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University of Calcutta, Kolkata 700 019, India

Etiology of fungal leaf spot of greengram in Kashmir province of Jammu and Kashmir

FAROOQ AHMAD BHAT AND MOHAMMAD NAJEEB MUGHAL
Division of Plant Pathology, Faculty of Agriculture
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Wadura 193201, Jammu and Kashmir

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Cercosporaca nescens Ell.and Mart. an imperfect fungus belonging to class Hyphomycetes was found associated with the predominant fungal leaf spot of greengramin Kashmir. Conidia of the fungus were hyaline, straight to sub-straight or slightly curved, obclavate-cylindric, 40.2-180.3 × 2.5-3.4 μ with an average of 102.8 × 3 μ having 1-14 septa. The diagnostic symptoms comprised of roughly circular to irregular, white centered reddish brown to brown leaf spots measuring 2-12 mm in diameter.

Key words : Greengram, cercosporacanescens, leaf spot, etiology

INTRODUCTION

Pulses offer a cheaper source of dietary protein to common masses particularly the vegetarian group, besides being a delicious dietetic variety for upper class. Greengram (*Vigna radiata*) is the third important pulse crop of India and occupies 8 per cent of the total area under pulses in the country.National food Security Mission is under implementation in J&K to increase the disease free production of pulses and other crops, to increase the farmer's income by making the farm business management more profitable and to generate employability. Although Jammu and Kashmir relies on import of greengram seed and its value added products, it is an important *kharif* crop of the state where different pulses are grown over an area of 26.57 thousand hectares with an annual production of 8.41 thousand tonnes including about 34 per cent from Kashmir province (Anonymous, 2015). The crop experiences several stresses worldwide due to pathogenic fungi, bacteria, viruses and nematodes. However, the foliar fungal diseases including *Cercospora* leaf spot (CLS) are more destructive for causing qualitative and quantitative losses worldwide. The leaf spot caused by *Cercospora* spp. was declared threat to greengram cultivation in several countries for its devastating appearance in crop stands (Poehlman *et al*. 1973). It inflicts heavy yield losses ranging from 23 to 96 per cent under natural epiphytotic conditions (Kasno, 1990; Iqbal *et al*. 1995; Kaur, 2007). The yield losses vary depending upon how early the crop is infected in the season, crop variety and prevailing weather conditions (Bhat *et al*. 2015). It has been considered a predominant fungal foliar disease of greengram in the valley of Kashmir with 5.87-28.43 per cent (Av. 22.96 %) intensity during *kharif* 2008 (Bhat, 2011). Keeping in view the importance of greengram as well as associated disease, the present study was, therefore, intended to generate timely information with respect to etiology of the disease which is a prerequisite for devising a meaningful management program for any plant disease.

MATERIALS AND METHODS

The present investigations on fungal leaf spot of greengram were conducted in the laboratory and the experimental field of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, located at 34°472 north latitude and 74°522 east longitude at an elevation of 1591 meters above mean sea level (masl). However, survey for estimation of the disease in Kashmir was conducted in some important areas distributed from north to south of the valley at 1580 to 2000 masl.The causal fungus was identified on the basis of its morphology and disease symptomology. Morphological characters of the pathogen were recorded with respect to conidia, conidiophores and mycelium. The fungal

*Corresponding author : farooqbhat@skuastkashmir.ac.in

structures were stained and mounted in lactophenol-cottonblue solution (Weeks and Padhye, 1982), and the micrometry was conducted with a calibrated compound microscope. Symptomology was recorded under natural epiphytotic conditions for which fifteen greengram plants (cv. Shalimar Mung-1) were randomly selected and tagged in the crop stand kept unsprayed throughout. Observations with respect to size, shape and colour of lesions, and fructification were recorded as soon as the disease appeared and then repeated at two days interval for two weeks.

