Sclerotinia Sclerotiorum Causing Pigeonpea Stem Rot in India: A New Biotic Stress to the Pigeonpea Crop under Climate Change Scenario

Ashish Kumar Gupta[1], Ravish Choudhary[2] and Bishnu Maya Bashyal[3]

[1]ICAR-Indian Agricultural Research Institute RS, Pusa, Samastipur, Bihar, India
[2]ICAR-Indian Agricultural Research Institute Regional Station, Pusa, Samastipur 848125, Bihar, India.
[3]ICAR-Indian Agricultural Research Institute, New Delhi India.
E-mail: [1]ashish.pathology@gmail.com, [2]ravianu1110@gmail.com, [3]bishnumayabashyal@gmail.com

Abstract—*Sclerotinia sclerotiorum (Lib.) de Bary is highly devastating plant pathogen causing stem rot of pigeonpea in India. The symptoms of the disease first appeared as light brown water-soaked lesions on stem and branches and enlarged in both directions finally covered with white fluffy mycelium. Ultimately, the leaves, inflorescence and pods were covered with white mycelial growth of the pathogen. During, January month of 2012-13, 2013-14 and 2014-15, average minimum air temperatures were 7.3, 10.5, and 7.1^{0}C, whereas average maximum air temperatures were 19.5, 19.0 and 22.2 ^{0}C, respectively. During the month of November in 2012-13 and 2013-14, average minimum RH (%) was 48.9 and 52.5, whereas in 2014-15 the average minimum RH (%) was 49.0 in the month of October. Maximum rainy days were observed in the month of August during 2012-13, 2013-14 and 2014-15. However, during 2012-13 and 2013-14, November and December months were completely dry, while rainfall occurred again in January month of both the years. Surprisingly, during 2014-15, no rainfall was occurred after October month till February which is very sensitive months for spreading of this pathogen. December and January are the coldest months and above 93% relative humidity accompanied by 9.5-12.2 mm rainfall (2-3 rainy days) during the years 2012-13 and 2013-14, upholds ideal conditions for sclerotial germination and apothecial development. The disease was found to appear on pigeonpea crop during 2012-13 and 2013-14, while in 2014-15 crop season no disease incidence was recorded due to high temperature, low relative humidity and no rainfall in winter season checked the infection and occurrence of the disease on pigeonpea crop in the north eastern plain zone of India. This is a critical example of climate change mediated non-occurrence of plant disease in pigeonpea crop.*

1. INTRODUCTION

Pigeonpea is one of the most important pulse crops of India. It is known as red gram, arhar and tur in the country. It is an important source of proteins (22%) along with carbohydrates, fibre, certain minerals viz., iron, calcium, magnesium, zinc, iodine, potassium and Phosphorous and 'B' complex vitamins. Pigeonpea stalks are also a major source of firewood and live stock feed. India is the world's largest producer and consumer of pulses including pigeonpea. About 90% of the global pigeonpea area is in India contributing to 93% of the global production. It occupies 4.9 m ha area with 3.1 mt production which accounts for a productivity of 1145 kg/ha; ranking ninth in the world [1]. Kharif is the main growing season of pigeonpea in India and major pigeonpea growing states are Maharashtra, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, Tamil Nadu, Bihar and Chhattisgarh. Cultivation of pigeonpea on bunds in low land areas is becoming popular and profitable among the farmers. Traditional varieties of pigeonpea need about 6 to 9 months to mature, while the improved varieties developed can be harvested in 3-4 months. Both long duration (180 days) and medium duration (130-140 days) genotypes are grown in India under various cropping systems. This pulse crop is grown mostly as an intercrop between cereals crops and plays a unique role in enriching the soil, by adding 40-90 kilogram nitrogen per hectare over a given season. It has the ability to resist drought and to add large quantities of biomass to the soil in addition to nitrogen fixation makes it a good choice for rainfed as well irrigated production systems. The deep root system of the crop helps to recycle plant nutrients from deeper layers, and the acid secretions from its roots increase the availability of phosphorus in the soil. Limitation to the increasing productivity of pigeonpea is also due to abiotic and biotic stresses prevalent across the pulse growing regions. The crop is affected by more than 100 pathogens, including fungi, bacteria, viruses, phytoplasmas and nematodes [2]. Among biotic stresses diseases viz., *Fusarium* wilt, *Phytophthora* Blight, Sclerotinia stem rot, sterility mosaic and foliar diseases and, insect pests feeding on pods lead to significant yield losses. Management of these stresses can contribute to a yield recovery of 300-350 Kg/ha.

