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Resistance source detection against stalk rot (*Fusarium verticilliodes*) under different seasons by two disease assessment methods

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Abstract

Hundred maize genotypes along with two checks were grown in Maize and Millet Research Institute (MMRI) Sahiwal-Pakistan in autumn (2009) and spring (2010) to check severity of stalk rot (*Fusarium Verticilliodes*) under natural infection and artificial inoculation. The disease was recorded using SR (stalk rating) and LL (lesion length). Classification of genotypes was performed by using rank-sum analysis. Means of severity recorded on the basis of SR and LL in autumn were higher than spring under both methods of disease assessment. SR only exhibited significant results ($P < 0.05$) in autumn under natural infection while LL in natural infection and SR and LL in artificial inoculation showed highly significant results ($P < 0.01$) in both seasons. Genotype-season interaction was highly significant ($P < 0.01$) for both severity scale and seasons except for SR ($P < 0.05$) under natural infection. Means of SR for both seasons showed highly significant results ($P < 0.01$) under artificial inoculation. In natural infection, significant positive correlation ($r = 0.90$ to 0.93; $P < 0.01$) was noted between SR and LL while same correlation ($r = 0.82$ to 0.90; $P < 0.01$) was also studied under artificial inoculation between SR and LL under autumn and spring season respectively. Inbred lines (EL7, EL17, and Y11) showed highly resistance against stalk rot under both seasons and disease assessment methods. These highly resistance genotypes may be used as donor of resistant gene or genes in highly adaptive and productive susceptible varieties in further breeding programms.

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Introduction

Importance of any cash crop can be evaluated by its area of production, utilization and share in trade. The same benchmark can be utilized for maize to judge its importance among cereal crops. Maize (*Zea mays* L.) is grown as a multipurpose crop in subtropical, tropical and temperate regions and its production is above 600 metric tonnes (McDonald & Nicol, 2005) in the world. In different regions of the world, different pathogens are involved in causing stalk rot in maize (Thon *et al.*, 2002; Yang *et al.*, 2002a). Among these Fungus ranks second that is involved in causing destructive loss in maize (Galperin *et al.,* 2003). Kossou and Aho (1993) studied that fungi could cause up to 50 – 80 % losses in maize. Maize is the vital fodder as well as grain crop of the world whose quality of grain and stalk is directly affected by stalk rot disease (Jim, 1999). Different *Fusarium* species are involved in promoting stalk rot (Kommedahl & Windels, 1981) disease but *Fusarium verticilloides (F.moniliforme)* depicted more drastic effect (Neish *et al.,* 1983; Anonymous, 1985). Ahmad *et al.*, (1997) elaborated that *verticilloides* is the major damaging specie of *Fusarium* in Pakistan. *Fusarium* species make a path into maize plant through exposed plant parts like node or wounded parts created by insects or any mechanical damage (Gatch & Munkvold, 2002; Koehler, 1960). Its presence in leave and stem is neglected due to its Symptomless infection (Munkvold & Desjardins, 1997).Stalk rot promotes pre-maturity that is the source of plant lodging and reduction in yield (Sibale *et al.*, 1992a). Lodging not only causes harvesting difficulties but also create environment for insect invadation. Different biotic and abiotic stresses that affect the photosynthesis process are the major sources of severe stalk rot (Dodd, 1980). Being a fodder crop, most of the livestalk feed depends upon it (Lenne & Thomas, 2006)). Due to increase in population, rangelands are being replaced into cropland to increase production of the crops for food and feeds (Romney *et al.*, 2003). Under this situation, it is necessary to develop stalk rot resistant maize cultivar in order to minimize the losses by *Fusarium* stalk rot (Munkvold, 1996). Many studies (Hooker, 1973; Hooker & Draganic, 1980) focus on the use of artificial inoculation techniques in order to evaluate the germplasm. But Koehler (1960) believes that disease symptoms are more clear through natural infections. Some shows the importance of both natural infection as well as artificial inoculation (Ledencan *et al.*, 2003). Warm and dry conditions are favorable for stalk rot disease (White, 1999). However warm temperature and low rainfall followed by inoculation create environment for the disease enhancement (Dodd, 1980; Afolabi *et al.*, 2008). So objective of study is to evaluate the performance of genotypes under natural infection and artificial inoculation under different seasons and resistant source detection.

Material and methods

The research work elaborated in this manuscript was performed at Maize and Millet Research Institute (MMRI) Yousafwala -Pakistan during the year 2009-10. The place is situated at 30.34° North, 71.91° South and at altitude of 213 meters above sea level.

