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Screening of onion (*Allium cepa*) genotypes to find out novel resistant source against purple blotch (*Alternaria porri*)

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ABSTRACT

Purple blotch caused by *Alternaria porri* (Ellis) Cif., 1930 causes huge losses to bulb crop and seed crop of onion (*Allium cepa* L.). This is a major disease affecting successful onion production in majority of the onion growing areas. To identify resistant source against purple blotch, 34 onion genotypes with good marketable yield based on field evaluation trial in 2017 and allied species Pusa Soumya were screened under protected conditions during *kharif*, 2018 at insect proof net house facility of ICAR-Indian Agricultural Research Institute, New Delhi. The pure culture of *Alternaria porri*, isolated from infected leaf samples of onion crop raised under natural epiphytotic conditions during *kharif*, 2017. Highest change of disease severity index was observed between 1–2 weeks post inoculation (WPI) while highest disease severity index was recorded at 5 WPI suggesting that preventive measures must be employed as soon as disease symptoms appear in the field. The disease severity index ranged from 0.10–33.33 at 1 WPI, which increased to 9.33–89.33 at 5 WPI. The cultivated allied species Pusa Soumya was identified as novel resistance source with DSI value less than 10.0% at 5 WPI. Four genotypes exhibited moderately susceptible reaction, while 20 genotypes were susceptible and 10 genotypes were highly susceptible. The area under disease progress curve (AUDPC) value ranged from 22.33–240.17. Pusa Soumya had the lowest value and among onion lines Arka Kirtiman was found promising. Three onion genotypes had low relative AUDPC value and can effectively be utilized in resistance breeding programme. The average daily temperature between 20–25°C and relative humidity above 75% was found ideal for disease development.

Keywords: AUDPC, DSI, Purple blotch, Pusa Soumya, rAUDPC

Onion (*Allium cepa* L.) is one of the most important vegetables having year round demand. In India, onion is grown on an area of 1.43 million ha and production is 26.74 million tonnes (NHB Advanced Estimate 2019–20). Onion cultivation is affected by many biotic and abiotic stresses which prevent realization of potential yield at farmers' field. Among biotic stresses, *Alternaria porri* (Ellis) Cif., 1930 a fungus of the Pleosporaceae family has been regarded as a major threat for successful onion cultivation globally (Kareem *et al*. 2012). The disease symptoms appear as water-soaked lesions with white centre and brown to purple edges. As the disease progress, the leaves turn yellow above and below the infection and dark brown to black concentric rings form throughout the lesions. The pathogen breaks the stimulus for bulb initiation and delay bulb formation and maturation (Dar *et al*. 2020). The disease can be observed in almost all the growing stages across different growing season. However, maximum day temperature around 28–32°C with maximum relative humidity above 85% is considered ideal for highest infection (Khamari *et al.* 2017). The loss also varies in different crop growth stages. The loss due to this disease is highest (100%) in seed crop (Abo-Elyousr *et al.* 2014) followed by *kharif* onion bulb crop (87.8%) (Chethana *et al.* 2011) and *rabi* onion bulb crop (25.0%) (Gupta and Pathak 1988). The diseases are mostly controlled by use of chemical fungicides. However, success of chemical control primarily depends on high frequency of spraying. Excess use of chemicals leads to development of pathogen resistant strains and accumulation of residues of harmful chemicals in produce and environment, resulting in risks to environment, health and non-target organisms. Therefore, management of disease through chemicals is not always effective in long run and resistant breeding is the need of the hour to manage the menace of the disease in sustainable manner. Keeping in view the lack of resistance breeding work in onion against purple blotch, the present study was carried out to identify resistant/tolerant source to purple blotch.

MATERIALS AND METHODS

Genotypes, their source and description: An experiment

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Fig 1 (A) Purple blotch infected leaves.

