Identification of Novel Source of Resistance and Differential Response of *Allium* Genotypes to Purple Blotch Pathogen, *Alternaria porri* (Ellis) Ciferri

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Purple blotch, caused by *Alternaria porri* (Ellis) Ciferri, is a serious disease incurring heavy yield losses in the bulb and seed crop of onion and garlic worldwide. There is an immediate need for identification of effective resistance sources for use in host resistance breeding. A total of 43 *Allium* genotypes were screened for purple blotch resistance under field conditions. *Allium cepa* accession ‘CBT-Ac77’ and cultivar ‘Arka Kalyan’ were observed to be highly resistant. *In vitro* inoculation of a selected set of genotypes with *A. porri*, revealed that 7 days after inoculation was suitable to observe the disease severity. *In vitro* screening of 43 genotypes for resistance to *A. porri* revealed two resistant lines. An additional 14 genotypes showed consistent moderate resistance in the field as well as *in vitro* evaluations. Among the related *Allium* species, *A. schoenoprasum* and *A. roylei* showed the least disease index and can be used for interspecific hybridization with cultivated onion. Differential reaction analysis of three *A. porri* isolates (*Apo-Chiplima, Apn-Nasik, Apg-Guntur*) in 43 genotypes revealed significant variation among the evaluated *Allium* species (*P = 0.001*). All together, the present study suggest that, the newly identified resistance sources can be used as potential donors for ongoing purple blotch resistance breeding program in India.

**Keywords**: *Allium* spp., *Alternaria porri*, purple blotch

Onion (*Allium cepa* L.) of the family Alliaceae is a high value spice cum bulbous vegetable crop with large commercial and medicinal significance. It is attributed to diverse medicinal properties and often used in the treatment of influenza, measles, chicken pox, cardiovascular disorders etc (Smith et al., 2003). Onion contains a lachrymatic agent, quercitin which, along with other phytochemicals such as flavonol, exhibit bactericidal, anti-cancer and antioxidant activities (Corzo-martinez et al., 2007; Javadzadeh et al., 2009). India is the second largest producer of onion in the world with an annual output of 6.50 million tonnes from an area of 0.52 million ha (FAOSTAT, 2014). Garlic (*Allium sativum* L.) is the second most important bulb crop after the onion and have significant importance for its culinary and medicinal properties. The presence of allicin, an important organosulphur compound and other essential oils in the bulbs and leaves of garlic makes it a traditional stimulant, diuretic, diaphoretic and expectorant (Smith et al., 2003). Although, India has the largest area under onion and garlic cultivation, their productivity is very low (only 10.16 t/ha for onion and 4.32 t/ha for garlic) as compared to global productivity (FAOSTAT, 2014). Various elements lead to the low productivity of onion and garlic, the most striking being the diseases caused by phytopathogens. Both onion and garlic are equally susceptible to numerous foliar, root and bulb pathogens that tremendously reduce the yield and quality of the crops (Cramer, 2000).
Purple blotch, caused by *Alternaria porri* (Ellis) Cifferi is the most destructive foliar disease, prevalent in all *Allium* growing countries of the world (Kareem et al., 2012). It is responsible for causing severe yield losses ranging from 2.5% to 97% in both the bulb and seed crop (Gupta and Pathak, 1998; Lakra, 1999). Under favourable condition, the pathogen develops brownish-purple necrotic lesions in the leaf tissues which breaks the stimulus for bulb initiation, thereby delaying bulb formation and maturation (Black et al., 2012). Severe attack on flowering *Alliums* causes complete girdling of the flower stalks with necrotic tissues, leading to their collapse and loss of seed production capacity. Bulbs are infected through the neck by forming a bright yellow to red infected area leading to complete drying and decay of the bulb scales (Black et al., 2012).