RESULTS AND DISCUSSION

Morphology of the causal pathogen

The morphology of mycelium, conidiophores and conidia of the pathogen as recorded during the investigation is presented Fig.1. Mycelium was sub-hyaline and 3.1 µm in average diameter with 6.8-12.2 septa per 100 µm hyphal length and irregular branching. The stromata were indistinct, if formed at all. Conidiophores were light to olivaceous brown, straight or slightly curved, geniculate, unbranched, cylindric, 32-119 µm × 3.3-4.8 µm in dimension with an average of 77 × 4 µm having 0-8 septa and borne in fascicles of 5-17. The increased average length (163 µm) and

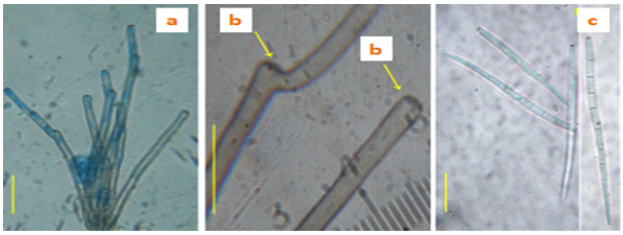

Fig. 1 : (a) Conidiophores, (b) scars on conidiophore apex and at geniculation, and (c)conidia of *C. canescens* (Scale-20 µm)

septation (upto 10) was recorded on sporulating leaf spots under high humid conditions at room temperature. Conidiogenous cells were apical as well as intercalary with one to several conidia formed on a single conidiophore and prominent scars were left on both conidia and conidiogenous cells following their separation. Conidia were hyaline, straight to sub-straight or slightly curved, obclavate-cylindric, 40-180 × 2.5-3 µm with an average of 103 × 3 µm having 1-14 septa and borne solitarily. The increased average length (177 µm) and diameter (4 µm) of conidia was recorded on sporulating lesions under high humid conditions at room temperature. The inter-septa distance was not uniform and varied from 6.6 to 21 µm. Moreover, the conidia were found germinating

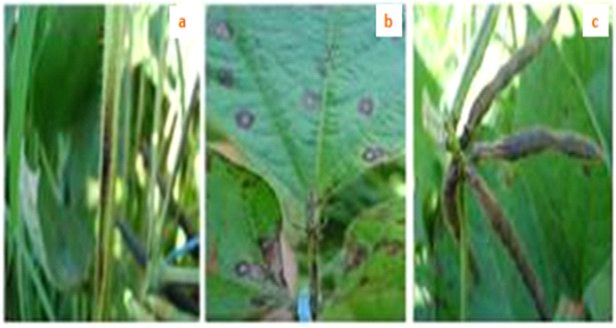

Fig. 2 : Symptoms due to *C. canescens* on (a) petiole, (b) leaves and (c) pods of greengram.

through basal, apical and intercalary cells under saturated conditions. The colour and shape of conidia and conidiophores were no different from the descriptions earlier maintained for *C. canescens* (Ellis and Martin, 1982; Saccardo, 1886; Chupp, 1953; Ellis, 1976; Thirumalachar and Chupp, 1948; Arya *et al*. 1997). However, some variations were found in the physical dimensions of pathogen. The conidia were slightly smaller than those reported earlier by some of above authors, though the description given by Solheim and Stevens (1931) and Arya *et al*. (1997) for the same pathogen were close to present findings. The pathogen responded to high humid conditions and produced larger conidiophores and conidia. This

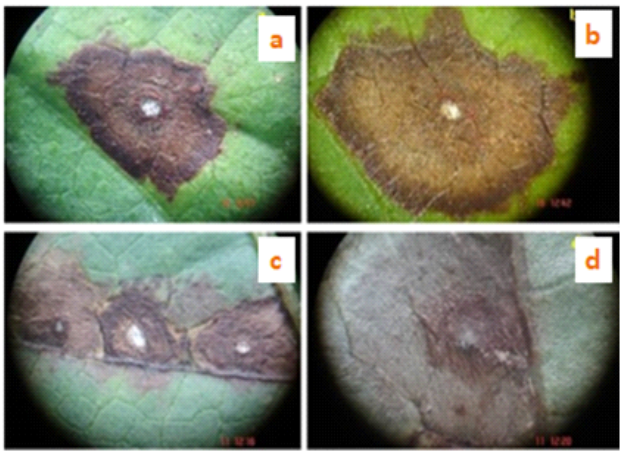

Fig. 3 : Magnified view of CLS of greengram: (a & b) fructification on upper surface and (c & d) lower surface

kind of variation was also reported by Ragunathan (1969) when *C. canescens* was subjected to different environments. Moreover, Joshi *et al*. (2006) reported that genetic variability existed in *C. canescens* isolates of the same geographical

region. These findings collectively supported our identification aspect by confirming the role of environment in determining the size of conidiophores and conidia. The existence of *C. canescens* in Kashmir was earlier reported by Dar and Ghani (1997). However, they reported it as a pathogen of faba bean. Moreover, the pathogen has a significant distribution occurring on greengram and allied crops across the world including most of the Indian states (Butler and Bisbey, 1931; Chupp, 1953; Munjal *et al*. 1960; Poehlman *et al*. 1973; Rewal and Bedi, 1976; Khandar *et al*. 1983; Kasno, 1990; Mittal, 1991; Iqbal *et al*. 1995).

