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Review Article

Purple Blotch Disease of Onion (*Allium cepa*): Perspective and Prospects

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Abstract

Purple blotch disease is a major threat to the onion crop and the disease is caused by the pathogens *Alternaria porri* (Ellis) Cif. and *A. allii* Nolla, thus these pathogens hamper onion cultivation across the worldwide. Hence, the crop is protected by the spraying of chemical fungicides, thus chemicals hamper the environment and incur additional costs to onion production and biological agents are effective to control the pathogens under certain environmental conditions. Hence, the development of varieties or hybrids possessing purple blotch disease resistance is encouraged for sustainable onion cultivation. Thus, insight into the understanding of pathogen causing purple blotch disease is important to develop a resistant variety. The knowledge of gene action and molecular markers linked to the resistant genes are essential for breeders for accurate selection phenotype at early stages through indirection selection. Thus, the available genomic sources permit for the precise mapping of resistant genes, markers associated with the *ApR1* gene would be a tool for accelerating the breeding for purple blotch disease resistance. In view, this review confers the perspective knowledge on purple blotch disease, causal organism, symptomatology, epidemiology, and etiology of the pathogen, genetics of purple blotch disease resistance and breeding prospects in onion.

Keywords: *Alternaria porri* (Ellis) Cif.; Breeding; Genetics; Pathogen; Resistance

Introduction

A leaf spot and blight disease of onion were first reported by Ajrekar (1923) from the Bombay state of India and it was considered that disease caused by a species of *Macrosporium sp*., the *Macrosporium porri* was first described as blight causing pathogen of *Allium* species (Cooke and Ellis 1879). Thus, the taxonomy of *Alternaria* species on *Allium* crops causing leaf spot and blight disease was confused, it was first described as the pathogen *M. porri*, later it was classified as the taxonomy of *Alternaria* species by *A. allii* Nolla, based on the symptomatology of pathogen (Nolla 1927), the pathogen maintains its identity with *M. porri* similarity, thus suggested the appropriate name as purple blotch for the disease due to the presence of large size lesions on leaves and seed stalks (Angell 1929) and the further name changed to *A. porri* (Cifferi 1930). The name *A. allii* was resurrected by Simmons (2007) in his identification manual, where it is described as five large spored and long-beaked species from *Allium*, and thus spores could distinguish based on morphology, the number of beaks and branches, the *A. porri* and *A. allii* are closely related and form two distinct clades differ by 8 nucleotides in their RPB2 sequences (Simmons 2007; Woudenberg *et al*. 2014). Taxonomy of purple blotch disease-causing pathogen has been classified in the Kingdom: Fungi, Division: Ascomycota, Class: Dothideomycetes, Order: Pleosporales, Family: Pleosporaceae, Genus: *Alternaria*, the *Alternaria* sect. *Porri* contained 82 *Alternaria* species, sect. *Porri* of *A. porri* and *A. allii* cause purple blotch disease in onion had characterized by broadly ovoid, or obovoid, ellipsoid, sub-cylindrical, or obclavate medium to large conidia, and were disto and euseptate, single or in small chains with a simple or branched extended filamentous beak. Conidia enclose multiple transverse, slightly constricted and longitudinal septa and secondary conidiophores can form apically, or laterally (Lawrence *et al*. 2013). The *A. porri* isolates are unable to differentiate on conidia, conidiophores shape and size, beak and septa of conidia. Whereas, the isolates are differentiated based on colony color on Sabouraud's medium and Brown's medium, and were differ significantly for aggressiveness, incubation period, disease incidence, and disease severity. The Czapek's medium was found best for growth of isolates, the

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isolates do not sporulate on culture media and sporulate poorly on the host plant (Gupta *et al*. 1987).

Epidemiology of *Alternia* species causing purple blotch disease

An epidemic attack by *A. porri* on onions was occurred at Baringo, Kenya occurred in the year 1961, and it causes distinct lesions on plant leaf blades, a) purple or brown blotches (Fig. 2) white, irregular spots or flecks with varying proportion (Bock 1964).