Purple blotch control often involves frequent application of mencozeb, propineb and difenconazole fungicides (Chethana et al., 2012; Priya et al., 2015). However, this is mostly time consuming, costly and often ineffective due to the emergence of resistant races of the pathogen. Biological control of purple blotch by inoculation of antagonistic fungi and bacteria isolated from suppressive soils has been considered as an alternative approach to the use of fungicides (Prakasham and Sharma, 2012). Nevertheless, this method is not fully effective and only have a minimal contribution towards purple blotch management. Genetic engineering could be an option towards purple blotch resistance in onion (Eady et al., 2003), but the industry and the consumers do not currently accept such plant varieties. In these circumstances, host resistance breeding could be the most effective way to control purple blotch disease. However, there is only a limited source of naturally available host plants that exhibit resistance against purple blotch. A few onion lines have been identified that exhibit resistance or moderate resistance to purple blotch in field screening conditions (Behera et al., 2013; Kale and Ajjappalavara, 2014; Tripathy et al., 2013). Yet, most of these lines exhibit only partial reduction in infection in bulb and seed crop and haven’t been commercialized as purple blotch resistance sources. Further, there is no report on the resistance effect of any related wild *Allium* species that can be used as a resistance source through interspecific hybridization in purple blotch resistant breeding program. Thus, it is essential to identify more effective resistance sources to solve the economically important purple blotch problem in cultivated *Alliums*. Moreover, there is no report on the pathotype variations within *A. porri* exhibiting differential reactions with *Allium* genotypes in targeted area which could be fundamental in managing the purple blotch problem. In the present study, we screened a selected set of onion, garlic and related *Allium* species towards identification of novel purple blotch resistance sources and differential reactions on *Allium* genotypes by three pathotypes of *A. porri*.

### Materials and Methods

#### Plant material.
Forty-three *Allium* genotypes (26 *A. cepa*, 7 *A. sativum*, 4 *A. tuberosum*, 1 *A. ampeloprasum*, 1 *A. schoenoprasum*, 1 *A. roylei*, 1 *A. fistulosum*, 1 *A. clarkei*, and 1 *A. griffithianum*) including landraces, cultivated varieties, wild genotypes and hybrids were screened against purple blotch disease under field and controlled conditions during September 2014 to March 2015 (Table 1).

#### Screening for purple blotch resistance under field conditions.
The field screening experiment was carried out at the research farm of Centre of Biotechnology, Siksha O Anusandhan University, Bhubaneswar, Odisha, located at 85° 49’ 28.3440” E and 20° 17’ 45.8124” N at an altitude of 57.11 m from sea level in eastern India. Eight weeks old seedlings were transplanted into plots with sizes of 3 × 2 m and spacing of 15 × 10 cm. Genotypes were grown in randomized block design in three replications with 20 plants per replication. The screening was done under normal epiphytotic conditions through natural infestation of the pathogen. Susceptible line Agrifound Rose was planted all around the experimental plot to ensure spread of purple blotch in the main field. Disease intensity was assessed 60 days after transplantation. Based on typical symptoms expressed and spore characteristics of *A. porri*, the number of infected and non-infected plants were calculated to determine the percentage of infection. An empirical scale consisting of 5 classes of intensity (Sharma, 1986) was employed to determine the disease severity when the disease was developed to its maximum extent. Based on the observed infection percentage of leaf area, the plants were categorized in different disease reaction groups and assigned 0 = no disease symptom, 1 = a few tip spots with 10% infected leaf area, 2 = purplish brown patches with 20% infected leaf area, 3 = multiple merging patches with 40% infected leaf area, 4 = streaking leaves with 75% infected leaf area, and 5 = complete drying and breakage of leaves. The percent disease index (PDI) was calculated by dividing the sum of the individual numerical rating with total observations, multiplied by the maximum disease rating scale and expressed in percentage (Wheeler, 1969). On the basis of PDI values, genotypes were classified into six disease reactions; immune (I, < 5), resistant (R, 5.1–10), moderately resistant (MR, 10.1–20), moderately susceptible (MS, 20.1–40), susceptible (S, 40.1–60), and highly susceptible (HS, > 60) (Pathak et al., 1986).
### Table 1. Evaluation of *Allium* genotypes for purple blotch resistance under field and controlled conditions