Fig 1 (B) Single spore of *Alternaria porri.*

was conducted at Research Farm of ICAR-Indian Agricultural Research Institute, New Delhi. The experimental material consisted of 8 varieties from Directorate of Onion and Garlic Research, Pune, Maharashtra; 4 varieties from Indian Institute of Horticultural Research, Bengaluru, Karnataka; 5 varieties, 18 breeding lines and one allied species *Allium fistulosum* cv. Pusa Soumya from Indian Agricultural Research Institute, New Delhi. The pure inoculum of *Alternaria porri* for challenge inoculation was isolated from infected leaf samples of onion crop consisted of some susceptible genotypes raised at field under natural epiphytotic conditions during rainy (*kharif*) season of 2017. During *kharif* 2018, total 35 onion genotypes were sown in insect proof net house (40 mesh) at ICAR-Indian Agricultural Research Institute, New Delhi and screened by challenge inoculation using pure inoculum of *Alternaria porri*.

Pathogen: The pathogen was isolated from infected leaf samples of *kharif* 2017 (Fig 1A) through single spore culture method (Fig 1 B). The identity of the pathogen was established through typical morphological features viewed under compound microscope (40X magnification). The pathogen was cultured in potato dextrose agar medium and incubated at 28°C in BOD incubator. 2–3 days after sub-culturing, sterile distilled water (approximately 10 ml) was poured on the fungus colonies and agitated with sterile glass rod and filtered with two layers of sterilized muslin clothes and volume was made up to 50 ml with autoclaved and cooled distilled water. The spore counting was done and a spore concentration of 10^5/ml was used for challenge inoculation purpose.

Challenge inoculation: The genotypes were sown in insect proof net house at Division of Vegetables Science, ICAR-Indian Agricultural Research Institute, New Delhi. The genotypes were evaluated under randomized block design. Each genotype had 3 replications, each replication consisted of 3 rows of plant and in each row 8 plants were maintained after thinning. The healthy growth of the seedlings was ensured by nutrition and irrigation. The spore suspension (1×10^5 propagules/ml) was sprayed along with stickers 5 ml/L to ensure effective dissemination of the spore to the leaf surface during evening hours. Following inoculation, the irrigation channels were filled to field capacity and the net house was covered with transparent polythene sheet to maintain high humidity (>85%) for initial 48 h. The weekly average temperature and humidity for the experimentation period is presented in Fig 2.

Disease scoring: The disease symptoms were assessed on 5 randomly selected plants in each genotype per replication. The plants were tagged for recording of

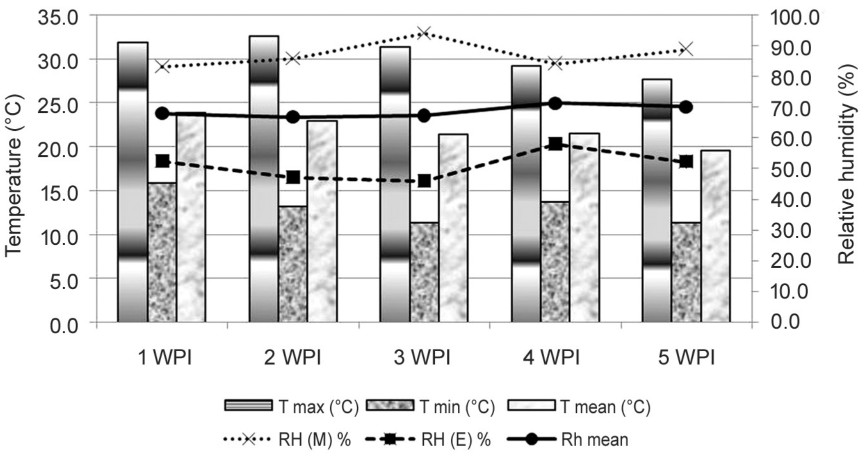

Fig 2 Temperature and humidity during experimentation period (WPI, Weeks post inoculation).

