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DISTRIBUTION OF STALK ROT OF MAIZE IN PUNJAB, PAKISTAN

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ABSTRACT

Surveys of 8 districts of Central Punjab, Pakistan namely Sialkot, Narowal, Gujranwal, Hafizabad, Shekhupura, Lahore, Okara and Sahiwal; and five districts of South Punjab namely Khanewal, Multan, Muzaffargarh, Vehari and Lodhran were carried out during April 2014 to assess the distribution of stalk rot of maize (*Zea mays* L.). In each district three sampling sites were selected. From each site data were collected from three fields well apart from each other. Disease prevalence (DP) was calculated on the basis of presence or absence of disease at each visited site. Disease incidence (DI) was calculated on the basis of number of infected plants out of total plants. Likewise, disease severity index (DSI) was recorded on the basis of plant area infected or damaged. Stalk rot disease was found in all the surveyed areas showing 100% prevalence. Disease incidence varied from 41 to 51% in different districts. The highest DI was recorded in Sialkot and Sahiwal. DSI ranged from 17.4% in Hafizabad to 30.2% in Sahiwal. Different species of *Fusarium* namely *F. vericeiliodes* (Sacc.) Nirenberg, *F. oxysporum* Snyder & Hansen, *F. prolifiratum* Nirenberg ex Gerlach & Nirenberg, *F. moniliforme* J. Sheld., *F. bullatum* Sherb., *F. thapsinum* Klittich, Leslie, Nelson & Marasas, *F. incarnatum* (Desm.) Sacc. and *F. acchari* (Butler & Khan) Gams were found associated with maize plants infected with stalk rot disease.

Key-words: Central Punjab, *Fusarium* spp., Maize, Stalk rot.

INTRODUCTION

Maize (*Zea mays* L.) is a short duration cross pollinated crop cultivated in spring and summer for grain as well as for fodder purpose in Pakistan. It is economically important cereal crop next to wheat and rice, widely grown in the world due to its adaptability to a wide range of climatic conditions (Gerpacio and Pingali, 2007; Mubeen *et al.*, 2017). The crop is utilized as a food for human consumption, as a fodder for livestock, as a feed for poultry and as a raw material in industry (Mahuku, 2010). It contains 72% starch, 10% proteins, 4.8% oil, 8.5% fiber, 3% sugar and 1.7% ash (Farhad *et al.*, 2009). It is cultivated on an area of 140 million hectares with 577 million metric tons production in the world (Ahmad *et al.*, 2015).

Maize is successfully cultivated in nearly all agro ecological zones of Pakistan and is cultivated in all of its provinces. Punjab and Khyber Pakhtunkhwa are the major maize growing provinces, contributing 39 and 56% of the total area under maize and 30 and 63% of total production. The total cropped area occupied by the maize in Pakistan for year 2016-17 was 1334 thousand hectares (Anonymous, 2017). Sialkot, Narowal, Gujranwala, Hafizabad, Sheikhupura, Lahore, Okara, Sahiwal are major maize growing districts in central Punjab. Pakistan is far behind in term of average yield per hectare than developing countries like America, Ireland, Australia, China. Both biotic and abiotic factors are constraints that reduce the maize yield per hectare and deteriorate the quality of the crop even in the presence of high yielding cultivars. Stalk rot is a serious biotic constraint (Fig.1), reported in all major maize growing countries in the world (Mir *et al.*, 2018). Stalk rot of maize is caused by a complex of several fungal pathogens and secondary colonizers (Afolabi *et al.*, 2008). In the Asian tropics, *Fusarium* stalk rot, *Macrophomina* stalk rot and late wilt are prevalent (Khokhar *et al.*, 2014). Stalk rot disease attacks vascular bundles and causes internal decay and discoloration by blocking translocation of water, nutrients and can result in death and lodging of the plant during the cropping season. In South East Asia, hot and humid weather favors the disease development (Khokhar *et al.*, 2014). Geographical location and cultural practices including planting date, crop rotation, tillage and fertilizer application also affect the disease index of all *Fusarium* species causing stalk rot of maize (Munkvold, 2003). Stalk rot of maize in Pakistan received little attention and scanty information are available on occurrence and distribution of the disease in Central Punjab, Pakistan. Therefore, the objective of present study was to carry out an extensive survey to document the distribution of stalk rot disease in maize growing areas of Central Punjab, Pakistan.