| Genotype            | Observation under field condition | Observation under controlled condition |
|---------------------|----------------------------------|----------------------------------------|
|                     | PDI* Disease index score Reaction | PDI* Disease index score Reaction |
| CBTAc11 (a)         | 23.61<sup>a</sup> 3 MS          | 26.22<sup>i</sup> 3 MS                |
| CBTAc17 (a)         | 32.54<sup>n</sup> 3 MS          | 37.32<sup>m</sup> 3 MS                |
| CBTAc46 (a)         | 63.21<sup>b</sup> 4 HS          | 64.21<sup>b</sup> 4 HS                |
| CBTAc77 (a)         | 9.23<sup>e</sup> 1 R            | 9.83<sup>e</sup> 1 R                 |
| CBTAc96 (a)         | 17.21<sup>j</sup> 2 MR          | 18.89<sup>a</sup> 2 MR                |
| CBTAc128 (a)        | 14.78<sup>h</sup> 2 MR          | 16.37<sup>+</sup> 2 MR                |
| CBTAc132 (a)        | 19.54<sup>y</sup> 2 MR          | 24.56<sup>y</sup> 3 MS                |
| CBTAc156 (a)        | 31.73<sup>mn</sup> 3 MS         | 33.62<sup>mn</sup> 3 MS               |
| CBTAc169 (a)        | 17.21<sup>s</sup> 2 MR          | 18.89<sup>s</sup> 2 MR                |
| CBTAc176 (a)        | 43.81<sup>i</sup> 4 S           | 45.59<sup>a</sup> 4 S                 |
| CBTAc181 (a)        | 43.81<sup>i</sup> 4 S           | 46.81<sup>j</sup> 4 S                 |
| CBTAc203 (a)        | 16.89<sup>+</sup> 2 MR          | 17.99<sup>i</sup> 2 MR                |
| CBTAc211 (a)        | 61.31<sup>s</sup> 3 MS          | 63.27<sup>s</sup> 3 MS                |
| CBTAc218 (a)        | 57.93<sup>s</sup> 4 S           | 63.27<sup>s</sup> 4 HS                |
| CBTAc132 (a)        | 53.56<sup>e</sup> 4 S           | 56.19<sup>e</sup> 4 S                 |
| CBTAc176 (a)        | 51.82<sup>+</sup> 4 S           | 66.13<sup>d</sup> 4 HS                |
| CBT-Ac178 (b)       | 47.31<sup>i</sup> 4 S           | 38.22<sup>i</sup> 3 MS                |
| CBT-Ac203 (b)       | 53.56<sup>e</sup> 4 S           | 56.19<sup>e</sup> 4 S                 |
| NHRDF Red (a)       | 49.61<sup>+</sup> 4 S           | 50.16<sup>s</sup> 4 S                 |
| NHRDF Red 2 (a)     | 47.31<sup>i</sup> 4 S           | 48.32<sup>i</sup> 4 S                 |
| Agrifound Rose (a)  | 65.67<sup>+</sup> 4 HS          | 62.54<sup>+</sup> 4 HS                |
| Agrifound dark red (a) | 51.82<sup>+</sup> 4 S         | 52.38<sup>f</sup> 4 S                |
| Agrifound White (a) | 53.56<sup>e</sup> 4 S           | 56.19<sup>e</sup> 4 S                 |
| Bhima Super (a)     | 32.54<sup>n</sup> 3 MS          | 27.83<sup>p</sup> 3 MS                |
| Bhima Shakti (a)    | 25.87<sup>p</sup> 3 MS          | 26.22<sup>+</sup> 3 MS                |
| Arka Kirtiman (a)   | 16.89<sup>+</sup> 2 MR          | 18.89<sup>s</sup> 2 MR                |
| Arka Kalyan (a)     | 10.21<sup>+</sup> 1 R           | 9.36<sup>+-</sup> 1 R                 |
| Arka Pitambar (a)   | 15.81<sup>+</sup> 2 MR          | 17.63<sup>+</sup> 2 MR                |
| Arka Lalima (a)     | 14.63<sup>+</sup> 2 MR          | 18.89<sup>s</sup> 2 MR                |
| Arka Niketan (a)    | 14.78<sup>+</sup> 2 MR          | 17.63<sup>+</sup> 2 MR                |
| Yamuna Safed (b)    | 23.61<sup>+</sup> 4 S           | 27.83<sup>p</sup> 3 MS                |
| Bhima Omkar (b)     | 16.89<sup>+</sup> 2 MR          | 18.89<sup>s</sup> 2 MR                |
| IC-353524 (c)       | 41.29<sup>s</sup> 4 S           | 45.59<sup>+</sup> 4 S                 |
| IC-353536 (c)       | 17.21<sup>+</sup> 2 MR          | 17.63<sup>s</sup> 2 MR                |
| IC-353535 (c)       | 21.32<sup>+</sup> 3 MS          | 24.56<sup>s</sup> 3 MS                |
| N-151 (c)           | 14.63<sup>+</sup> 2 MR          | 15.49<sup>s</sup> 2 MR                |
| PI576881 (d)        | 47.31<sup>i</sup> 4 S           | 51.79<sup>s</sup> 4 S                 |
| Pf664902 (e)        | 12.31<sup>+</sup> 2 MR          | 13.47<sup>s</sup> 2 MR                |
| IC-353540 (f)       | 11.68<sup>s</sup> 2 MR          | 12.53<sup>s</sup> 2 MR                |
| NIC 20231 (g)       | 34.27<sup>s</sup> 2 MS          | 45.59<sup>s</sup> 4 S                 |
| IC-383446 (h)       | 59.47<sup>s</sup> 4 S           | 61.63<sup>d</sup> 4 HS                |
| IC-255676 (i)       | 51.82<sup>s</sup> 4 S           | 52.38<sup>f</sup> 4 S                 |

(a), *A. cepa*; (b), *A. sativum*; (c), *A. tuberosum*; (d), *A. ampoloprasum*; (e), *A. schoenoprasum*; (f), *A. roylei*; (g), *A. fistulosum*; (h), *A. clarkei*; (i), *A. griffithianum*; PDI, percent disease index; R, resistance; MR, moderate resistance; MS, moderate susceptible; S, susceptible; HS, highly susceptible.