subsequent disease progress to avoid individual plant variation in subsequent disease recording. Individual plant rating was done on a 0–5 scale as suggested by Sharma (1986). The rating correspond to percent leaf infection, where '0' correspond to no visible disease symptoms. The subsequent higher grades, namely '1', '2', '3' and '4' correspond to leaf infection symptoms covering less than 10%, 20%, 40% and 75% leaf area. The rating '5' correspond to the most severe symptom which manifested as complete drying of the leaves or breaking of the leaves. Following individual plant scoring, disease severity index (DSI) on a percentage basis was calculated following Chiang *et al.* (2017) where:

disease severity index on a percentage basis DSI (%) = [sum (class frequency × score of rating class)]/[(total number of plants) × (maximal disease index)] × 100

Based on DSI value, the genotypes were categorized into 6 categories, namely immune (DSI = <5%); resistant (DSI = 5–10%); moderately resistant (DSI = 11–20%); moderately susceptible (DSI = 21–40%); susceptible (DSI = 41–60%) and highly susceptible (DSI = >61%) (Pathak *et al*. 1986).

The disease intensity over time as a result of interaction of host, pathogen and the biological and physical environment was measured by calculating the area under disease progress curve (AUDPC) following midpoint (Trapezoidal) rule (Madden *et al*. 2007).

$$AUDPC=\sum_{i=1}^{n-1}(y_i+y_{i+1})/2\times(t_{1+1}-t_i)$$

where y_i is an assessment of a disease (percentage) at the i^{th} observation, t_i is time (in weeks) at the i^{th} observation and n is the total number of observations.

The relative AUDPC (rAUDPC) of individual line was calculated as percentage of the mean of theoretical maximum AUDPC value (Feng *et al*. 2018). AUDPC and r AUDPC value were estimated using excel based calculator (Simko 2021).

Data analysis: The differences among genotypes for DSI value, AUDPC value and rAUDPC value were analyzed through ANOVA following Panse and Sukhatme (1967) using SPAR -2.0.

RESULTS AND DISCUSSION

The genotypic variations with respect to disease severity index became significant from one weeks post inoculation (Table 1). The average disease severity index at 1 WPI was 25.90% and it ranged from 0.0 (Pusa Soumya) to 33.33% (Bhima Shakti). Among onion genotypes, 3 genotypes namely Bhima Super, 2017 KhSel-1 and 2017KhBSel-17 were found moderately resistant with DSI value 20.0% and the remaining 31 genotypes exhibited moderately susceptible reaction. At 2 weeks post inoculation, the temperature was above 30°C and maximum humidity above 90% and a steep progress in DSI was observed. The average DSI (%) of the genotypes (51.85%) represented susceptible reaction. Three onion genotypes namely Bhima Red (34.67%), Arka Kirtiman (37.53%) and Bhima Super (40.0%) had least disease infection and exhibited moderately susceptible reaction and 1 genotype, 2017 KhB Sel-11 exhibited highly susceptible reaction with DSI value of 64.0. The *Allium fistulosum* genotype Pusa Soumya exhibited resistant reaction with DSI value of 9.33. Between 2–3 WPI there were slight recoveries in disease severity of 21 genotypes. This resulted in reduction in average DSI from 51.85 to 50.78%. However, at 3 WPI, the range of DSI (10.67–74.67%) was higher compared to DSI of 2 WPI (9.33–64.0%). At 3 WPI, Pusa Soumya exhibited moderately resistant reaction with DSI value of 10.67%. Five onion genotypes (Arka Kirtiman, Arka Niketan, Bhima Shakti, 2017 KhB Sel-15 and 2017 KhB Sel-17) were categorized as moderately susceptible and 9 genotypes exhibited highly susceptible reaction with DSI value above 61%. Pusa Soumya exhibited highest DSI value (20.0%) at 4 WPI. There was disease symptom recovery in Pusa Soumya post 4 WPI and at 5 WPI the DSI was 9.33%. The disease severity index (%) of onion genotypes ranged from 37.3–88.0% at 4 WPI, which increased to 38.67–89.33% at 5 WPI. The average disease severity index was above 60% both at 4 WPI and 5 WPI suggesting highly susceptible reaction. The categorization of genotypes based on DSI (%) value at different time intervals post inoculation is presented in Fig 3. The analysis of disease severity index over time suggested that at 1 WPI most of the genotypes exhibited moderately susceptible reaction while at subsequent observations, majority of the genotypes exhibited susceptible reaction. In the present study we observed symptom development and disease progress in all the genotypes upon challenge inoculation with pure culture under high temperature and humidity condition. The plant defense system was activated upon inoculation and symptom recovery was observed between 2–3 WPI. However, the pathogen overpowered the resistance mechanism in plants and symptom development progressed at faster rate between 3–5 WPI. This suggests that plant age negatively correlates with purple blotch resistance and the growers should follow preventive as well as control measures to prevent disease progress and subsequent yield losses. The weekly progress of purple blotch disease symptom development was reported by Islam *et al.* (2020) showed highest disease index of 76.12% which is less than the present study. We observed varietal differences in disease response and could not locate resistant sources against purple blotch in the available onion genotypes. Four genotypes namely Bhima Red Bhima Super, Arka Kirtiman and Arka Niketan (Table 1) were found moderately susceptible while the other fell in susceptible (20) and highly susceptible (10) category. Onion is affected by many diseases under field condition which reduce the yield and increases the cost of cultivation due to plant protection chemicals. Genetic resistance is the best method to control the disease menace in economical and sustainable manner. Onion is a highly cross-pollinated crop and exhibits high genetic variability for most of the traits. Alves *et al.* (2018) reported varietal differences in 46 onion genotypes against downy mildew caused by *Peronospora*

