RO-59, Pusa Madhavi, RO-1 x Bhima Shakti, RO-59 x Bhima Shakti, RO-59 x Pusa Shobha, Bhima Kiran x Pusa Shobha, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Madhavi, Pusa Shobha x Pusa Madhavi, Pusa Shobha x Kashi No. 1, Pusa Shobha x Pusa Red, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red were grouped under in moderately resistant group and the grade scale was 2 with 11-20 per cent leaf area infection. Fifteen genotypes Bhima Kiran, Bhima Shakti, Pusa Red, RO-1 x Bhima Kiran, RO-1 x Pusa Shobha, RO-1 x Pusa Madhavi, RO-1 x Pusa Red. RO-59 x Bhima Kiran, RO-59 x Pusa Madhavi, RO-59 x Pusa Red, Bhima Kiran x Pusa Madhavi, Bhima Kiran x Kashi No. 1, Bhima Shakti x Kashi No. 1, Bhima Shakti x Pusa Red and Pusa Madhavi x Kashi No. 1 were grouped under in moderately susceptible group and the grade scale was 3 with 21-40 per cent leaf area infection and three genotypes Kashi No. 1, RO-1 x Kashi No. 1 and Bhima Kiran x Pusa Red were susceptible with grade scale of 4. The per cent leaf area infection ranged from 41-60 per cent and among the genotypes none of them were found highly susceptible group. In E2, among the genotypes none of them were found immune, while one genotype viz., RO-59 were found resistant with grade scale of 1. The per cent leaf area infection ranged from 1-10 per cent. Twelve genotypes RO-1, Pusa Shobha, RO-1 x RO-59, RO-1 x Bhima Shakti, RO-59 x

Bhima Shakti, RO-59 x Pusa Madhavi, RO-59 x Pusa Red. Bhima Shakti x Pusa Madhavi. Pusa Shobha x Pusa Madhavi, Pusa Shobha x Kashi No. 1, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red were grouped under in moderately resistant group and the grade scale was 2 with 11-20 per cent leaf area infection. Fifteen genotypes Bhima Shakti, Pusa Red, RO-1 x Bhima Kiran, RO-1 x Pusa Shobha, RO-1 x Pusa Madhavi, RO-59 x Bhima Kiran, RO-59 x Pusa Shobha, RO-59 x Kashi No. 1, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha, Bhima Kiran x Pusa Madhavi, Bhima Shakti x Pusa Shobha, Bhima Shakti x Kashi No. 1, Pusa Shobha x Pusa Red and Pusa Madhavi x Kashi No. 1 were grouped under in moderately susceptible group and the grade scale was 3 with 21-40 per cent leaf area infection and six genotypes Bhima Kiran, Pusa Madhavi, Kashi No. 1. RO-1 x Kashi No. 1. RO-1 x Pusa Red. Bhima Kiran x Kashi No. 1 and Bhima Shakti x Pusa Red were susceptible with grade scale of 4. The per cent leaf area infection ranged from 41-60 per cent and among the genotypes none of them were found highly susceptible group. In E<sub>3</sub>, among the genotypes none of them were found immune and resistant, while five genotypes RO-59, RO-1 × RO-59, Bhima Kiran × Pusa Shobha, Bhima Kiran x Pusa Madhavi, and Bhima Shakti x Pusa Shobha were grouped under in moderately resistant group and the grade scale was 2 with 11-20 per cent leaf area infection. Twenty four genotypes RO-1, Bhima Shakti, Pusa Shobha, Pusa Madhavi, Pusa Red, RO-1 x Bhima Shakti, RO-1 x Pusa Shobha, RO-59 x Bhima Kiran, RO-59 x Pusa Madhavi, RO-59 x Bhima Shakti. RO-59 x Pusa Shobha. RO-59 x Pusa Red. Bhima Kiran x Bhima Shakti. Bhima Kiran x Kashi No. 1, Bhima Kiran x Pusa Red, Bhima Shakti x Pusa Madhavi, Bhima Shakti x Kashi No. 1, Bhima Shakti x Pusa Red, Pusa Shobha x Pusa Madhavi, Pusa Shobha x Kashi No. 1. Pusa Shobha x Pusa Red. Pusa Madhavi x Kashi No. 1, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red were grouped under in moderately susceptible group and the grade scale was 3 with 21-40 per cent leaf area infection and four genotypes RO-1 x Bhima Kiran, RO-1 x Pusa Madhavi, RO-1 x Pusa Red and RO-59 x Kashi No. 1 were susceptible with grade scale of 4. The per cent leaf area infection ranged from 41-60 per cent and three genotypes Bhima Kiran, Kashi No. 1 and RO-1 x Kashi No. 1 were found highly susceptible group and the grade scale was 4 with 61 per cent and more leaf area infection. According to Alimousavi et al. [15] Kumari et al. [16] Tripathy et al. [17], Suhas et al.