*Values were arc sine transformed before analysis. In a column, a mean followed by common letters are not significantly different at 5% level by Duncan’s multiple range test.*
Detection and identification of *A. porri*. The single spore isolation method (Hoey et al., 1996) was used for the isolation of the causal organism from the leaf of an infected plant. The isolate was maintained on potato dextrose agar (PDA) medium for 8 days, following which, spore suspension was prepared and sprayed on a fresh host plant. The symptoms observed in the inoculated plant were found similar as detected in the original infected plant. The pathogen was re-isolated and compared with the original isolate and was found to have similar morphological characteristics. Further, the identity of the pathogen isolate was confirmed through PCR amplification using species specific primers (Pavón et al., 2010).

Development of purple blotch progress curve. To establish the progress of purple blotch disease in *Allium* spp., plants of representative genotypes from five disease reaction categories (R, Arka Kalyan; MR, Arka Niketan and CBT-Ac77; MS, Bhima Super; S, NHRDF Red; HS, CBT-Ac211 and Agrifound Rose) were artificially inoculated with *A. porri* isolate *Apo-Chiplima*. In this process, 4 plants were selected for each genotype and the assessment was done for three replications. Plants were allowed to develop for a period of 8 weeks in the pots inside a growth chamber. Before inoculation, the leaves were cleaned with sterilized distilled water, dried using tissue paper and predisposed to 95% humidity for 24 h. Spore suspension of *A. porri* isolate was harvested from a eight day old PDA culture and adjusted to $10^6$ spores/ml. One plant of each genotype was kept as control and inoculated with distilled water. The leaves of the remaining three plants of each genotype were pin-pricked and sprayed until run-off with the spore suspension. The inoculated pots were maintained in a growth chamber with average daily temperature of $25 \pm 1^\circ$C, 12 h photoperiod and relative humidity of 85%. The observation on disease incidence was made by calculating the lesion length along the leaf area as well as the number of lesions per plant at five different stages (0, 2, 5, 7, 9 days after inoculation [DAI]) of disease development.

Screening for purple blotch resistance under controlled conditions. All the 43 *Allium* genotypes used in the field screening were also tested under *in vitro* conditions in a climate controlled green house. The seeds and bulbs (*A. sativum*) were surface sterilized with 10% sodium hypochlorite for 10 min followed by twice submersion and washing with 70% alcohol for 30 s. Seedlings were raised in earthen pots with sterilized soil and transplanted into fresh pots with the same soil substrate 60 days after sowing. The leaves of the plants were cleaned and artificially inoculated with *A. porri* as described previously. Observation on disease severity was recorded at 7 DAI. The specific disease reaction and PDI for each genotype was evaluated as per the method described in the field screening experiment.

Differential response of *Allium* genotypes to *A. porri* isolates. The 43 *Allium* genotypes were artificially inoculated with three virulent isolates of *A. porri* collected from Sambalpur (*Apo-Chiplima*), Nasik (*Apn-Nasik*) and Guntur (*Apg-Guntur*) districts of India and were used to evaluate the differential response of *Allium* genotypes to purple blotch fungal isolates. Disease severity was recorded at 7 DAI and the genotypes were categorized based on the disease reaction as described previously.

Statistical analysis. The statistical analysis was carried out with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The values calculated in percentage (%) were trans-
formed into the arcsine value before analysis. The means were separated by Duncan’s multiple range test \( (P = 0.05) \). The possible two-way interaction between and among the three \( A. porri \) isolates and 43 \( Allium \) genotypes were tested with a general linear model approach. A mean separation test was performed using Fisher’s distribution analysis at \( P = 0.001 \) for \( Allium \) genotypes and \( A. porri \) isolates.