Sources of inoculums

Diseased debris containing pathogen fruiting body with conidia in the farm field, or nearby farm is the primary source of inoculum for succeeding bulb crop followed by seed crop of onion (Pandotra 1964), fungus remain as mycelium in onion leaf debris from diseased plants (Muimba-Kankolongo 2018) and spread by the punctures made by the thrips, opening of stomata pores and epidermal layers. The fruiting body with conidia of leaf lesions fabricated as principal source of inoculum for infections and for disseminating the disease. The frequent wind blows, and rain splashes disperse the conidia and mycelium among the stalks and leaves of plants are the secondary source of inoculums (Fig. 1). The pathogen prolong their existence on leaves, seed stalks and on soil surface, hence burring of diseased debris in the soil about 2 to 6 inches deep below the soil surface, thus lead to the complete knock out of pathogen by breaking on existence of primary source of inoculum (Pandotra 1964, 1965).

Etiology

The conidium germinate to form pre-penetration structure and penetrate into the leaf surface through stomata as well as through epidermis, the maximum conidia germinate at 25°C within 24 h (h) of inoculation. The conidium produce several germ tubes and nurture across the leaf surface, further form bulbous appressorium on epidermal cell (52.4% of appressoria) and on stomata (48.6% of appressoria). The bulbous primary hyphae developed under the appressoria, it could lead to for secondary hyphae development within 48 h after inoculation, hyphae grew and penetrate into the intercellular spaces of mesophyll cells (Aveling *et al*. 1994), then turned to reddish brown septate non-sporulating mycelium (Datar 1994) and cause purple blotch lesions favored by high relative humidity (70% RH), while low RH (40%) resulted in white flecking (often sterile) after prolonged periods of infection. The appressoria and lesions are form in broad optimum temperatures range from 21 to 30°C (Bock 1964).

Alternaria porri is a potentially important pathogen in winter grown *Allium* crops. The conidia of *A. porri* germinate within 2 h (4°C), advances for production of terminal and intercalary appressoria (10°C). The maximum counts of appressoria produces after 24 h at 25°C. Pathogens penetrate via epidermis and stomata of leaves with high frequency of stomata penetration. Prominently, infection occur after 16 h of leaf wetness at 15°C and 8 h of leaf wetness at 10–25°C, and severity of infection increases with increasing leaf wetness over 24 h at all temperatures, and cause lesions (Suheri and Price 2000). The moist weather causes lesions to cover with brown mold sporangia. The suitable periods of rain, or heavy dew and favorable environmental condition promote the disease development (Muimba-Kankolongo 2018).

Symptomatology

An epidemic of purple blotch at Baringo, Kenya was revealed by purple or brown blotches, and white, irregular spots or flecks with varying proportion (Bock 1964). Symptomatic expression of purple blotch disease first appears on leaves with 2–3 mm in diameter of whitish water soaked lesions, these lesions enlarge, coalesce, zonate and lesions turn brown to purplish color under favorable conditions (Fig. 2). Seldom, lesion surface covered by black fruiting bodies under humid conditions (Verma and Sharma 1999). Onion plants showing purple blotch symptoms mainly due to the colonization of *A. porri* and *Stemphylium vesicarium* (onion leaf blight disease), consequently as on disease advancement the pathogens manly infect on leaves and floral stalks shows typical purple blotch lesion symptoms, the pathogens *A. porri*, *S. vesicarium* or mixtures of both accounted for 2.6, 39.8 and 57.6%, respectively. Hence, purple blotch disease is a complex disease caused by two pathogens by the synergistic association, *S. vesicarium* initiate the infection and facilitate the task of *A. porri* for causing purple blotch symptoms (Abdel-Rahim *et al*. 2017). The older plant tissues more susceptible than younger plants leaf blades to the fungus infestation, infection cause small elliptic tan colored water-soaked lesions that soon turn brownish color and later form purplish lesions with darker margin covered by yellow zone of necrotic tissue, as on disease advances the lesions enlarged to form concentric rings, girdling of leaf and stem cause down fall of plant shoot (Muimba-Kankolongo 2018).