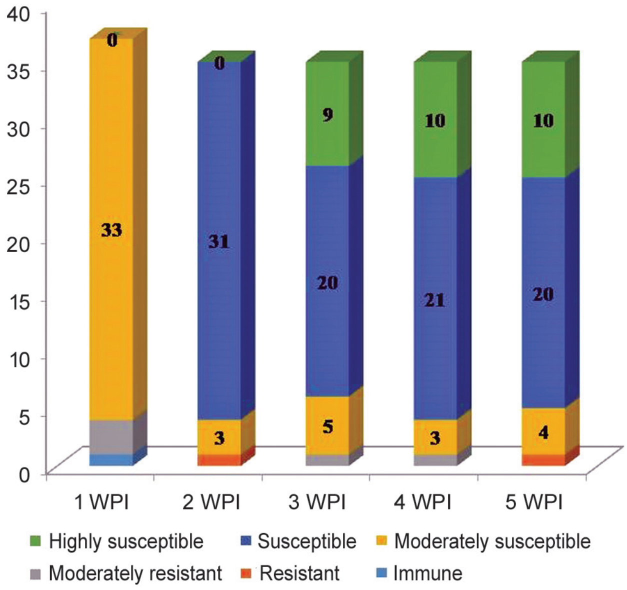

Fig 3 Categorization of genotypes based on DSI (%) value.

destructor under natural epiphytotic condition and located resistance source in the available germplasm.

Dangi *et al*. (2019) reported genotypic differences for Stemphyllium blight tolerance in onion genotypes and observed Pusa Soumya possessing resistance. Behera *et al.* (2013), Nanda *et al.* (2016) and Chand *et al.* (2018) reported varietal differences in disease response against purple blotch and stemphyllium blight and observed resistant/moderately resistant genotypes in onion accessions, which is contrary to our findings, however, our results support the findings of Yadav *et al.* (2017). They reported moderately susceptible reaction as the initial level of resistance in onion accessions. We found Pusa Soumya as a novel source of resistance against purple blotch. Dangi *et al.* (2019) reported Pusa Soumya resistant against Stemphyllium blight. Therefore, Pusa Soumya is a suitable donor for resistance trait to major diseases affecting onion production. Kudryavtseva *et al*. (2019) reported that *Allium fistulosum* accessions possess numerous resistances that are not found in the bulb onion (*Allium cepa* L.). They made interspecific hybrids between *A. cepa* and *A. fistulosum* and observed that advanced generations of the hybrid posses' resistance against Stemphylium leaf blight (SLB). Besides locating resistant source against purple blotch, the study also confirmed that purple blotch disease progress faster at average temperature of 20–25°C (Islam *et al.* (2020).