**Results**

**Screening of onion genotypes for \( A. porri \) in field condition.** Among the 43 genotype tested, 26 were \( A. cepa \) genotypes and the majority were categorized into moderately resistant (9) followed by susceptible (7), moderately susceptible (5) and highly susceptible (3) (Table 1). Two genotypes, CBT-Ac77 and Arka Kalyan were found to be resistant against purple blotch. Among the \( A. sativum \) genotypes, three were susceptible, two were moderately susceptible and another two were moderately resistant. Screening of the wild \( Allium \) genotypes revealed that, \( A. schoenoprasum \) and \( A. roylei \) were moderately resistant, \( A. ampeloprasum \), \( A. clarkei \), and \( A. griffithianum \) were susceptible and \( A. fistulosum \) was moderately susceptible.

A two step PCR was performed with primers targeting the \( Alt 1 \) gene to confirm the isolated pathogen from the original host (Pavón et al., 2010). The first duplex PCR resulted in an expected band size of 195 bp which is common in all \( Alternaria \) spp. (Fig. 1A). This was followed by a semi nested PCR performed using species primers, which resulted in the amplification of a 118 bp fragment specific to \( A. porri \) (Fig. 1B).

**Purple blotch disease progress curve under in vitro condition.** A disease progress curve was generated to standardize the minimum time required for making observation on purple blotch disease in control condition. Representative onion genotypes showing resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reactions were artificially inoculated with \( A. porri \) and individual lesion lengths and number of lesions per plants were examined. Results demonstrated that, the disease development started at 2 DAI and progressed subsequently in all the classes (Fig. 2). Although, the disease progression was highest at 9 DAI, it was found at par with 7 DAI. Therefore, 7 DAI was considered more appropriate to observe purple blotch disease progression.

**Screening of \( Allium \) genotypes for \( A. porri \) under controlled condition.** Among the 43 \( Allium \) genotypes, two genotypes were resistant, 14 genotypes were moderately resistant, 10 genotypes were moderately susceptible, 11 genotypes were susceptible, and 6 genotypes were highly susceptible (Table 1). The results were similar with the observations obtained in the field screening experiment.

**Response of \( Allium \) genotypes to different isolates of \( A. porri \).** Disease reaction of 43 genotypes against the three different isolates of \( A. porri \), \( Apo-Chiplima \), \( Apn-Nasik \), and \( Apg-Guntur \) were studied at 7 DAI (Table 2). The three isolates were capable of inducing infection in all the \( Allium \) genotypes. However, CBT-Ac77 (PDI, 8.77) and Arka Kalyan (PDI, 6.83) showed the lowest level of disease infection with \( Apo-Chiplima \) isolate. Similarly, Arka Kirtiman (PDI, 9.42) and CBT-Ac77 (PDI, 9.89) exhibited least infection with \( Apg-Guntur \) isolates. None of the genotypes were resistant to \( Apo-Nasik \) and 16 genotypes

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**Fig. 2.** (A, B) Purple blotch disease progression curve on selected \( Allium \) genotypes with variable response to \( Alternaria porri \). DAI, days after inoculation.
Table 2. Differential reaction of *Allium* genotypes artificially inoculated by three isolates (*Apo-Chiplima, Apn-Nasik, and Apn-Guntur*) of *Alternaria porri*.