Effect of purple blotch disease incidence on plant growth and bulb yield

Onion is susceptible to numerous foliar diseases, those reduces bulb yield and quality (Cramer 2000) and purple blotch is an important disease of onion across the world (Chaput 1995; Cramer 2000; Schwartz *et al*. 2005) especially in warm and humid environments (Suheri and Price 2001). The fungus infestation cause on both leaves and flower stalks (Bock 1964) and reduce onion tops production by 62–92% (Suheri and Price 2001), cause bulb yield loss of 30% (Everts and Lacy 1990) and 10% losses in

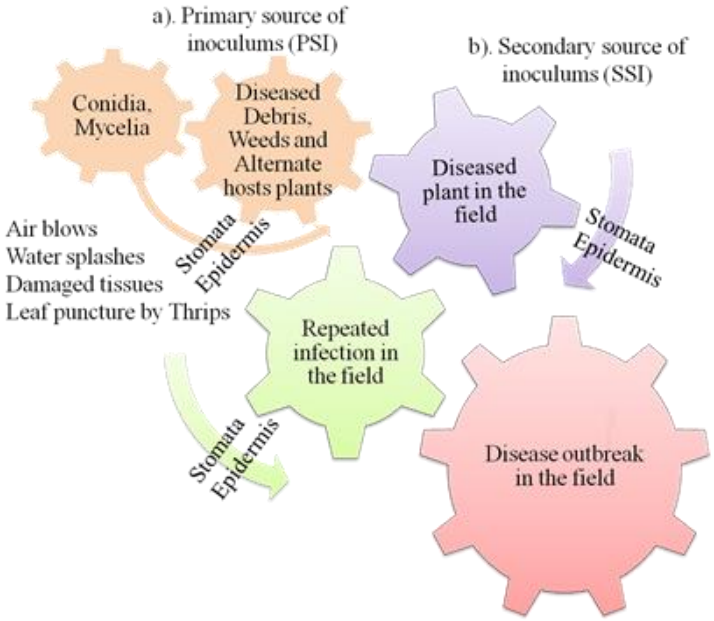

Fig. 1: Life cycle of *Alternaria porri* Cifferi causing purple blotch disease in onion, **a**) conidia mycelia present in debris, weeds and alternate host plants as act as primary source of inoculums, **b**). Diseased plants in field act as Secondary source of inoculums cause repeated infection and lead to the disease outbreak in the crop

Fig. 2: a) Conidium, **b**) Hyphae with conidiospores and **c**) Purple blotch infestion on onion crop stand

s eed crop under congenial environmental conditions (Daljeet *et al*. 1992; Schwartz 2004). Purple blotch disease could cause heavy yield losses ranging from 2.5 to 87.8 per cent during *kharif* season (Srivastava *et al*. 1994), with maximum percentage in Karnataka (60%) and Maharashtra (90%) states of India in *kharif* and *rabi* seasons respectively (Gupta *et al.* 1994). *A. porri* spores present in the air are responsible for increase the disease incidence in onion crop, maximum incidence does occur with adequate leaf wetness duration at 5°C for 16 h and 8 h at 10–25°C. The numbers of lesions are increases with increasing leaf wetness duration and temperature (Suheri and Price 2001). The older leaves are more susceptible than younger leaves for purple blotch disease, infestation reduce the photosynthetic activity of leaves, thus lead to reduction in plant growth, bulb yield and seed yield (Verma and Sharma 1999).

Management and control of purple blotch disease – chemical and biological agents

The losses of bulb and seed yield of onion cause by purple blotch disease could prevent either by protective sprays fungicides or biological agents which are antogonistic effects on *A. porri* during crop cultivation. Despite several limitations in the field conditions like frequent or unexpected rainfall, weather modulation mainly humidity could favor to the pathogen outbreak and thus pathogen cause severe damage on the standing crop in the field under favorable condition. Under those circumstances the fungicide sprays are an effective method to control the disease to maintain the crop stand, several researchers standardized the optimum dose of sprays for the control of

Table 1: Effective chemicals to control purple botch disease under field conditions

Brand name	Chemical composition	Concentration	References
Dithane M-45	Mancozeb 75% WP	0.2%	Wanggikar *et al*. (2014)
Rovral WP	Iprodione 41.6%	20 g per 10 liters of water (0.2%)	Akter *et al*. (2015)
Ridomil Gold WP	Mancozeb (64% W/W) + Metalaxyl-M (4% W/W)		
Dithane M-45	Mancozeb 75% WP		
AGEENT, Custom, DOZAN, Katyayani, DuoGuard	Cymoxanil 8% + Mancozeb 64% WP	2500 ppm	Rao *et al*. (2015)
Folicur	Tebuconazole 25 EC	0.1%	Yadav *et al*. (2017)
Score	Difenoconazol 25EC	0.1%	
Score	Difenconazole 25 EC	0.1%	Kavitha *et al*. (2017)
Nativo 75 WG	Tebuconazole 50% + Trifloxystrobin 25%	0.05%	
Roshan plus	Hexaconazole 5% SC	0.1%	Nisha *et al*. (2020)
Real-mil	Mancozeb 64%WP +Cymoxanil 8% WP	0.3%	

disease are presented in Table 1, however it adds additional cost to the cost of cultivation.