Fungus isolation and identification

Pathogen was separated from diseased plant debris including leaves, nodes and stalks. Infected material was cut into small pieces (0.5 to 1 cm) and surface sterilized with 2 % Sodium-hypo chloride for two minutes. These cut pieces were then washed at least thrice with sterilized distilled water and placed on half strength Potato sucrose Agar (PSA) nutrient medium plates (Johnston and Booth, 1968) with 200 mg L^{-1} streptomycin. The plates were incubated at 25 C° with 12 hours photoperiod. A fungus was identified by Nikon light microscope in the front of Laminar air Flow cabinet after 3 and 7 days. Fungi were marked at species level by considering their growth traits, spore size and formation. Different isolates of stalk rot were separated from the samples and single spore cultures were maintained. Correct identification of fungus on apparent traits was notified by placing each culture on simple nutrient agar medium (Nirenberg, 1976). Culture was stored in refrigerator after proper identification.

Selection of plant genotypes.

Hundred maize genotypes along with two checks varieties (Sahiwal-2002,Agatti-2002)were selected from MMRI and grown in its experimental fields in autumn (2009) and spring (2010) with two disease assessment methods (natural infection and artificial inoculation).

Experimental design

The experiment was conducted in MMRI fields to identify the resistance/tolerance source in the germplasm by natural infection as well as artificial inoculation method. Separate fields were maintained for each assessment method in each season. Seeds were grown in augmented experimental design with no replication. Planting for each field was performed manually in a single row plot having 10 meter in length with 2 seeds per hill later on thinned to one by maintaining plant to plant distance 20 cm and row to row distance 75cm. A standard doze of fertilizer 200-100-60 (autumn) and 250-125-60 (spring) NPK per hectare was applied to each trial. The crop was given normal hoeing, irrigation, earthling up and 2-3 foliar insecticidal application to protect the crop against maize borer and other sucking insects.

Pollination procedure

In maize male and female parts are located on the same plant (monoecious) and naturally favored by cross pollination. Tassels and shoots of parents were covered to prevent them from contamination by wind circulated pollens. For this purpose, apron with pockets, tassel bags, ear shoots bags, paper clips, knife of suitable size and lead pencil were used.

Plants grown in the experimental fields were checked daily in the morning at flowering stage to cover the tassels and shoots. Each ear shoot was covered before silk emergence to avoid the fertilization from unknown pollen. Similarly tassels were also covered to prevent pollen dehiscence. Pollination was performed after 24 hours of tassel and shoot preparation. Pollens are mostly released after 3 hours of sun rising but it may occur earlier or later depending on the temperature and humidity. Tassel bag was gently shaken for pollen dehiscence before removing to collect the viable pollens. In order to check the resistance and maintain the purity level, pollens of plants were selfed on the same plant silk in each row. Tassel bags containing viable pollens were placed over the ear shoot until harvesting was performed.

Inoculation technique

Toothpicks colonized by *Fusarium Verticilliodes* were utilized as inoculum material to study the behavior of stalk rot disease under artificial infection as elaborated by **(**Drepper & Renfro, 1990). Preparation of inoculum for toothpicks was performed as described by Jardine and Leslie (1992). Sterilized nail having 1.8mm diameter fitted on wooden handles were utilized to make hole in the stalk without harming the stalk tissues at first elongated internode after anthesis. Inoculated toothpicks were injected into the holes and properly waxed after injection to avoid other fungi to enter the hole and disturb the action of inoculant.

Disease scoring

Stalks of five plants randomly selected in each row were split longitudinally below the ear after 40 and 45 days of the inoculation at physiological maturity in autumn and spring respectively. Each spilt stalk rating (SR) was rated visually by Hooker (1956) scale method where 1(25% inoculated inter node discolored)= H.R, 2(26-50% inoculated inter node discolored) = R, 3(51-75% inoculated inter node discolored)= M.R, 4(71-100% inoculated inter node discolored) = M.S, 5(50% of adjacent internodes discolored)= S, 6-10(More than 50% of adjacent internodes discolored and above)= H.S. Disease was also assessed quantitatively by calculating the length of discoloration as a proportion of the length of four internodes multiplied by 100 and named as lesion length (LL). Weather data (maintained manually) was obtained from the registers of MMRI for both seasons.