| Genotype          | PDI* at 7 DAI |
|-------------------|---------------|
|                   | *Apo* Reaction | *Apn* Reaction | *Apg* Reaction |
| CBT-Ac111 (a)     | 21.61<sup>s</sup> | MS | 26.61<sup>s</sup> | MS | 22.61<sup>s</sup> | MS |
| CBT-Ac171 (a)     | 27.32<sup>s</sup> | MS | 37.32<sup>j</sup> | MS | 39.32<sup>m</sup> | MS |
| CBT-Ac466 (a)     | 64.27<sup>b</sup> | HS | 61.44<sup>k</sup> | HS | 62.45<sup>d</sup> | HS |
| CBT-Ac77 (a)      | 8.71<sup>j</sup> | R  | 12.23<sup>s</sup> | MR | 9.89<sup>ac</sup> | R |
| CBT-Ac96 (a)      | 18.39<sup>j</sup> | MR | 23.06<sup>s</sup> | MS | 24.81<sup>t</sup> | MS |
| CBT-Ac128 (a)     | 17.83<sup>j</sup> | MR | 19.03<sup>f</sup> | MR | 20.61<sup>s</sup> | MS |
| CBT-Ac132 (a)     | 23.54<sup>j</sup> | MS | 18.69<sup>j</sup> | MR | 23.49<sup>j</sup> | MS |
| CBT-Ac156 (a)     | 32.39<sup>j</sup> | MS | 51.29<sup>f</sup> | S  | 53.68<sup>g</sup> | S |
| CBT-Ac169 (a)     | 17.83<sup>j</sup> | MR | 21.12<sup>g</sup> | MS | 23.49<sup>j</sup> | MS |
| CBT-Ac176 (a)     | 46.82<sup>e</sup> | S  | 61.44<sup>k</sup> | HS | 63.71<sup>e</sup> | HS |
| CBT-Ac181 (a)     | 46.82<sup>e</sup> | S  | 38.46<sup>e</sup> | MS | 34.46<sup>e</sup> | MS |
| CBT-Ac203 (a)     | 18.39<sup>j</sup> | MR | 17.48<sup>e</sup> | MR | 27.39<sup>g</sup> | MS |
| CBT-Ac211 (a)     | 63.43<sup>e</sup> | HS | 61.44<sup>k</sup> | HS | 52.79<sup>b</sup> | S |
| CBT-Ac218 (a)     | 61.29<sup>e</sup> | HS | 63.87<sup>c</sup> | HS | 61.33<sup>e</sup> | HS |
| CBT-Ac111 (b)     | 54.28<sup>f</sup> | S  | 46.84<sup>g</sup> | S  | 49.84<sup>i</sup> | S |
| CBT-Ac163 (b)     | 66.41<sup>h</sup> | HS | 63.87<sup>c</sup> | HS | 46.82<sup>k</sup> | S |
| CBT-Ac103 (b)     | 37.89<sup>e</sup> | MS | 18.69<sup>g</sup> | MR | 36.22<sup>e</sup> | MS |
| CBT-Ac153 (b)     | 16.92<sup>e</sup> | MR | 18.69<sup>g</sup> | MR | 18.23<sup>w</sup> | MR |
| CBT-Ac171 (b)     | 31.27<sup>b</sup> | MS | 41.29<sup>b</sup> | S  | 46.82<sup>k</sup> | S |
| NHRDF Red (a)     | 49.86<sup>d</sup> | S  | 48.96<sup>g</sup> | S  | 48.36<sup>e</sup> | S |
| NHRDF Red 2 (a)   | 49.86<sup>d</sup> | S  | 36.28<sup>f</sup> | MS | 43.51<sup>i</sup> | S |
| Agrifound Rose (a)| 63.43<sup>g</sup> | HS | 61.44<sup>f</sup> | HS | 66.17<sup>g</sup> | HS |
| Agrifound dark red (a) | 52.11<sup>g</sup> | S  | 58.15<sup>c</sup> | S  | 62.45<sup>d</sup> | S |
| Agrifound White (a) | 57.83<sup>e</sup> | S  | 33.48<sup>g</sup> | MS | 37.81<sup>e</sup> | MS |
| Bhima Super (a)   | 28.33<sup>e</sup> | MS | 29.38<sup>g</sup> | MS | 19.73<sup>r</sup> | MR |
| Bhima Shakti (a)  | 27.32<sup>e</sup> | MS | 17.48<sup>e</sup> | MR | 19.73<sup>r</sup> | MR |
| Arka Kirtiman (a) | 18.39<sup>j</sup> | MR | 11.47<sup>s</sup> | MR | 9.42<sup>ig</sup> | R |
| Arka Kalyan (a)   | 6.83<sup>j</sup> | R  | 18.69<sup>j</sup> | MR | 13.20<sup>d</sup> | MR |
| Arka Pitambar (a) | 17.83<sup>j</sup> | MR | 19.03<sup>f</sup> | MR | 16.38<sup>g</sup> | MR |
| Arka Lalima (a)   | 16.92<sup>e</sup> | MR | 21.12<sup>e</sup> | MS | 17.89<sup>e</sup> | MR |
| Arka Niketan (a)  | 17.83<sup>j</sup> | MR | 15.78<sup>s</sup> | MR | 12.62<sup>ab</sup> | MR |
| Yamuna Safed (b)  | 28.33<sup>g</sup> | MS | 51.29<sup>g</sup> | S  | 57.23<sup>f</sup> | S |
| Bhima Omkar (b)   | 18.39<sup>j</sup> | MR | 18.69<sup>j</sup> | MR | 31.83<sup>i</sup> | MS |
| IC-353524 (c)     | 44.72<sup>g</sup> | S  | 51.29<sup>g</sup> | S  | 24.81<sup>e</sup> | MS |
| IC-353583 (c)     | 17.83<sup>c</sup> | MR | 23.06<sup>s</sup> | MS | 17.89<sup>e</sup> | MR |
| IC-353535 (c)     | 23.54<sup>g</sup> | MS | 18.69<sup>j</sup> | MR | 31.83<sup>q</sup> | MS |
| N-151 (c)         | 15.53<sup>s</sup> | MR | 18.69<sup>j</sup> | MR | 16.38<sup>g</sup> | MR |
| PI576881 (d)      | 52.11<sup>g</sup> | S  | 27.21<sup>s</sup> | MS | 34.46<sup>e</sup> | MS |
| PI664902 (e)      | 13.39<sup>j</sup> | MR | 17.48<sup>e</sup> | MR | 18.23<sup>w</sup> | MR |
| IC-353540 (f)     | 10.73<sup>e</sup> | MR | 14.92<sup>s</sup> | MR | 12.62<sup>ab</sup> | MR |
| NIC-20231 (g)     | 44.72<sup>g</sup> | S  | 48.96<sup>g</sup> | S  | 63.71<sup>e</sup> | HS |
| IC-383446 (h)     | 61.29<sup>g</sup> | HS | 53.28<sup>e</sup> | S  | 57.23<sup>f</sup> | S |
| IC-255676 (i)     | 52.11<sup>g</sup> | S  | 61.44<sup>h</sup> | HS | 64.13<sup>b</sup> | HS |