[18] and Lakshmipathi [19] the field screening provided us the genotypes with variable responses to purple blotch disease incidences could leads to the identification of resistant sources. It is required to be further confirmed through artificial inoculation and molecular assay and their genetic inheritance need to be revealed with use of identified resistant lines.

### 3.2 Screening of Different Genotypes of Onion against Thrips (*Thrips tabaci*)

The different genotypes of onion were also grouped into four different categories of resistance viz., highly resistant, resistant, moderately resistant and susceptible are presented in Table 1.

Table 1. Screening of different genotypes of onion against purple blotch disease

Genotypes	Plant Disease Index			
••	E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>	
RO-1 (P <sub>1</sub> )	10	20	25	
RO-59 (P <sub>2</sub> )	15	10	20	
Bhima Kiran (P <sub>3</sub> )	35	45	65	
Bhima Shakti (P <sub>4</sub> )	30	40	40	
Pusa Shobha (P <sub>5</sub> )	10	20	25	
Pusa Madhavi (P <sub>6</sub> )	20	30	25	
Kashi No. 1 (P <sub>7</sub> )	45	55	65	
Pusa Red (P <sub>8</sub> )	30	40	35	
$P_1 \times P_2$	10	15	20	
P <sub>1</sub> x P <sub>3</sub>	25	35	50	
P <sub>1</sub> x P <sub>4</sub>	15	15	25	
P <sub>1</sub> x P <sub>5</sub>	30	25	35	
P <sub>1</sub> x P <sub>6</sub>	30	40	60	
P <sub>1</sub> x P <sub>7</sub>	45	55	65	
P <sub>1</sub> x P <sub>8</sub>	40	45	50	
$P_2 \times P_3$	25	30	35	
$P_2 \times P_4$	15	20	25	
$P_2 \times P_5$	20	25	30	
P <sub>2</sub> x P <sub>6</sub>	25	15	30	
$P_2 \times P_7$	45	40	50	
$P_2 \times P_8$	25	20	30	
P <sub>3</sub> x P <sub>4</sub>	10	25	30	
P <sub>3</sub> x P <sub>5</sub>	15	25	20	
P <sub>3</sub> x P <sub>6</sub>	30	35	20	
P <sub>3</sub> x P <sub>7</sub>	40	45	30	
P <sub>3</sub> x P <sub>8</sub>	45	50	35	
P <sub>4</sub> x P <sub>5</sub>	15	25	20	
P <sub>4</sub> x P <sub>6</sub>	20	15	25	
P <sub>4</sub> x P <sub>7</sub>	25	35	40	
P <sub>4</sub> x P <sub>8</sub>	30	45	40	
P <sub>5</sub> x P <sub>6</sub>	15	20	25	
P <sub>5</sub> x P <sub>7</sub>	20	15	25	
P <sub>5</sub> x P <sub>8</sub>	20	25	30	
$P_6 \times P_7$	35	40	30	
P <sub>6</sub> x P <sub>8</sub>	20	15	25	
P <sub>7</sub> x P <sub>8</sub>	10	15	20	

Scale: 0 = (I) Immune

1 = (R) Resistant (1-10%)

2 = (MR) Moderately resistant (11-20%)

3 = (MS) Moderately susceptible (21-40%)

4 = (S) Susceptible (41-60%)

5 = (HS) Highly susceptible (61% and above)