2. STUDY DETAIL

Field studies were conducted at the experimental farm of ICAR-IARI Regional Station, Pusa, Bihar (India) during the

years, 2012-13 and 2013-14. The farm site (25^0 59' N, 85^0 40' E and 52 m above mean sea level) is located in Samastipur district of Bihar (India) on southern bank of Budhi Gandak river. The soil type at the experimental site was calcareous clay in texture, pH (8.1), low in organic carbon, nitrogen, zinc, boron, sulphur and intermediate in phosphorus and potash content. The pigeonpea crop was sown in the month of August in RBD with three replicates in plots of size 6.0 x 4.0 m spaced at 75.0 x 25.0 cm.

3. SYMPTOM

The symptoms of the disease first appeared as light brown water-soaked lesions on stem and branches. These lesions girdled the entire stem or branches and enlarged in both directions and became notably covered with white fluffy mycelium (Fig. 1). Ultimately, the leaves, inflorescence and pods were covered with white mycelial growth of the pathogen. Affected leaves lost turgidity and ultimately were blighted on the stem [4].

4. THE PATHOGEN

Sclerotinia rot of pigeonpea caused by *Sclerotinia sclerotiorum* (Lib.) de Bary in India (Gupta et al., 2015). *Sclerotinia sclerotiorum* (Lib.) de Bary is a necrotrophic pathogen with a broad host range worldwide. It is a simple interest plant pathogen using ascospores as primary source of inoculum and spreads horizontally through contact of infected plant to nearby healthy plants. The pathogen causes root, stem, twig and head rots in oilseeds, pulses, vegetables, ornamentals, medicinal and fodder crops, leading to crop failures with 60 - 80% disease incidence and up to 100% yield loss worldwide [3]. Isolation studies from diseased tissues and sclerotia consistently produced white fluffy mycelial colonies and abundant large black sclerotia on PDA. Sclerotial formation started after 72 h of incubation and appeared fully on the surface of mycelial growth in the form of a ring at the periphery of the plate after 6 days [4].

5. CULTURAL, MORPHOLOGICAL MOLECULAR CHARACTERISTICS"

Fungal colony colour creamy white to light brown with sparse, fluffy type of growth was observed. Hyphae was hyaline in colour, colour of sclerotia whitish to blackish and appeared in form of ring at the periphery or scattered in the centre of the plate. The internal transcribed spacer (ITS) regions 1 and 2, including the 5.8S ribosomal DNA (rDNA) region, were amplified by polymerase chain reaction (PCR) using universal primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [6] synthesized by Sigma Aldrich House, Suffolk, UK. The PCR amplification reaction was carried out in a final volume of 25 µL, which consisted of 1X PCR assay buffer, 200 mM dNTPs mixture, 2.0 mM $MgCl_2$, 0.1 µM primer, 1.5 U Taq DNA Polymerase and 50 ng template DNA. PCR reactions were carried out in an Icycler, Bio-rad (96 well system) under initial denaturation at 94 ^{0}C for 4 min, annealing at 57 ^{0}C for 1 min, 72 ^{0}C for 1 min, and final extension at 72 ^{0}C for 7 min. The PCR fragments were sequenced and the sequences were aligned using the multiple sequence alignment program, Clustal W [6]. The phylogenetic analysis was performed using MEGA 4.0 (Tamura et al. 2007) with a bootstrap of 1000 replicates. *Fusarium oxysporum* strain ATCC-MYA 3931 was used as the outgroup taxon. The internal transcribed spacer (ITS) region of the pathogen was amplified using the primers ITS1 and ITS4 and sequenced. The BLAST searches in NCBI database revealed 100% query coverage and 99% identity with *S. sclerotiorum* [3].

6. HOST RANGE"

The fungus infects more than 500 plant species belonging to 278 genera and 75 plant families spread over gymnosperms and angiosperms including monocots and dicots [3]. *Bidens pilosa*, *Capsella bursa pastoris* and *Vernonia cinerea* new weed hosts were also recorded for this pathogen. These weed hosts were not observed earlier in this area for the same pathogen. These weed hosts may act as source of primary inoculum for the disease development [5].