(a), *A. cepa*; (b), *A. sativum*; (c), *A. tuberosum*; (d), *A. ameloprasum*; (e), *A. schoenoprasum*; (f), *A. roylei*; (g), *A. fistulosum*; (h), *A. clarkei*; (i), *A. griffithianum*; PDI, percent disease index; DAI, days after inoculation; R, resistance; MR, moderate resistance; MS, moderate susceptible; S, susceptible; HS, highly susceptible.

*Values were arcsine transformed before analysis. In a column, a mean followed by common letters are not significantly different at 5% level by Duncan’s multiple range test.*
were found moderately resistant including CBT-Ac77 (PDI, 12.23), Arka Kalyan (PDI, 18.49), and Arka kirtiman (PDI, 11.47). Among the 43 genotypes, thirty one showed similar reactions against the three isolates. The remaining twelve (CBT-Ac96, CBT-Ac128, CBT-Ac132, CBT-Ac169, CBT-Ac203, CBT-As103, Bhima Shakti, Arka Lalima, Bhima Super, Bhima Omkar, IC-353536, and IC-353535) demonstrated qualitative differences in their reaction to the three isolates (Table 2). For example, CBT-Ac96 and CBT-Ac169 were moderately resistant to Apo-Chiplima isolate, but moderately susceptible to Apn-Nasik and Apg-Guntur isolates. Likewise, CBT-Ac128, CBT-Ac203, and Bhima Omkar genotypes were moderately susceptible to Apg-Guntur but moderately resistant to Apo-Chiplima and Apn-Nasik isolates (Table 2).

Statistical analyses revealed that, the three A. porri isolates have no significant variation over the disease index scores. Nevertheless, a significant variation in the level of disease severity was noted in the overall dispersion of plants in all Allium accessions (Table 3).

**Discussion**

In our effort to identify resistance sources against purple blotch disease, 43 Allium genotypes were screened under artificial and field conditions. The PDI under field conditions indicated that among the genotypes, 4.6% were resistant, 32.5% were moderately resistant, 32.5% were susceptible, 20.9% were moderately susceptible, and 6.9% were highly susceptible. None of the 43 varieties screened for purple blotch were free from disease. Hence, no variety could be admitted in the category I (immune; < 5 PDI). The highest purple blotch disease severity was recorded for Agrifound Rose (65.6%) indicating very high disease pressure. Our results are in agreement with previous reports which demonstrated that, only a few lines are resistant while the majority have moderate resistance to purple blotch under natural infestation in open field condition (Pathak et al., 1986). Sugha et al. (1992) evaluated 94 onion genotypes under natural conditions and designated just two varieties, IC39178 and IC49371 as resistant to purple blotch. In the same context, Behera et al. (2013) observed VG-18 cultivar as resistant and another 12 lines as moderately resistant to purple blotch. The specific disease reaction of different genotypes against purple blotch as evident from this survey could be highly useful for researchers in disease forecasting and pest management programs.