The temporal increase of disease was quantified through disease progress curve (AUDPC) and it ranged from 22.33–240.17 with a mean value of 148.58. The lowest value was recorded in the resistant genotype Pusa Soumya while highest was recorded in 2017 KhBSel.-3- a genotype selected for *kharif* onion breeding. The genotype 2017 KhBSel.-4, which had highest DSI (%) at 5 WPI had AUDPC value of 231.67. Among the three moderately susceptible genotypes, Arka Kirtiman had the lowest disease progress followed by Bhima Red. Arka Kirtiman is a CGMS based hybrid and we can consider that hybrids may give better protection against purple blotch as Chand *et al*. (2018) suggested that purple blotch resistance is controlled by single dominant gene. The majority of the genotypes (16) had AUDPC value between 125–150 and based on DSI, they were categorized as susceptible with DSI value above 60%. Three genotypes namely Arka Kirtiman, Arka Niketan and Bhima Red had AUDPC value between 75–100 suggesting least disease progress in these genotypes and can be utilized for enriching the North Indian genotypes with respect to resistance trait against purple blotch. Among the North Indian genotypes, Pusa Madhavi had the slowest disease progress with AUDPC value of 124.33 and among the breeding lines 2017 KhB Sel.-17 had the slowest disease development. However, based on DSI (%) value, they were considered susceptible. As the disease development was low, in these two genotypes, they can be effectively utilized for resistant breeding programme. The theoretical maximum AUDPC value was calculated as 400 and the rAUDPC value of the genotypes ranged from 0.06 to 0.60. The lower values of the genotypes suggest their fold superiority over the susceptible lines. The relative superiority of DOGR, Pune genotypes ranged from 0.23 to 0.54. Three genotypes namely Bhima Red, Bhima Super and Bhima Shweta was found promising based on rAUDPC value. Among the IIHR, Bengaluru genotypes, Arka Kirtiman was found most promising with lowest rAUDPC value (0.20). The rAUDPC value ranged from 0.31–0.57, in IARI, New Delhi released onion varieties while in breeding lines it ranged from 0.27–0.60. The breeding line 2017 KhB Sel-17 was found most promising with low rAUDPC value (0.27).

In onion resistance breeding programme is lagging behind. It may be partly due to diverse pathogens affecting onion production across different geographical regions and it is very difficult to find resistance sources in single or few genotypes across all major pathogens. Therefore, regular screening of the germplasm resources is needed to identify novel resistance/tolerance source and integrate them into resistance breeding programme. In this study, we identified novel resistance source against purple blotch in Pusa Soumya which can be used to introgress the disease resistance traits in onion genotypes. Earlier *Allium roylei* was used as bridge species in crossing work between *Allium fistulosum* and *Allium cepa.* However, we received success in direct hybridization work between Pusa Soumya and onion variety Pusa Riddhi and this can be advanced in introgression of purple blotch resistance trait from Pusa Soumya to onion varieties. We studied disease reaction differences across onion genotypes collected from different geographical regions of India and observed that onion varieties from South India performed better in terms of disease resistance against purple blotch. We identified onion lines with lower rate of disease progress (Arka Kirtiman, Bhima Red, 2017 KhB Sel-17) and they can be utilized in imparting resistance against *Alternaria porri* in onion genotypes. The study also confirmed that allied species (*A. fistulosum*) posses desirable traits and must be explored for