7. PREDISPOSITION FACTOR"

The experimental location, Pusa (Bihar) is characterized by a humid sub-tropical climate under irrigated and non-limiting soil moisture conditions in NEPZ of India. Cool humid weather along with heavy dew occurred during the months of November to February. Relative humidity is usually higher than elsewhere due to its specific location on southern and western bank of Budhi Gandak river. During December and January of 2012-13 and 2013-14, average minimum air temperatures were 10.1 and 8.4 ^{0}C, whereas average maximum air temperatures were 22.2 and 19.3 ^{0}C, respectively. The average minimum RH (%) during December and January was 62.3 and 67.2, whereas maximum RH (%) was 93.0 and 92.3, respectively, for both the years. December and January are the coldest months of the year, and above 93% relative humidity accompanied by 9.5-12.2 mm rainfall (2-3 rainy days) upholds ideal conditions for sclerotial germination and apothecial development (Gupta et al., 2016). Pigeonpea crop was planted in the month of August and developed a closed canopy till November and December. However, flowering occurs in December which coincides with cool, wet and humid weather conditions along with dead flowers providing favourable conditions for disease initiation and spread. Pathogen infection, development and spread is being highly affected by high temperature during the months of December and January. The cool sub-humid climate with average minimum (9.2 ^{0}C) and maximum temperatures (21.0 ^{0}C) along with more than 93% relative humidity play an decisive role for stem rot disease development and spread in pigeonpea crop [5]. The disease was found to appear on pigeonpea crop during 2012-13 and 2013-14, while in 2014-15 crop season no disease incidence was recorded due to high

temperature in winter season (Fig. 2). The three months' i.e., November, December and January of 2014-15, minimum temperature (15.2, 9.36, 7.1^{0}C), maximum temperature (29.6, 24.1, 22.2^{0}C), minimum relative humidity (53.8, 56.5, 56.5%) and (89, 88.7, 91%) has vast effect on appearance of disease on crop (Figures 2 and 3). Maximum rainy days were observed in the month of August during 2012-13, 2013-14 and 2014-15. However, during 2012-13 and 2013-14, November and December months were completely dry, while rainfall occurred again in January month of both the years. Surprisingly, during 2014-15, no rainfall was occurred after October month till February which is very sensitive months for spreading of this pathogen (Fig. 4). Sunshine hours were also lowest in the months of December and January during 2012-13, 2013-14 and 2014-15 (Fig. 5). Probably higher maximum temperature and low relative humidity checked the infection and occurrence of the disease on pigeonpea crop in the NEPZ region of India. This is a critical example of climate change mediated non-occurrence of plant disease in pigeonpea crop.

8. CONCLUSION"

The disease and pathogen highly affected by high temperature during the months of December and January. The cool sub-humid climate with average minimum (9.2 ^{0}C) and maximum temperatures (21.0 ^{0}C) along with more than 93% relative humidity play an decisive role for stem rot disease development and spread in pigeonpea crop. However, the disease has been found to appear on crop during 2012-14, while in 2014-15 crop season no disease incidence was recorded due to high temperature, low relative humidity and no rainfall in winter season resulting checked the infection and occurrence of the disease on pigeonpea crop. This is a typical menace to the pigeonpea crop production under climate change scenario occurred in North Eastern Plain Zone of India.

REFERENCES"

[1] GOI, Government of India, "Agricultural Statistics at a Glance-2015", Directorate of Economics and Statistics, Ministry of Agriculture, Government of India, 2015.

[2] Nene, Y.L., Sheila V.K. and Sharma, S.B., "A world list of chickpea and pigeonpea pathogens", 5th edn. *International Crops Research Institute for the Semi-Arid Tropics*, Patancheru 502 324, Andhra Pradesh, India, 7, 1996, pp. 1–27.

[3] Mehta, N., "*Sclerotinia* stem rot-an emerging threat in mustard", *Plant Disease Research*, 24, 2009, pp. 72–73.

[4] Gupta, A.K., Bashyal, B.M., Choudhary, R., Kumar, M. and Solanki, I.S. First report of *Sclerotinia* rot of pigeonpea caused by *Sclerotinia sclerotiorum* Lib. de Bary in India. *Canadian Journal of Plant Pathology*, 37, 4, 2015, pp. 514-518.

[5] Gupta, A.K., Choudhary, R., Bashyal, B.M. and Solanki I.S., "Incidence and characterization of pigeonpea stem rot disease caused by *Sclerotinia sclerotiorum* (lib.) de Bary in North Eastern Plain Zone of India", (*In press*), *Legume Research*, 2016.

[6] White, T.J., Bruns, T., Lee, S. and Taylor, J., "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics", In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors, PCR protocols: a guide to methods and applications, New York (NY): Academic Press; 1990, pp. 315–322.