Table 2. Screening of different genotypes of onion against thrips

Category of resistance	Scale (Thrips population)	Screening of different genotypes of onion against thrips		
Highly resistant in E₁	< 22.90	RO-1, RO-59, RO-1 x RO-59, RO-1 x Pusa Madhavi, RO-59 x Pusa Madhavi and RO-59 x Kashi No.		
Resistant in E <sub>1</sub>	> 22.90 <23.83	RO-1 x Bhima Kiran, RO-59 x Pusa Red. Bhima Shakti x Kashi No. 1, Pusa Shobha x Pusa Madhavi and Pusa Madhavi x Pusa Red		
Moderatey resistant in E₁	>23.83 < 27.10	Bhima Kiran, Bhima Shakti, Pusa Shobha, Pusa Madhavi, Pusa Red, RO-1 x Bhima Shakti, RO-1 x Pusa Shobha, RO-1 x Kashi No. 1, RO-1 x Pusa Red, RO-59 x Bhima Kiran, RO-59 x bhima Shakti, RO-59 x Pusa Shobha, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha, Bhima Kiran x Kashi No. 1, Bhima Kiran x Pusa Red, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Madhavi, Bhima Shakti x Pusa Red, Pusa Shobha x Kashi No. 1, Pusa Shobha x Pusa Red, Pusa Madhavi x Kashi No. 1 x Pusa Red		
Susceptible in E <sub>1</sub>	>27.10	-		
Highly resistant in E <sub>2</sub>	< 24.69	RO-1, RO-59, RO-1 x RO-59, RO-1 x Bhima Kiran, RO-1 x Bhima Shakti, RO-59 x bhima Shakti, RO-59 x Pusa Red, Bhima Shakti x Pusa Madhavi and Pusa Madhavi x Kashi No. 1		
Resistant in E <sub>2</sub>	> 24.69 <25.61	RO-59 x Bhima Kiran, RO-59 x Kashi No. 1, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Red and Pusa Shobha x Pusa Red		
Moderatey resistant in E <sub>2</sub>	>25.61 <29.31	Bhima Kiran, Bhima Shakti, Pusa Shobha, Pusa Madhavi, Kashi No. 1, Pusa Red, RO-1 x Pusa Madhavi, RO-1 x Kashi No. 1, RO-1 x Pusa Red, RO-59 x Pusa Shobha, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha, Bhima Kiran x Pusa Madhavi, Bhima Kiran x Pusa Red, Pusa Shobha x Pusa Madhavi, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red		
Susceptible in E <sub>2</sub>	>29.31	Bhima Kiran x Kashi No. 1, Bhima Shakti x Kashi No. 1 and Pusa Shobha x Kashi No. 1		
Highly resistant in E <sub>3</sub>	< 23.76	RO-1 x RO-59		
Resistant in E <sub>3</sub>	> 23.76 <27.08	RO-1, RO-59, Bhima Kiran, Bhima Shakti, Kashi No. 1, RO-1 x RO-59, RO-1 x Bhima Kiran, RO-1 x Pusa Red, RO-59 x Bhima Kiran, RO-59 x bhima Shakti, RO-59 x Pusa Shobha, RO-59 x Pusa Madhavi, RO-59 x Kashi No. 1, RO-59 x Pusa Red, Bhima Kiran x Pusa Madhavi, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Madhavi, Bhima Shakti x Kashi No. 1, Pusa Shobha x Pusa Madhavi, Pusa Madhavi x Kashi No. 1, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red		
Moderatey resistant in E <sub>3</sub>	>27.08< 28.24	RO-1 x Bhima Shakti, RO-1 x Kashi No. 1 and Bhima Kiran x Pusa Red		
Susceptible in E₃	>28.24	Pusa Shobha, Pusa Madhavi, Pusa Red, RO-1 x Pusa Shobha, RO-1 x Pusa Madhavi, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha, Bhima Kiran x Kashi No. 1, Bhima Shakti x Pusa Red, Pusa Shobha x Kashi No. 1 andPusa Shobha x Pusa Red		