Statistical analysis

Data collected in each season (autumn and spring)

with two assessment methods about stalk rot severity (SR and LL) was analyzed through SigmaXL software. Effect of genotypes as well as their interaction with each season was also calculated separately. Correlation between SR and LL was described to see the association between severity scales under each assessment methods in two seasons. Rank-sum method elaborated by Onyeka *et al.*, (2005) was used to separate the lines in to different resistant/susceptible levels by using the mean of SR and LL under two different assessment methods. Genotypes ranked on the basis of rank-sum method were also tested for the normality. Ranking of the mean of SR and LL was preformed manually from smallest to largest one. Sum of the rank for each genotype (y_n) was calculated and compared with sum of ranks for all genotypes (G). Deviation of each genotype from the grand mean was estimated by the formula ([(y_n -G)/ standard deviation] x 2). Genotypes depicting positive deviation (towards the right of grand mean) on mean distribution curve were considered as susceptible one while genotypes showing negative deviation (towards the left of grand mean) were rated as resistant one. Genotypes having 1,2 and 3 deviations were ranked as moderately susceptible, susceptible, and highly susceptible while genotypes having -1,-2 and -3 deviations were classified as moderately resistant, resistant and highly resistant respectively (Onyeka *et al.,* 2005).

Results

Effect of stalk rot under natural infection

Effect of *Fusarium* stalk rot was recorded in both years (2009-10) during autumn (July-October) and spring (February-June) season under natural conditions. Stalk rating (SR) ranged from 1.2 to 8.2 with the mean of 4.56 in autumn while 1.1 to 8.2 with the mean of 4.09 in spring season. lesion length (LL) varied from 2.6 to 18.5% with mean of 11.15% in autumn while 2.4 to 18.2% with the mean of 10.13% in spring (table 1).

Table 1. Effect of stalk rot observed on maize genotypes under natural conditions during autumn (2009) and spring (2010).

Autumn (2009)						Spring (2010)			
Treatments	SR	LL%	Treatments	SR	LL%	SR	LL%	SR	LL%
DR10	3.6	8.9	DR74	3.5	8.9	2.1	4.4	3.1	8.6
DR14	7.5	13.9	DR75	5.7	17.2	4.8	11.1	5.6	15.1
DR16	7.6	14.4	DR76	5.5	16.5	7.3	15.8	5.7	15.3
DR17	5.4	14.3	DR77	5.5	14.6	3.7	8.8	4.8	12.4
DR19	5.3	12.4	DR78	5.6	15.1	4.7	13.3	3.5	7
DR20	7.4	14.3	DR79	4.1	9.7	7.4	15.3	3.6	7
DR26	6.5	16.1	DR80	5.4	16.6	7.3	17.4	5.3	15.8
DR27	4.1	12.3	DR81	5.7	15.4	3.8	8.7	5.8	15.2
DR30	6.5	15.4	DR82	6.4	14.3	5.8	16.2	6.3	14.1
DR31	4.6	12	DR83	4.5	10.9	4.8	13.4	3.4	7.9
DR32	5.9	15.6	DR84	5.4	15.5	5.9	15.1	5.4	16.9
DR33	5.8	12.8	DR85	3.3	10.1	5.7	13.7	3.2	9.6
DR34	3.5	8.2	DR9	4.4	14.3	3.1	9.7	4.2	12.6
DR35	4.3	12	EL17	1.2	2.9	4.1	11.6	1.2	2.6
DR36	4.8	12.8	EL7	1.2	3.1	5.5	15.3	1.1	2.8
DR38	4.5	13.3	sahiwal-2002	7	13.7	3.7	9.4	7.1	13.3
DR39	5.3	15.2	agatti-2002	6.9	13.2	4.7	10.9	7.1	13.2
DR40	4.2	12.7	Y11	1.4	2.7	3.7	9.7	1.2	2.5
DR41	5.8	14.8	Y12	2.1	5.3	5.8	14.2	1.2	2.6
DR42	5.5	16.8	Y13	2.2	4.6	4.1	12.1	1.5	2.4
DR43	6.8	15.6	Y14	2	4.6	6.9	17.3	1.6	2.4
DR44	4.3	11.9	Y15	3.2	8.5	3.6	8.1	1.4	2.9
DR46	5.6	15.5	Y18	3.3	8	5.5	15.3	1.3	2.7
DR48	6.3	15.5	Y19	1.3	2.9	6.2	14.5	2.2	4.1