Table 1 Average DSI (%), AUDPC and rAUDPC of the genotypes

Genotype	DSI (%)					AUDPC	rAUDPC
	1 WPI	2 WPI	3 WPI	4 WPI	5 WPI		
Pusa Soumya	0.10	9.33	10.67	20.00	9.33	22.33	0.06
Bhima Shakti	33.33	48.00	40.00	60.00	60.00	131.33	0.32
Bhima Raj	32.00	54.67	45.33	56.00	60.00	139.17	0.35
Bhima Dark Red	28.00	54.67	48.00	54.67	56.00	135.0	0.34
Bhima Super	20.00	40.00	50.67	45.33	38.67	105.0	0.26
Bhima Red	22.67	34.67	52.00	40.00	38.67	93.33	0.23
Bhima Kiran	30.67	56.00	46.67	56.00	54.67	130.0	0.33
Bhima Safed	29.33	57.33	45.33	58.67	57.33	146.0	0.37
Bhima Shweta	25.33	52.00	45.33	45.33	45.33	110.83	0.28
Bhima Shubhra	28.00	60.00	70.67	77.33	84.00	216.0	0.54
Arka Pragati	32.00	56.00	48.00	56.00	60.00	138.33	0.35
Arka Kirtiman	25.33	37.33	30.67	37.33	38.67	79.5	0.20
Arka Niketan	25.33	49.33	34.67	40.00	40.00	94.5	0.24
Arka Lalima	28.00	56.00	42.67	57.33	57.33	139.67	0.35
Early Grano	21.33	52.00	41.33	60.00	60.00	134.0	0.34
Sel-153-1	32.00	52.00	46.67	60.00	60.00	138.5	0.35
Pusa Red	29.33	60.00	66.67	73.33	81.33	205.33	0.51
Pusa Madhvi	22.67	52.00	46.67	52.00	56.00	124.33	0.31
Pusa Shobha	28.00	60.00	74.67	78.67	85.33	227.17	0.57
Pusa Riddhi	32.00	60.00	74.67	80.00	84.00	229.33	0.57
2017 Kh onion Sel-1	20.00	54.67	41.33	58.67	58.67	128.67	0.32
2017 Kh Sel-2	25.33	54.67	48.00	52.00	52.00	135.83	0.34
2018 KPBS-50	22.67	56.00	42.67	53.33	56.00	125.0	0.31
2017 KhB Sel-3	25.33	60.00	74.67	88.00	88.00	240.17	0.60
2017 KhB Sel-4	28.00	60.00	74.67	80.00	89.33	231.67	0.58
2017 KhB Sel-10	25.33	53.33	65.33	76.00	82.67	192.83	0.48
2017 KhB Sel-11	25.33	64.00	44.00	60.00	60.00	142.50	0.36
2017 KhB Sel-13	21.33	52.00	44.00	60.00	60.00	130.0	0.33
2017 KhB Sel-15	30.67	54.67	40.00	57.33	52.00	129.67	0.32
2017 KhB Sel-17	20.00	45.33	40.00	52.00	50.67	107.17	0.27
2017 KhB Sel-19	28.00	56.00	68.00	76.00	88.00	208.17	0.52
2017 KhB Sel-20	29.33	49.33	61.33	76.00	84.00	192.33	0.48
2017 KhB Sel-21	28.00	56.00	73.33	84.00	86.67	229.0	0.57
2017 KhB Sel-23	26.67	41.33	54.67	57.33	58.67	129.83	0.32
2017 KhB Sel-25	25.33	56.00	44.00	58.67	52.00	137.67	0.34
Mean	25.91	51.85	50.78	59.92	61.30	240.17	0.60
Range	0.10 - 33.33	9.33 - 64.0	10.67 - 74.67	20.0 - 88.0	9.33 - 89.33	22.33 - 240.17	0.056 - 0.60
P value	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00

genetic improvement programme particularly in resistance breeding.

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