Fig. 1. Pigeonpea branches showing the critical symptoms of the stem rot disease under field condition

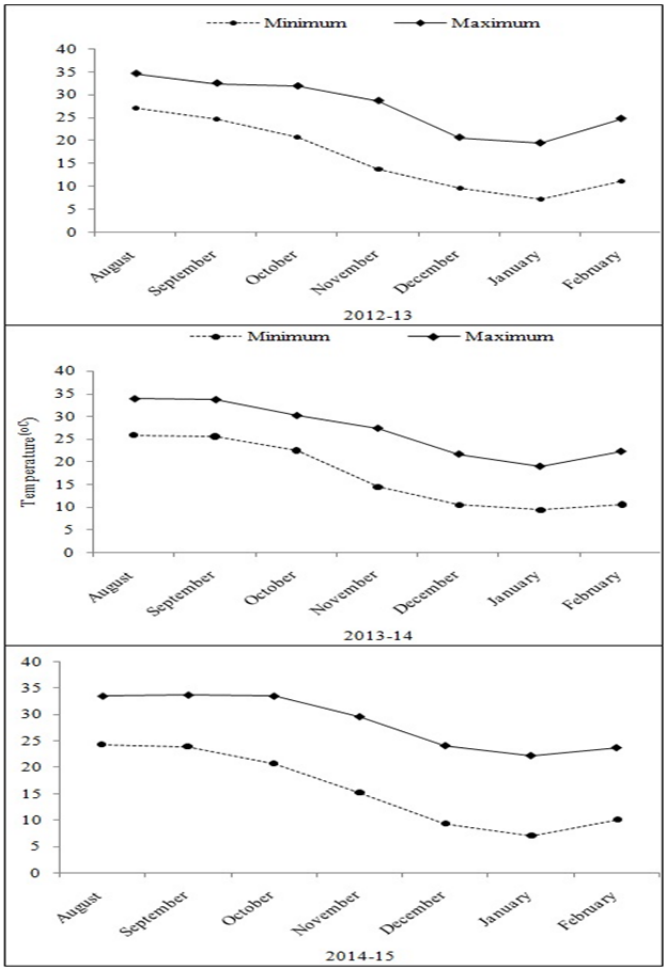

Fig. 2. Average maximum and minimum monthly temperature (°C) during the years, 2012-2015