DR49	5.7	14	Y2	2.7	4.8	5.6	14.1	3.2	9.7
DR5	4.2	12.8	Y20	2.5	4.4	3.8	7.7	3.3	8.5
DR50	6.2	16.1	Y22	1.5	2.6	5.8	15.8	2.1	4
DR51	6.4	15.6	Y24	2.1	5.3	6.1	18.2	2.1	3.9
DR52	5.6	17.7	Y25	2.1	4.4	5.5	18.1	2.1	4.7
DR53	5.5	16.1	Y26	3.2	7.9	3.8	8	3.2	7.5
DR54	6.7	17.6	Y27	3.3	8.9	6.3	16.9	3.2	8.5
DR55	6.5	14.8	Y29	2.1	4.6	3.1	10.4	2	3.9
DR56	6.4	18.5	Y3	2.8	5.7	6.4	14.5	2.3	4.5
DR57	8	14.8	Y30	3.2	9	7.5	15	3.1	7.5
DR58	6.6	17.2	Y31	3.1	8.9	3.1	8.9	3	8.6
DR59	6.6	18.3	Y32	2.1	5	6.6	15.8	2.3	4.1
DR60	6.3	16.1	Y35	2	5.2	6.3	14.8	2.1	4.4
DR61	4.4	11.7	Y36	3.2	9	3.5	8.7	3.4	7.7
DR62	3.5	7.8	Y37	3.1	8.2	3.1	9.3	3.1	8.8
DR63	6.4	15.2	Y38	3.3	8.9	3.3	8.7	2.2	4.6
DR64	5.6	14.9	Y5	2.1	5.1	5.6	12.5	2.1	4.4
DR65	4.9	11.1	Y6	2.6	5.3	5.2	14.5	3.1	8.1
DR66	6.5	15	Y7	3.2	9.3	3.4	9.2	3	7.6
DR67	5.5	17.7	Y8	3.1	10.2	4.7	11.3	3.2	7.8
DR68	6.2	14.8	Y81	4.8	10.5	6.1	14.5	3.1	8.5
DR69	8.2	15.6	Y83	3.8	8.7	8.2	15.4	3.1	8.3
DR7	4.3	13.2	Y85	4.7	12.1	4.1	11.4	3.2	8.5
DR70	4.2	12.8	Y9	2.1	5	3.5	8.5	2.2	4.7
DR71	6.2	15.6	Y93	3.8	7.8	6.1	15.3	3.2	7.8
DR72	5.4	17.8	Y95	3.7	8.1	5.3	13.8	3.1	7.4
DR73	5.8	16	Y97	3.7	9.2	3.2	9.4	3.2	8.5
			Mean	4.56	11.55			4.09	10.13
			Max	8.2	18.5			8.2	18.2
			Min	1.2	2.6			1.1	2.4

SR= stalk rating and LL= lesion length, Max=maximum value; Min=minimum value.

Table 2. Effect of stalk rot observed on maize genotypes under artificial conditions during autumn (2009) and spring (2010).

Autumn (2009)						Spring (2010)			
Treatments	SR	LL%	Treatments	SR	LL%	SR	LL%	SR	LL%
DR10	3.6	9	DR74	3.7	10	2.3	5.8	3.1	10.7
DR14	7.7	17.6	DR75	6.3	16.7	5.1	17.6	6.2	14.9
DR16	7.8	14.5	DR76	5.5	17.6	7.5	18.7	4.2	15.8
DR17	5.6	17.5	DR77	8.6	16.5	4.1	13.8	7.9	17.4
DR19	5.7	18.8	DR78	5.8	17	4	17.9	4.4	13.3
DR20	7.7	16.7	DR79	4.4	11.3	7.6	15.6	3.8	8.1
DR26	7	15.9	DR80	8.6	18.8	7.7	18.9	7.9	18.7
DR27	3.4	14.4	DR81	6.5	17.6	3.1	14.3	8	18.6
DR30	5.7	18.1	DR82	6.6	16.2	5.3	14.6	8	14.5
DR31	5.2	19.4	DR83	4.7	12.8	5.2	18.1	3.6	9.1
DR32	8.5	19.4	DR84	5.6	17.5	8.2	17.6	5.6	17.4
DR33	6.9	18.6	DR85	3.5	10.2	6.5	19.6	3.3	11
DR34	3.8	9.2	DR9	4.7	16.7	3.2	9.7	4.3	12.8
DR35	4.5	14	EL17	1.2	3.8	4.2	11.9	1.1	3.1
DR36	5.5	16.3	EL7	1.3	4	5.6	14.8	1.2	3.3
DR38	5.4	16.2	sahiwal-2002	7.9	14.5	4.1	11.8	7	19
DR39	5.6	14.1	agatti-2002	8	14.3	5	15.3	7.2	17.6
DR40	4.4	14.8	Y11	2	7.1	4.2	10.3	1.3	2.9
DR41	6.4	17.4	Y12	2.4	6.9	6	15.6	2.1	6.6
DR42	5.7	17.2	Y13	2.5	8.1	4.3	12.6	2.3	6.1
DR43	7.1	15.7	Y14	2.2	7.5	7	15.8	2.4	5.9
DR44	4.5	13.9	Y15	3.4	11.7	4.2	13.9	2.1	5.8