The bio-control agents are effective to control purple blotch disease, the bio agents namely *Trichoderma* spp., *Penicillium* spp., *Aureobasidium pullulens*, *Sporobolomyces roseus* and *Cryptococcus luteolus* were effective to control *A. porri*. The seed treatment with *Trichoderma harzianum* reduces the purple blotch disease incidence thus increase the bulb yield of onion (Chethana *et al*. 2012; Mishra and Gupta 2012). The seed treatment, seedling dip and three foliar sprays of bacteria namely *Pseudomonas fluorescens*, *P. aeruginosa*, and *Bacillus subtilis*, and fungi namely *T. viride* and *T. harzianum* could control the purple blotch disease in field conditions (Yadav *et al.* 2013). The botanical like clove extract of *Allium sativum* (10%) *Aloe vera* (10%), neem oil (20%) and pongamia oil (20%) resulted in inhibition of *A. porri* (Chethana *et al*. 2012).

Development of resistance varieties to purple blotch

The development of purple blotch disease resistant cultivars, varieties or hybrids is another approach to control the disease, and it is an economical and environmentally friendly method as it reduces the ecological problems caused by the use chemicals to control purple blotch disease. However, the fungicides available in the market have low potential to manage onion purple blotch disease (Uddin *et al.* 2006; Abdel-Hafez *et al.* 2014). In this context, there is a need of hunting resistance source for purple blotch disease to improve the bulb production and productivity. Thus, at present there were no potential onion varieties or hybrids succeed in onion acreage showing resistance to purple blotch disease. Nevertheless, several researchers are hunting for purple blotch disease resistance source of resistance lines from past several years.

The onion hybrids cross *viz*., Red Creole × Kaharda and Kaharda × Red Creole were resistant to purple blotch disease, these hybrids are performed better than their parents and other hybrids in terms of disease incidence and bulb yields, negative environmental correlation was noted between disease incidence and bulb yield significantly, higher disease incidence could lower bulb yield was due to environmental effects rather than the genotypes (Abubakar and Ado 2008).

Selves of second-generation mutant ($M_{2)}$ onion plants under epiphytotic conditions, revealed the disease resistance against purple blotch with 7.60% M_2 plants (1–10 PDI), while 12.80% M_2 plants with moderately resistance (11–25 PDI), these disease resistance plants was associated with more than two times higher chlorophyll content (95.8–108.10 mg/100 g) with dark waxy leaves than the normal green foliage (Patil *et al*. 2008). The disease resistance (1–10 PDI) was noticed in M_4 white onions (15.80%) than the M_4 red onions (2.20%). Furthermore, majority of M_4 population (about 70–75%) were moderate resistance (11–25 PDI) against the purple blotch disease in both red and white onions. Therefore, further crop improvement is essential for incorporation of disease resistance by advancement of 3–4 selection cycles (Patil *et al*. 2009).

Purple blotch disease resistance attributed by cuticle thickness; thus resistance could break by wounding and it is naturally associated with sand storm blast (Bock 1964). The cultivars screened in search of resistance to purple blotch disease, thus the varieties VL-1, PBR-1, PBR-5 and PRR are found resistant to purple blotch disease (Daljeet *et al*. 1992). The onion accession CBT-Ac77 and the variety Arka Kalyan was found highly resistant to purple blotch resistance among 43 *Allium* genotypes screened under field conditions, thus suggested the newly identified resistance sources were the potential donors for purple blotch resistance breeding (Nanda *et al*. 2016).

Genetics of purple blotch resistance

The purple blotch disease resistance line PBR-287 was identified as a good source of resistance, and hence it was used in the cross with susceptible parent. Parents, F_1 and individual F_2 progenies were subjected for genetics of resistance and RAPD marker analysis. The results reveal that F_2 individuals segregated in 3:1 ratio for resistance (Ganesh and Veeregowda 2005). The molecular markers linked to purple blotch disease resistance was developed by using F_1, F_2, and BC_1 populations, which were developed from resistant (R) parent Arka Kalyan and the susceptible (S) parent Agrifound Rose. The inheritance of purple blotch disease revealed that the F_1 was resistant, while 498 F_2

plants and 128 BC_1 lines segregated in 3R:1S and 1R:1S ratio. Hence, *A. porri* resistance (*ApR*) is controlled by a single dominant gene and thus designated as *ApR1* gene (Chand *et al*. 2018).