Although, the field screening results provided us with different genotypes with variable response to purple blotch, it is required to be further confirmed through artificial inoculation. Based on the disease progress curve, a quantitative variation for infection was observed in all the genotypes except Arka Kalyan by 7 DAI. Thus, it was concluded that, 7 DAI was the most desirable time to observe disease severity. The artificial inoculation with Apo-Chiplima isolate (A. porri) resulted in infection and lesion development in all 43 genotypes (Table 1) and a very high percentage of genotypes (48.8%) were classified as susceptible and moderately susceptible. The disease reaction assigned to 41 genotypes based on artificial inoculation correlated with the reactions based on field screening except for six genotypes. Four of them were susceptible (CBT-Ac218, CBT-As63, CBT-As103, IC-383446), one moderately susceptible (NIC 20231) and one moderately resistant (CBT-Ac132) under field screening, but showed highly susceptible, susceptible and moderately susceptible after inoculation study, respectively (Table 1). This indicates that, the field resistance category may break in controlled condition under heavy disease pressure. Therefore, screening should be carried out under both artificial and natural conditions before assigning disease categories to the genotypes. Additionally, it is also possible that, the Apo-Chiplima isolate of A. porri could be highly virulent as compared to the isolate(s) of the pathogen in the field. Therefore, a detailed investigation of the pathogenic variants that are prevalent in the region using molecular approaches could be useful in facilitating purple blotch host resistance breeding.

Inoculation with a virulent isolate or high concentration of the inoculum often result in the shifting of disease severity from resistant to susceptible category during arti-

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**Table 3. Analysis of variance for the distribution over percentage of infection by three Alternaria porri isolates**

| Sources of variation | df | Sum of squares | Mean squares | F  |
|----------------------|----|----------------|--------------|----|
| Between A. porri isolates | 2  | 35.49          | 17.75        | 0.38* |
| Between Allium accessions | 42 | 37949.08       | 903.55       | 19.56 |
| Residual             | 84 | 3880.93        | 46.20        |     |
| Total                | 128| 41865.50       |              |     |

*Significant at 1% level.
ficial inoculation (Garg et al., 2013). However, two genotypes (CBT-Ac77 and Arka-Kalyan) showed resistant reaction for both field and in vitro inoculation. CBT-Ac77 is a land race collected from Koraput, Odisha (Rout et al., 2015) while Arka Kalyan is an improved line developed through vigorous mass selection of IHR-145 bulbs at Indian Institute of Horticulture research, Bangalore, India (IHR Annual Report, 1995). Previous reports have demonstrated that, Arka Kalyan exhibits moderate resistance to purple blotch (Ambresh and Gowda, 2013; Chethana et al., 2011). In addition to this, three wild genotypes (A. schoenoprasum (PI 664902), A. roylei (IC-353540), and A. tuberosum (IC-353536) also had the lowest level of infection in both field and in vitro conditions. These three wild genotypes were also resistant to Fusarium oxysporum f. sp cepae (Rout et al., 2015), thereby acting as resistance sources for two important fungal diseases in Alliums. Taken together, it is essential that, these few purple blotch resistant lines are properly listed in their usage in resistant selection breeding programs and integrated pest management packages.

Pathotype variation within the species is critical to the virulence of a pathogen (Pariaud et al., 2009). Pathogens may adapt to their compatible host under experimental field and natural conditions resulting in the change of their virulence. Thus far, there has been no report on the existence of different strains of A. porri with different degrees of virulence. However, it is apparent from the present study that, the host response of the three A. porri isolates varied among the Allium genotypes. Therefore, it is likely that Apo-Chiplima, Apg-Guntur, and Apn-Nasik isolates are three different pathotypes of A. porri. Further research on the genetic characterization of A. porri isolates will provide more insights into the evolutionary history and the pathogenic nature of this important phytopathogen.

Overall, none of the Allium accessions and cultivars were found completely immune to any of the A. porri isolates used in the study. However, the identified two resistant A. cepa genotypes (CBT-Ac77 and Arka Kalyan) may offer better choices for breeding purple blotch resistant onion. CBT-Ac77 and Arka Kalyan with best yield characteristics may be recommended for wider cultivation and evaluated in multi-location hotspot for purple blotch in order to determine their efficiency as pre-breeding lines. Besides, A. schoenoprasum and A. roylei with moderate resistance and low PDI may be used as potential donors for purple blotch resistance through interspecific hybridization. A previous work on interspecific hybridization between A. schoenoprasum and A. cepa did not result in viable offspring (Van Raamsdonk et al., 2003). However, A. roylei has been predicted as a suitable candidate for hybridization with cultivated onion due to high genetic similarity and taxonomic affinity and may be definitely exploited for this purpose (Kik, 2002). Alternatively, these lines could be used as starting materials towards molecular isolation and characterization of genes associated with A. cepa–A. porri interaction. Further, the different reactions of the Allium genotypes to three different isolates signify the presence of multiple strains of A. porri. Therefore, there is an urgent need for molecular diversity analysis in this economically important phytopathogen to understand their nature of aggressiveness and pathogenicity.

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