DR46	5.8	19	Y18	3.5	9.7	5.7	16.8	2	6.1
DR48	6.5	16.3	Y19	2	7.3	6.4	15.7	2.3	5.6
DR49	6.2	16.2	Y2	3.2	9.3	6	16.3	3.2	11.1
DR5	3.3	15	Y20	3.2	9.2	3.2	11.3	3.4	9.7
DR50	6.4	18.2	Y22	3.7	7.1	6.2	15.8	2.2	5.3
DR51	8.5	17.4	Y24	3.4	7.5	8.2	17.9	2.5	5.1
DR52	6.6	17.6	Y25	3.6	6.6	6.5	17.8	2.2	6.1
DR53	4.9	17.5	Y26	3.3	9.1	4.1	12.8	3.2	9.9
DR54	6.9	17.5	Y27	3.5	10.2	6.8	16.4	3	9.7
DR55	8.5	18.2	Y29	3.4	6.5	8	17.6	2.4	5.1
DR56	8.6	17.6	Y3	3.4	9.9	8.1	18.7	2.4	6.1
DR57	8.7	20	Y30	3.4	10.4	8	18.6	3.1	9.9
DR58	6.7	19.9	Y31	3.2	10.2	3.2	10.2	3	8.9
DR59	8.5	16.7	Y32	2.2	6.2	8.2	19	2.5	5.4
DR60	8.5	20.8	Y35	2.1	7.5	8	14.9	2.3	5.8
DR61	3.5	13.7	Y36	3.5	10.4	3	10	3	9.7
DR62	3.3	10.6	Y37	3.3	9.4	3.2	11.1	3.2	8.6
DR63	6.6	20.7	Y38	3.5	10.2	3.5	9.9	2.4	6
DR64	5.9	18.2	Y5	3.4	7.5	5.6	15.7	2.1	5.6
DR65	5.5	16.8	Y6	3.4	10.6	5.4	15.8	3.1	9.2
DR66	6.7	19.4	Y7	3.3	11	3.6	10.6	3	8.7
DR67	5.7	20	Y8	3.3	10.6	5.3	12.9	3.2	8.7
DR68	8.5	16.7	Y81	5	15.4	8	18.6	3.1	8.8
DR69	8.4	18.2	Y83	3.3	15.5	8.3	18.5	3.2	10.1
DR7	4.6	15.4	Y85	5	17.6	4.2	12.9	3.3	9.5
DR70	4.3	15	Y9	3.2	6.9	3.7	9.7	2.3	5.6
DR71	6.4	17.2	Y93	4.1	12.5	6.3	15.2	3.4	9.7
DR72	5.6	20.1	Y95	4	12	4.1	14.9	3.2	8.9
DR73	6.6	17	Y97	4	13.6	3.5	10.7	3.3	8.5
			Mean	5.08	13.94			4.48	12.06
			Max	8.7	20.8			8.3	19.6
			Min	1.2	3.8			1.1	2.9

SR= stalk rating and LL= lesion length, Max=maximum value; Min=minimum value.

Severity recorded on the basis of SR and LL was higher in autumn than spring season.SR exhibited significant results ($P < 0.05$) in autumn while highly significant results ($P < 0.01$) in spring. LL showed highly significant results ($P < 0.01$) in both season. Highly significant genotype-season interaction was recorded on the basis of SR ($P < 0.01$) in spring while significant results ($P < 0.05$) were obtained in autumn. LL recorded on the basis of genotype-season interaction showed highly significant results ($P < 0.01$) in both seasons. Mean of SR in both season showed non-significant results ($P > 0.05$) while mean of LL showed highly significant response ($P < 0.01$). Maximum value of SR was observed in DR69 (8.2) while minimum value of SR was observed in EL7 and EL17 (1.2) in autumn. Similarly maximum value of LL was observed in DR56 (18.5) while minimum value was noted in Y22 (2.6). In spring season, maximum SR and LL was observed in DR69 (8.2) and DR51 (18.2) while minimum value of SR and LL was found 1.1(EL7) and 2.4(Y13, Y14) respectively (table 1). Varietal checks including agatti-2002 and sahiwal-2002 showed SR 6.9 and 7 while LL 13.2 and 13.7 in autumn season respectively. In spring SR 7.1 and 7.1 while LL 13.2 and 13.3 were recorded in agatti-2002 and sahiwal-2002 respectively. Significant positive correlation ($r = 0.90$ to 0.93; $P < 0.01$) was studied between SR and LL under autumn and spring season respectively (table 2). Coefficient of determination (R^2) showed strong association between SR and LL (0.82 to 0.87) under autumn and spring season respectively (Fig 1, 2).Classification of genotypes on the basis of rank-sum analysis (table 3) elaborated that 8 genotypes were highly resistant, 14 were resistant, 28 were moderately resistant, 24 were moderately susceptible, 23 were susceptible (including agatti-2002 and sahiwal-2002) and 5 were highly susceptible (Fig 3). Frequency distribution of all genotypes ranked on the basis of their resistance level in natural infection appeared to be continuous and Anderson-Darling test ($P < 0.05$) showed the normality of data.