MATERIALS AND METHODS

Survey

The survey for stalk rot of maize was conducted during April 2014, in 8 maize growing districts of Central Punjab, and 5 districts of South Punjab, Pakistan. These included Sialkot, Narowal, Gujranwala, Hafizabad,

Sheikhupura, Lahore, Okara, Sahiwal (Central Punjab), and Khanewal, Multan, Muzaffargarh, Vehari and Lodhran (South Punjab). In each district, three sites were selected which were 2-10 km apart (Table 1). At each selected site, three maize fields were randomly selected. In this way, a total of 24 locations in 8 districts covering 72 fields were visited.

Sampling procedure

Each of 72 fields was arbitrarily divided into five parts. Each part consisted of a row of 50 plants. In this way a total of 250 maize plants were observed from each field and disease incidence was recorded using the following formula:

$$\text{Disease Incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

On the basis of presence or absence of disease in different fields, disease prevalence was calculated as follows:

$$\text{Disease Prevalence (\%)} = \frac{\text{No. of infected fields}}{\text{Total No. of fields}} \times 100$$

Ten maize plants showing characteristic *Fusarium* stalk rot symptoms were randomly sampled from each part (Mohammadi *et al.,* 2016). Following 1-9 disease rating scale was used to measure disease severity (ICAR 2012).

1. Healthy or slight discoloration at the sight of inoculation
2. Up to 50% of infected internode discolored
3. 51 to 75% of infected internode discolored
4. 76 to 100% of infected internode discolored
5. Less than 50% of infected internode discolored
6. More than 50% of infected internode discolored
7. Discoloration of three internodes
8. Discoloration of four internode s
9. Discoloration of five or more internodes and premature death of plant

Disease severity index was calculated by applying following formula:

$$\text{Disease Severity Index (\%)} = \frac{\text{Sum of all rating}}{\text{Total no. of plants observed} \times \text{Rating scale}} \times 100$$

Tissue specimens were taken from both diseased and asymptomatic plants. Surveys were typically conducted when crop was at physiological maturity and stalk rot symptoms were likely to appear (Kelly *et al.,* 2017). Diseased stalk sections were packed in paper bags, labeled and brought to Laboratory for isolation and identification of associated fungal pathogens.

Isolation of associated fungi

Diseased samples were examined under stereoscope and infected stems with typical stalk rot symptoms were processed further. Three 5 mm diseased stalk sections were taken at 5, 10 and 15 cm from the first internode above the brace roots (Scauflaire *et al.,* 2011). Diseased sections were surface sterilized with 2% sodium hypochlorite for 2 min and rinsed thoroughly with sterilized water. These pieces were drained and placed on potato dextrose agar that was amended with Streptomycin sulphate (130 μg mL^{-1}) to inhibit bacterial growth (Gai *et al.,* 2017). Cultures were maintained at 25 °C for two days. The growing fungal hyphal tips were transferred to PDA and grown for two days at 25 °C. Conidia were isolated using single spore isolation method and incubated at 25 °C for 10 days. All the fungal isolates were stored at -75 °C in 50% glycerol (Shin *et al.,* 2014). Isolated fungi were identified on the basis of morphological characters (Leslie and Summerell, 2008).

Statistical analysis

All the data were analyzed by ANOVA followed by LSD test at 5% level of significance using computer software Statistix 8.1.

RESULTS AND DISCUSSION

In Central Punjab, disease prevalence was 100% in all the 8 districts as stalk rot disease was present in all the field of the surveyed areas (Fig. 2A).Disease incidence ranged from 41% to 53% in different districts. The highest disease incidence (51%) was recorded in Sialkot and Sahiwal that was significantly higher than disease incidence in other 6 districts. Disease incidence in Narowal, Gujranwala, Hafizabad, Sheikhupura, Lahore and Okara was 42, 45, 45, 42, 41 and 45%, respectively (Fig. 2B). The highest disease suavity index (DSI) was 30.2%, recorded in Sahiwal followed by 27% in Sheikhupura. DSI in other districts namely Narowal, Gujranwala, Hafizabad, Sialkot, Lahore and Okara was 20.1, 23.1, 17.4, 26.5, 20.8 and 22.8%, respectively (Fig. 2C). Variation in incidence and severity of stalk rot disease at different locations might be attributed to variation in various soil and environmental factors among the locations (Doohan *et al.*, 2003; Scauflaire *et al.*, 2011). Dorn *et al.* (2009) carried out a survey for stalk rot of maize in two areas of Switzerland for two consecutive years and found significant variation in disease occurrence between years and locations ranging from 24.2% to 83.8%. Variation in temperature, humidity etc were among the factors responsible for variation in disease with respect to years and locations.