Symptomology

The disease symptoms were observed on all above ground plant parts such as leaves (both upper and lower), petioles and pods (Fig. 2). On leaves, the disease initially appeared as small (0.5-1mm diameter) dark brown spots which increased in area and reached 1.5, 3, 5, 7.1 and 8.9 mm diameter in next 2, 4, 6, 8 and 10 days, respectively. The characteristic whitish centre appeared when spots were just about 2 mm in diameter. Spores could be harvested from young 3-4 days old spots of about 2 mm diameter. The leaf spots varied in shape from roughly circular to irregular and in diameter from 2-12 mm. The shape of leaf spot was usually determined by leaf veins which delimited pathogen's growth although these were also infected in some instances. The spots were reddish brown in colour with slightly darker periphery. Some older spots appeared brown around whitish centre with a definite dark brown margin. Moreover, some spots were uniformly dark brown or reddish brown around whitish centre without any distinctive border. The characteristic whitish center was mostly present on the upper side of leaves, although it was insignificantly visible on under side in some instances. Coalescing of two or more spots was frequently observed and the seriously infected leaves would usually dry and remained attached to the plant. Yellowing of leaves was also observed especially when petiole was invaded by the pathogen. Moreover, the disease appeared even on leaves of 20 days old plants growing underneath and in complete shade of 50 days old lodged plants under open field conditions. The low power stereoscopy revealed that the fructification was amphigenous and was both in and around whitish centre of leaf spots (Plate 3). The stereoscopy also revealed that fructification occurred throughout the diseased area except along extreme periphery and was not restricted to whitish center as thought earlier. Although actual cause that led to development of characteristic whitish center was not ascertained, it was assumed to be the result of total exhaustion of host cells in the infection court. The brown to reddish brown leaf spots with whitish center and with or without distinctive periphery and frequently delimited by veins as observed in the present investigation were more or less similar to the account of symptoms associated with *C. canescens* (Butler, 1918; Solheim and Stevens, 1931; Rewal and Bedi, 1976; Grewal, 1978; Arya *et al*. 1997). Amongst earlier descriptions the one by Arya *et al.* (1997) mentioned bigger spots and others such as that of Wells (1924), Solheim and Stevens (1931), and Grewal (1978) maintained that the spots associated with *C. canescens* were smaller than those produced by *C. cruenta.* Ellis and Martin (1882) had also reported the association of smaller leaf spots (2.5-5 mm) with type species of *C. canescens.* However, the frequent delimitation of spots by veins, the spots being more conspicuous on upper side with amphigenous fructification and reddish margins as observed in the present investigation were maintained by most of the authors for leaf spots due to *C. canescens* (Ellis and Martin, 1882; Chupp, 1953; Grewal, 1978; Arya *et al*. 1997). Moreover, the disease manifestation by shaded leaves as recorded during symptomatology revealed the insignificant influence of intensity of light rather than denying the fact that cercosporin is a photosensitive toxin (Daub and Ehrenshaft, 2000). On petioles, the spots were more elongated than circular, and whitish centre was not found except in older spots. The spots appeared as dark areas which gradually increased in size and retained the darker areas towards the center with indeterminate and light to reddish brown margin. Pods and seeds manifested the disease differently from other parts. On pods, the colour and area of diseased spots varied with pod age. Pods, infected when young, showed larger darkened areas and cracks along the suture. However, those which were infected later in the crop development stage depicted the restricted but significant darkened areas. Seeds manifested the disease as reddish brown to dark brown and black areas though shriveling of seeds due to infection was also observed. The disease manifestation by seeds, pods and petioles was less variable from those

reported earlier by Butler (1918), Solheim and Stevens (1931), and Dhingra and Asmus (1983) for *C. canescens*. However, the reddish brown areas on seeds, development of cracks on pods, and necrosis of radicle were in addition to the collective description of symptom so far.

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