At 75 DAP in E<sub>1</sub>, RO-1, RO-59, RO-1 × RO-59, RO-1 x Pusa Madhavi. RO-59 x Pusa Madhavi. RO-59 x Kashi No. 1 genotypes had showed thrips population less than 22.90 and highly resistant to thrips infestation. However. genotypes RO-1 x Bhima Kiran, RO-59 x Pusa Red. Bhima Shakti x Kashi No. 1, Pusa Shobha x Pusa Madhavi and Pusa Madhavi x Pusa Red showed thrips population more than 22.90 but less than 23.83 per plant were grouped into resistant. While, genotypes, Bhima Kiran, Bhima Shakti, Pusa Shobha, Pusa Madhavi, Pusa Red, RO-1 x Bhima Shakti, RO-1 x Pusa Shobha, RO-1 x Kashi No. 1, RO-1 x Pusa Red, RO-59 x Bhima Kiran, RO-59 x bhima Shakti, RO-59 x Pusa Shobha, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha. Bhima Kiran x Pusa Madhavi, Bhima Kiran x Kashi No. 1, Bhima Kiran x Pusa Red, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Madhavi, Bhima Shakti x Pusa Red, Pusa Shobha x Kashi No. 1, Pusa Shobha x Pusa Red, Pusa Madhavi x Kashi No. 1, Pusa Madhavi x Pusa Red, Kashi No. 1 x Pusa Red were found moderately resistant by recording thrips population more than 23.83 but less than 27.10 per plant. Whereas, none of the genotypes found susceptible by recording thrips population more than 27.10 per plants, In E<sub>2</sub>, RO-1, RO-59, RO-1 x RO-59, RO-1 x Bhima Kiran, RO-1 x Bhima Shakti, RO-59 x bhima Shakti, RO-59 x Pusa Madhavi, RO-59 x Pusa Red, Bhima Shakti x Pusa Madhavi and Pusa Madhavi x Kashi No. 1 genotypes had showed thrips population less than 24.69 and highly resistant to thrips infestation. However, genotypes RO-59 x Bhima Kiran, RO-59 x Kashi No. 1, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Red and Pusa Shobha x Pusa Red showed thrips population more than 24.69 but less than 25.61 per plant were grouped into resistant. While, genotypes, Bhima Kiran, Bhima Shakti, Pusa Shobha, Pusa Madhavi, Kashi No. 1, Pusa Red, RO-1 x Pusa Madhavi, RO-1 x Kashi No. 1, RO-1 x Pusa Red, RO-59 x Pusa Shobha, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha, Bhima Kiran x Pusa Madhavi, Bhima Kiran x Pusa Red, Pusa Shobha x Pusa Madhavi, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red were found moderately resistant by recording thrips population more than 25.61 but less than 29.31 per plant. Whereas, Bhima Kiran x Kashi No. 1, Bhima Shakti x Kashi No. 1 and Pusa Shobha x Kashi No. 1 genotypes found susceptible by recording thrips population more than 29.31 per plants. In E<sub>3</sub>, RO-1 x RO-59 genotype had showed thrips

population less than 23.76 and highly resistant to thrips infestation, However, RO-1, RO-59, Bhima Kiran, Bhima Shakti, Kashi No. 1, RO-1 x RO-59, RO-1 x Bhima Kiran, RO-1 x Pusa Red, RO-59 x Bhima Kiran, RO-59 x bhima Shakti, RO-59 x Pusa Shobha, RO-59 x Pusa Madhavi, RO-59 x Kashi No. 1, RO-59 x Pusa Red, Bhima Kiran x Pusa Madhavi, Bhima Shakti x Pusa Shobha, Bhima Shakti x Pusa Madhavi, Bhima Shakti x Kashi No. 1, Pusa Shobha x Pusa Madhavi, Pusa Madhavi x Kashi No. 1, Pusa Madhavi x Pusa Red and Kashi No. 1 x Pusa Red genotypes showed thrips population more than 23.76 but less than 27.08 per plant were grouped into resistant. While, genotypes, RO-1 x Bhima Shakti, RO-1 x Kashi No. 1 and Bhima Kiran x Pusa Red were found moderately resistant by recording thrips population more than 27.08 but less than 28.24 per plant. Whereas, Pusa Shobha, Pusa Madhavi, Pusa Red, RO-1 x Pusa Shobha, RO-1 x Pusa Madhavi, Bhima Kiran x Bhima Shakti, Bhima Kiran x Pusa Shobha, Bhima Kiran x Kashi No. 1, Bhima Shakti x Pusa Red, Pusa Shobha x Kashi No. 1 andPusa Red Shobha × Pusa genotypes susceptible by recording thrips population more than 27.10 per plants. The results are lined with the Alimousavi et al. [15] Kumari et al. [16] Tripathy et al. [17] and Lakshmipathi [19] this could be due the inherent capacity of the genotypes for thrips resistance, which could be attributed by the morphological characters like glossy foliage, compact growth habit, nonspreading of leaves correlates with the high bulb yields reveled by Suhas et al. [18,20] in onion.

#### 4. CONCLUSION

Based on the findings of this present experiment, the genotypes namely RO-1, RO-59, RO-1 × RO-59, RO-1 × Pusa Madhavi, RO-59 × Pusa Madhavi, RO-59 × Kashi No. 1 can further be deployed for commercial cultivation or may be used as parent in onion improvement programmes aimed at developing purple blotch resistant varieties. RO-1, Pusa Shobha, RO-1 × RO-59 and Bhima Kiran × Bhima Shakti were found resistant for thrips. The identified resistant genotypes can further be used for commercial cultivation or may be used as parents in future onion improvement programmes.

#### **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative Al technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of this manuscript.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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