Table 1.Surveyed areas of different areas of Central and South Punjab, Pakistan.

Districts	Locations	Latitude	Longitude
Sialkot	Daska	32.329	74.378
	Sambrial	32.468	74.354
	Chaprar	32.634	74.635
Narowal	Siran	32.121	74.858
	Mahianwala	32.109	74.855
	Zafarwal	32.115	74.87
Gujranwala	Jhangi	32.135	74.236
	Butala Road	32.1	74.23
	GondlanWala	32.19	74.125
Hafizabad	PindiBhattian	31.048	73.45
	Kiyara	31.935	73.562
	Pear da Khoo	31.938	73.516
Sheikhupura	KotJeewan Singh	31.671	73.906
	Kalokey	31.761	73.898
	Tahiwala	31.617	73.988
Lahore	Barki	31.473	74.51
	Gujran	31.531	74.519
	Wagah	31.598	74.549
Okara	RenalaKhurd	30.905	73.602
	DepalPur	30.679	73.629
	ChakSamundri 42 sp	30.418	73.669
Sahiwal	ChakNo90/6R	30.69	73.039
	Chak No 134/9-L	30.63	73.069
	Daudpur	30.615	73.133
Khanewal	Kabirwala	30.357	71.842
	Mianchannu	30.515	72.232
	Jahanian	30.061	71.906
Multan	19 kassi	30.126	71.58
	Shujaabad	29.803	71.326
	Shahkot	29.994	71.485
Muzaffargarh	Kot Addu	30.44	70.955
	Jatoi	29.514	70.869
	Qasba Gujrat	30.16	70.942
Vehari	Luddan	29.963	72.282
	Chak No 69 WB	29.757	72.187
	Mailsi	29.783	72.169
Lodhran	Thada Minor Rd	29.54	71.64
	KahrorPakka	29.624	71.894
	Dunyapur	29.794	71.712

Fig. 1. Stalk rot of maize in Punjab.

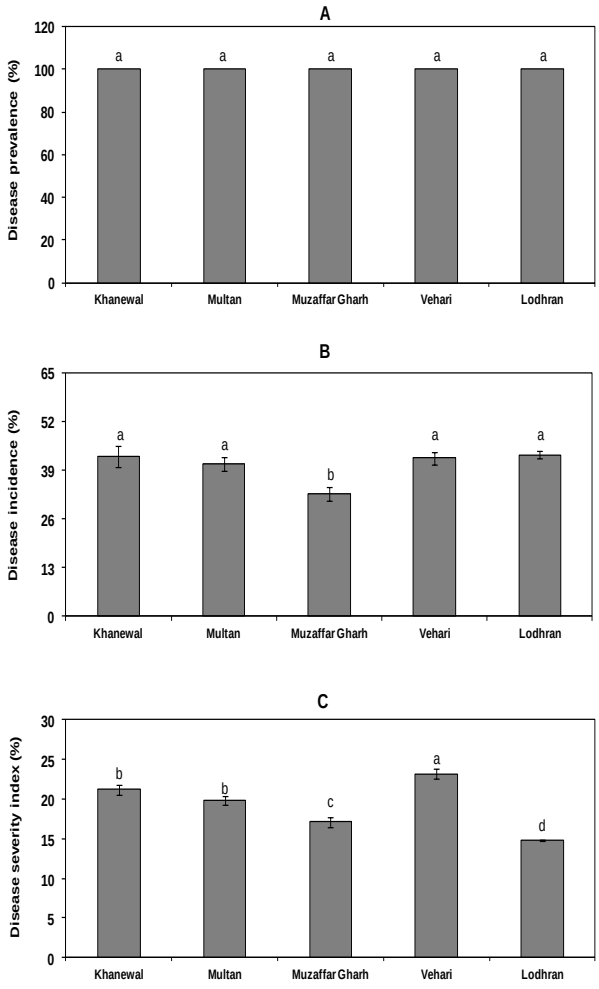

Fig.2. Prevalence (A), incidence (B) and severity index (C) of stalk rot disease of maize in different districts of Central Punjab. Vertical bars show standard deviation of means of 9 replicates. Different letters show significant difference at P = 0.05 as determined by LSD test.