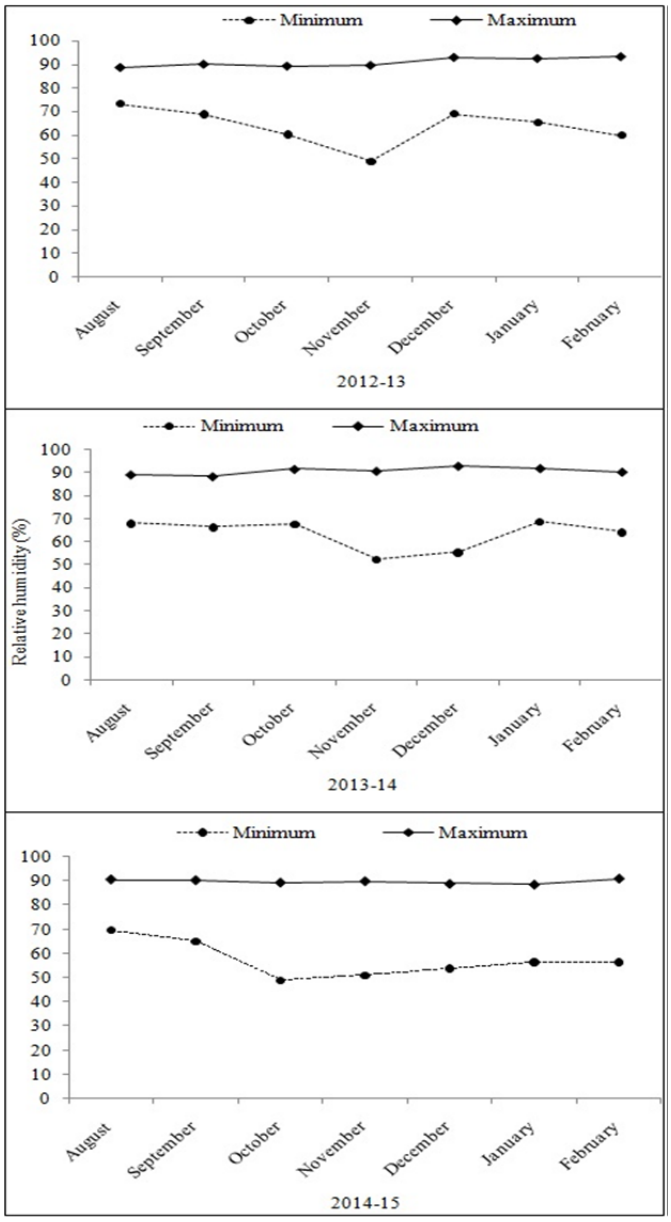

Fig. 2. Average relative humidity (%) during the years, 2012-2015

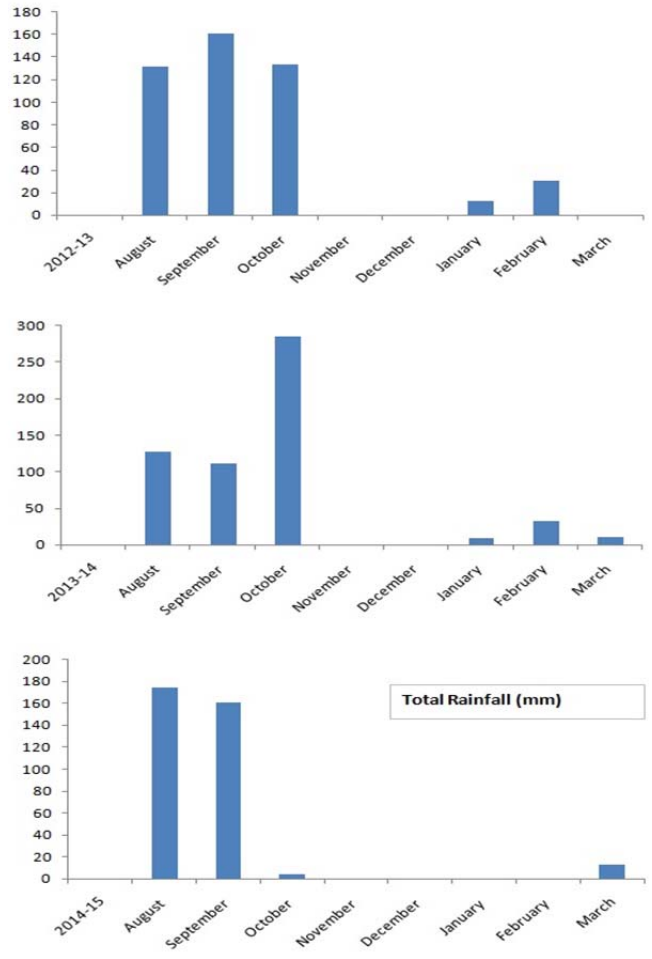

Fig. 2. Total monthly rainfall (mm) during the years, 2012-2015

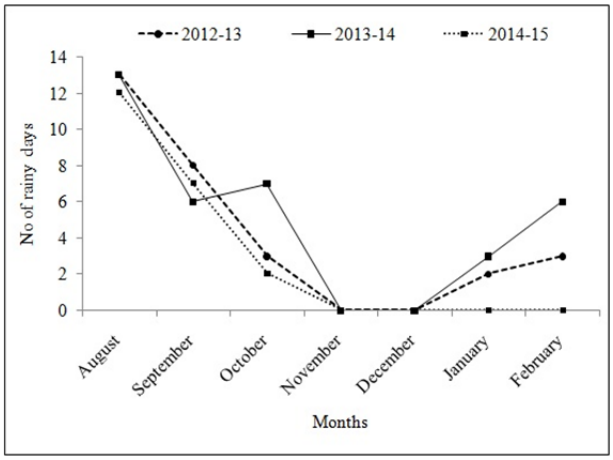

Fig. 4. No of Rainy days.

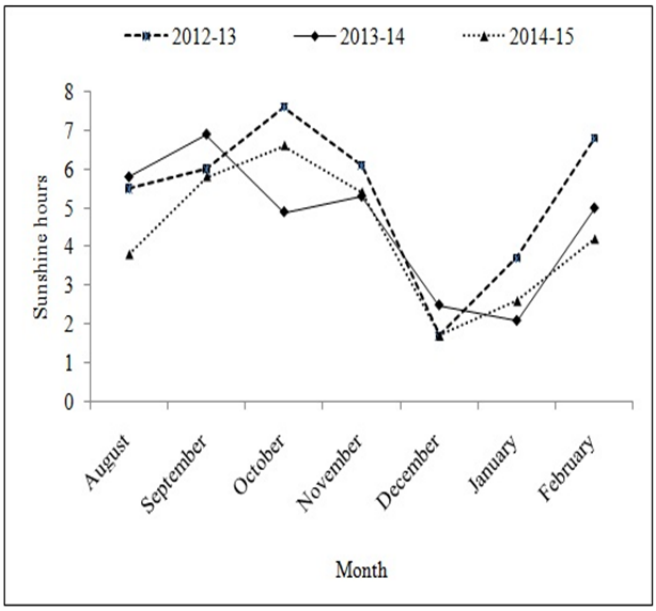

Fig. 5. Sunshine hours during wheat season 2012-13 and 2013-14 at Pusa, Bihar (India).