Molecular perspective

Three breeding lines PBR-287, MS-65-268 and Arka Kalyan-704 were identified as resistant to purple blotch disease, and these lines were subjected to random amplified polymorphic DNA (RAPD) analysis with 160 ten mer random primers for identification and estimation of genetic relationship among resistant and susceptible (Arka Niketan) varieties. Out of 160 primers 41 primers are exhibited polymorphism among the accessions, and DNA profile with 5 primers namely OPB02, OPB13, OPC09, OPC12 and OPF08 distinguished resistant lines from the susceptible line. The PCR products ranged from 300 bp to 2000 bp and were consistent, unambiguous and repeatable primer with an average of 2.65 polymorphic bands, 11.65 monomorphic bands were produced per primer (14.3 bands). Principle component analysis (PCA) confirmed the least genetic dissimilarity (40%) was recorded between PBR-287 and MS-65-268; whereas, the highest (65%) was found between lines Arka Niketan-709 and MS-65-268 (Ganesh and Veeregowda 2005). The nucleotide *rbcL* and *matK* were used and developed SSR markers to detect the purple leaf blotch (*PLB*) gene, and it does exist on shorter arm of eight chromosome at s1/s2 *locus*. The *PLB* gene conferred resistance to purple leaf blotch in onion mutant lines (BP2-75/2, BP2 -100/1, BP2-100/2) and mutant variety BARI Piaz-2 was successfully detected in the mutant lines using SSR markers (Chakraborty *et al*. 2015). The *ApR1* gene was linked with seven markers namely $AcISSR47_{1257}$, $AcISSR68_{1600}$, $AcISSR103_{1416}$, AcSSR7, AcSSR22, AcSSR31, and AcSSR33 showed polymorphism among resistant and susceptible bulks and were used in genotyping of mapping populations (F_2 and BC_1). The three inter simple sequence repeats (ISSR) were converted into sequence-tagged markers (STS), the single-copy status of resistant locus association confirmed by southern blotting. The markers linked closely at 1.3 centi Morgan (cM) distance of AcSSR7 (SSR) and ApR-450 at 1.1 cM (STS) to the *ApR1* locus. These findings could be recommended for facilitating the introgression of *ApR1* gene to desirable genotypic backgrounds (Chand *et al*. 2018).

Conclusion and Future Perspective

Purple blotch caused by *A. porri* is a serious disease, incur to heavy yield losses in the bulb and seed crop of onion. The yield losses can be controlled by efficient crop management practices, crop rotation, protective sprays with fungicides, use of biological agents in onion production could control the purple blotch disease, but these crop management activates add the additional cost to the production cost of onions. Thus, the identification of effective and stable resistance varieties are Arka Kalyan, VL Paiz-1 and breeding lines PBR-287, PBR-1, PBR-5 and PRR (Ganesh and Veeregowda 2005; Daljeet *et al*. 1992), hybrids are Red Creole × Kaharda and Kaharda × Red Creole (Abubakar and Ado 2008), accession namely CBT-Ac77 (Nanda *et al*. 2016) would be useful sources of resistance to purple blotch thus these are useful in breeding of onions resistance to purple blotch. Development of genomic SSR markers is a practical tool set for genetics studies in onion (Baldwin *et al*. 2012), thus *ApR1* gene flanking markers could be applicable in MAS with high efficiency (Chand *et al*. 2018). The chromosomal location of the *ApR1* gene is yet to be ascertain, thus fine mapping of resistant locus may be preceded by advanced DNA markers such as single nucleotide polymorphisms (SNPs) with more close linkage (Chand *et al*. 2018). The validation of *ApR1* gene flanking SSR and ISSR markers in other genotypes need to be focused for the identification and isolation of potential purple blotch disease resistance source.

Conflict of Interest

We the authors declare that have no conflict of interest.

Ethical Approvals

The manuscript was not submitted anywhere else, and results were presented without fabrication, falsification, or inappropriate data manipulation. Research does not pose any threat to public health or national security.

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