Table 3. Rank sum classification method for stalk rot rating of genotypes for autumn and spring under natural conditions.

Stalk Rating			Lesion Length			Genotype ranking	
Treatment	SR[n]	W	LL[n]	X	Y	Z	Classes[n]
EL17	1.2	2	2.75	2	4	-3.4	HR
EL7	1.15	1	2.95	3	4	-3.4	HR
Y11	1.3	3	2.6	1	4	-3.4	HR
Y12	1.65	4	3.95	6	10	-3.1	HR
Y19	1.75	5	3.5	5	10	-3.1	HR
Y22	1.8	6	3.3	4	10	-3.1	HR
Y14	1.8	6	3.5	5	11	-3.1	HR
Y13	1.85	7	3.5	5	12	-3.1	HR
Y29	2.05	8	4.25	7	15	-2.9	R
Y25	2.1	9	4.55	8	17	-2.8	R
Y24	2.1	9	4.6	9	18	-2.8	R
Y32	2.2	11	4.55	8	19	-2.7	R
Y35	2.05	8	4.8	11	19	-2.7	R
Y5	2.1	9	4.75	10	19	-2.7	R
Y9	2.15	10	4.85	12	22	-2.6	R
Y18	2.3	12	5.35	14	26	-2.4	R
Y3	2.55	13	5.1	13	26	-2.4	R
Y15	2.3	12	5.7	15	27	-2.4	R
DR10	2.85	15	6.65	17	32	-2.1	R
Y20	2.9	16	6.45	16	32	-2.1	R
Y38	2.75	14	6.75	19	33	-2.1	R
Y6	2.85	15	6.7	18	33	-2.1	R
Y2	2.95	17	7.25	20	37	-1.9	MR
Y26	3.2	21	7.7	21	42	-1.7	MR
Y30	3.15	20	8.25	24	44	-1.6	MR
Y7	3.1	19	8.45	26	45	-1.5	MR
Y37	3.1	19	8.5	27	46	-1.5	MR
Y95	3.4	24	7.75	22	46	-1.5	MR
Y31	3.05	18	8.75	30	48	-1.4	MR
Y36	3.3	23	8.35	25	48	-1.4	MR
Y93	3.5	26	7.8	23	49	-1.3	MR
DR62	3.3	23	8.55	28	51	-1.2	MR
Y27	3.25	22	8.7	29	51	-1.2	MR
DR79	3.85	27	8.35	25	52	-1.2	MR
Y83	3.45	25	8.5	27	52	-1.2	MR
DR74	3.3	23	8.75	30	53	-1.1	MR
Y8	3.15	20	9	33	53	-1.1	MR
DR34	3.3	23	8.95	32	55	-1	MR
Y97	3.45	25	8.85	31	56	-1	MR

DR85	3.25	22	9.85	36	58	-0.9	MR
DR83	3.95	28	9.4	34	62	-0.7	MR
Y81	3.95	28	9.5	35	63	-0.7	MR
DR44	3.95	28	10	37	65	-0.6	MR
DR61	3.95	28	10.2	38	66	-0.5	MR
DR5	4	29	10.25	39	68	-0.4	MR
Y85	3.95	28	10.3	40	68	-0.4	MR
DR27	3.95	28	10.5	41	69	-0.4	MR
DR70	3.85	27	10.65	42	69	-0.4	MR
DR40	3.95	28	11.2	44	72	-0.3	MR
DR38	4.1	30	11.35	45	75	-0.1	MR
DR78	4.55	34	11.05	43	77	0	MS
DR35	4.2	31	11.8	47	78	0	MS
DR17	4.55	34	11.55	46	80	0.1	MS
DR7	4.2	31	12.3	51	82	0.2	MS
DR53	4.65	35	12.05	49	84	0.3	MS
DR63	4.85	38	11.95	48	86	0.4	MS
DR73	4.5	33	12.7	54	87	0.4	MS
DR66	4.95	39	12.1	50	89	0.5	MS
DR31	4.7	36	12.7	54	90	0.6	MS
DR55	4.8	37	12.6	53	90	0.6	MS
DR9	4.3	32	13.45	60	92	0.7	MS
DR58	4.85	38	13.05	57	95	0.8	MS
DR19	5	40	12.85	56	96	0.9	MS
DR65	5.05	41	12.8	55	96	0.9	MS
DR39	5	40	13.05	57	97	0.9	MS
DR42	4.8	37	14.45	65	102	1.1	MS
DR77	5.15	43	13.5	61	104	1.2	MS
DR14	6.15	53	12.5	52	105	1.3	MS
DR36	5.15	43	14.05	63	106	1.3	MS
DR33	5.75	49	13.25	59	108	1.4	MS
DR67	5.1	42	14.5	66	108	1.4	MS
DR64	5.6	47	13.7	62	109	1.5	MS
DR49	5.65	48	14.05	63	111	1.6	MS
DR41	5.8	50	14.5	66	116	1.8	MS
DR46	5.55	46	15.4	74	120	2	S
DR68	6.15	53	14.65	67	120	2	S
DR82	6.35	56	14.2	64	120	2	S
agatti-2002	7	62	13.2	58	120	2	S
DR72	5.35	44	15.8	77	121	2	S
DR81	5.75	49	15.3	72	121	2	S
DR32	5.9	51	15.35	73	124	2.2	S
DR48	6.25	54	15	70	124	2.2	S