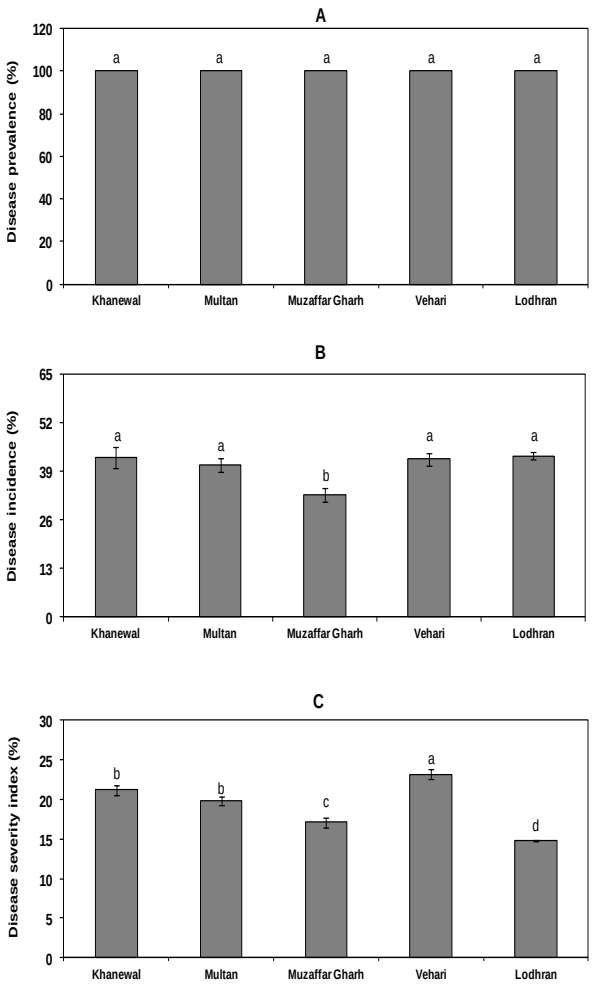

Fig.3. Prevalence (A), incidence (B) and severity index (C) of stalk rot disease of maize in different districts of South Punjab. Vertical bars show standard deviation of means of 9 replicates. Different letters show significant difference at P = 0.05 as determined by LSD test.

Like in Central Punjab, stalk rot disease was also found in all the surveyed fields of South Punjab showing 100% prevalence (Fig. 3A). Disease incidence ranged from 33-43% in different districts. The difference in disease incidence among the four out of five districts namely Khanewal, Multan, Vehari and Lodhran was insignificant where it ranged from 41-43%. By contrast, Muzaffargarh the disease incidence was only 33% and was significantly ($P = 0.05$) lower as compared to other surveyed districts of this region (Fig. 3B). DSI was highly variable in different districts of the South Punjab. It was the highest in Vehari (23.2%) that was significantly higher than DSI in all other districts. DSI in Khanewal, Multan, Muzaffargarh and Lodhran was 21.2, 19.8, 17.11 and 14.8%, respectively (Fig. 3C).

A total of 8 *Fusarium* spp. were isolated from diseased maize plants samples collected from different districts of Central Punjab. These included *F. vericeiliodes, F. oxysporum, F. prolifiratum, F. moniliforme, F. bullatum, F. thapsinum, F. incarnatum*and *F. sacchari.* Literature shows that in many other countries such as Belgium, China, South Africa, Switzerland different *Fusarium* spp. namely *F. verticillioides,F. subglutinans, F. prolifiratum, F. commune, F. graminearum, F. avenaceum, F. culmorum, F. equiseti,* and *F. crookwellense*are associated with stalk rot of maize (Dorn *et al.,* 2009; Scauflaire *et al.,* 2011; Yu *et al.,* 2017; Schoeman *et al.,* 2018). However, in contrast to findings of the present study, Ahmad *et al.* (2000) collected infected maize samples from all the four provinces of Pakistan and identified *Fusarium* spp. along with other fungal species also. They identified a total of 17 fungal species out of which *Macrophomina phaseolina* was the most abundant in all the provinces followed by *F. moniliforme* and *Rhizoctonia solani.* Other important species included *Fusarium graminearum, Pythium aphanidermatum, Fusarium roseum, Cephalosporium acremonium* and *F. oxysporum.* Likewise, Francis and Burgess (1975) also reported *Fusarium roseum*as major species along with other fungi namely *Diplodia maydis, Bipolaris sorokiniana, Rhizoctonia* sp. and *Macrophomina phaseolina* associated with stalk rot of maize Australia.

The present study concludes that generally stalk rot of maize is present in almost all the maize fields of Central and South Punjab with variable intensities. Vehari, Sialkot, Sheikhupura and Sahiwal are among the predominant areas with respect to severity of stalk rot of maize. Different *Fusarium* spp. are responsible for stalk rot of maize in Punjab.

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