Sahiwal 2002	7.05	63	13.5	61	124	2.2	S
DR76	5.6	47	15.9	78	125	2.2	S
DR80	5.35	44	16.2	81	125	2.2	S
DR84	5.4	45	16.2	81	126	2.3	S
DR71	6.15	53	15.45	75	128	2.4	S
DR75	5.65	48	16.15	80	128	2.4	S
DR30	6.15	53	15.8	77	130	2.4	S
DR60	6.3	55	15.45	75	130	2.4	S
DR50	6	52	15.95	79	131	2.5	S
DR20	7.4	64	14.8	68	132	2.5	S
DR52	5.55	46	17.9	88	134	2.6	S
DR57	7.75	66	14.9	69	135	2.7	S
DR16	7.45	65	15.1	71	136	2.7	S
DR51	6.25	54	16.9	85	139	2.9	S
DR56	6.4	57	16.5	83	140	2.9	S
DR43	6.85	60	16.45	82	142	3	HS
DR69	8.2	67	15.5	76	143	3.1	HS
DR26	6.9	61	16.75	84	145	3.1	HS
DR54	6.5	58	17.25	87	145	3.1	HS
DR59	6.6	59	17.05	86	145	3.1	HS
Mean	4.32		10.84		77.51^{G}		
Max	8.2		18.35				
Min	1.15		2.5				

SR^{n} = mean of stalk rating for autumn and spring under natural conditions, LL^{n} = mean of lesion length % for autumn and spring under natural conditions, w = genotype ranking using SR, x= genotype ranking using LL, y = rank-sum for (w + x) each genotype, z=deviation from grand mean of rank-sums ([z=(y-G)/ standard deviation]x2), G= grand mean of rank-sums, $classes^{n}$ = (HR= highly resistance, R=resistant,MR=moderately resistant, MS=moderately susceptible, S= susceptible, HS= highly susceptible),Max=maximum value, Min=minimum value.

Table 4. Rank sum classification method for stalk rot rating of genotypes for autumn and spring under artificial conditions.

Stalk Rating			Lesion Length		Genotype ranking		
Treatment	SR^{a}	W	LL^{a}	X	y	z	$classes^{a}$
EL17	1.15	1	3.45	1	2	-3.4	HR
EL7	1.25	2	3.65	2	4	-3.3	HR
Y11	1.65	3	5	3	6	-3.2	HR
Y29	2.9	11	5.8	4	15	-2.8	R
Y9	2.75	10	6.25	6	16	-2.8	R
Y22	2.95	12	6.2	5	17	-2.7	R
Y12	2.25	6	6.75	12	18	-2.7	R
Y14	2.3	7	6.7	11	18	-2.7	R
Y19	2.15	4	7.4	14	18	-2.7	R

Y32	2.35	8	6.65	10	18	-2.7	R
Y24	2.95	12	6.3	7	19	-2.7	R
Y25	2.9	11	6.35	8	19	-2.7	R
Y35	2.2	5	7.4	14	19	-2.7	R
Y5	2.75	10	6.55	9	19	-2.7	R
Y13	2.4	9	7.1	13	22	-2.5	R
Y18	2.75	10	7.9	15	25	-2.4	R
DR10	2.95	12	7.4	14	26	-2.3	R
Y3	2.9	11	8	16	27	-2.3	R
Y15	2.75	10	8.75	18	28	-2.3	R
Y38	2.95	12	8.1	17	29	-2.2	R
Y31	3.1	13	9.55	22	35	-2	R
Y37	3.25	16	9	19	35	-2	R
Y20	3.3	17	9.45	20	37	-1.9	MR
Y26	3.25	16	9.5	21	37	-1.9	MR
DR34	3.5	19	9.45	20	39	-1.8	MR
Y7	3.15	14	9.85	25	39	-1.8	MR
Y8	3.25	16	9.65	23	39	-1.8	MR
Y6	3.25	16	9.9	26	42	-1.6	MR
Y27	3.25	16	9.95	27	43	-1.6	MR
Y36	3.25	16	10.05	28	44	-1.6	MR
Y2	3.2	15	10.2	30	45	-1.5	MR
Y30	3.25	16	10.15	29	45	-1.5	MR
DR74	3.4	18	10.35	31	49	-1.3	MR
DR79	4.1	25	9.7	24	49	-1.3	MR
DR62	3.25	16	10.85	34	50	-1.3	MR
DR85	3.4	18	10.6	33	51	-1.2	MR
Y95	3.6	20	10.45	32	52	-1.2	MR
DR61	3.25	16	11.85	38	54	-1.1	MR
Y97	3.65	21	11.05	36	57	-1	MR
Y83	3.25	16	12.8	42	58	-0.9	MR
Y93	3.75	22	11.1	37	59	-0.9	MR
DR5	3.25	16	13.15	44	60	-0.8	MR
DR83	4.15	26	10.95	35	61	-0.8	MR
DR70	4	23	12.35	40	63	-0.7	MR
Y81	4.05	24	12.1	39	63	-0.7	MR
DR27	3.25	16	14.35	50	66	-0.6	MR
DR40	4.3	27	12.55	41	68	-0.5	MR
DR35	4.35	28	12.95	43	71	-0.4	MR
Y85	4.15	26	13.55	45	71	-0.4	MR
DR44	4.35	28	13.9	47	75	-0.2	MR
DR7	4.4	29	14.15	49	78	-0.1	MR

DR38	4.75	31	14	48	79	0	MS
DR73	5.05	35	13.85	46	81	0.1	MS
DR9	4.5	30	14.75	52	82	0.1	MS
DR53	4.5	30	15.15	56	86	0.3	MS
DR42	5	34	14.9	53	87	0.3	MS
DR58	4.95	33	15.05	55	88	0.4	MS
DR39	5.3	39	14.7	51	90	0.5	MS
DR66	5.15	37	15	54	91	0.5	MS
DR17	4.85	32	15.65	60	92	0.6	MS
DR63	5.05	35	15.3	57	92	0.6	MS
DR78	5.1	36	15.15	56	92	0.6	MS
DR36	5.55	42	15.55	59	101	1	MS
DR76	4.85	32	16.7	71	103	1.1	MS
DR65	5.45	40	16.3	67	107	1.2	MS
DR72	4.85	32	17.5	76	108	1.3	MS
DR30	5.5	41	16.35	68	109	1.3	MS
DR75	6.25	47	15.8	62	109	1.3	MS
DR67	5.5	41	16.45	69	110	1.4	MS
DR49	6.1	45	16.25	66	111	1.4	MS
DR19	4.85	32	18.35	82	114	1.5	MS
DR48	6.45	51	16	63	114	1.5	MS
DR71	6.35	49	16.2	65	114	1.5	MS
DR41	6.2	46	16.5	70	116	1.6	MS
DR64	5.75	44	16.95	72	116	1.6	MS
DR82	7.3	58	15.35	58	116	1.6	MS
DR43	7.05	56	15.75	61	117	1.7	MS
DR84	5.6	43	17.45	75	118	1.7	MS
DR31	5.2	38	18.75	83	121	1.8	MS
DR50	6.3	48	17	73	121	1.8	MS
DR46	5.75	44	17.9	80	124	2	S
DR20	7.65	62	16.15	64	126	2.1	S
DR54	6.85	55	17.05	74	129	2.2	S
DR52	6.55	52	17.7	78	130	2.2	S
DR14	6.4	50	18.15	81	131	2.3	S
DR33	6.7	53	19.3	84	137	2.6	S
DR51	8.35	64	17.65	77	141	2.7	S
DR81	7.25	57	19.3	84	141	2.7	S
DR60	8.25	63	17.85	79	142	2.8	S
agatti-2002	7.6	61	18.15	81	142	2.8	S
DR26	7.35	59	19.3	84	143	2.8	S
DR55	8.25	63	18.15	81	144	2.9	S
DR77	8.25	63	18.15	81	144	2.9	S

DR80	8.25	63	18.15	81	144	2.9	S
sahiwal-2002	7.45	60	19.3	84	144	2.9	S
DR32	8.35	64	18.15	81	145	2.9	S
DR56	8.35	64	18.15	81	145	2.9	S
DR16	7.65	62	19.3	84	146	3	HS
DR68	8.25	63	19.3	84	147	3	HS
DR57	8.35	64	19.3	84	148	3	HS
DR59	8.35	64	19.3	84	148	3	HS
DR69	8.35	64	19.3	84	148	3	HS
Mean	4.78		13.18		79.15^{G}		
Max	8.5		20.2				
Min	1.15		3.35				

SR^{a} = mean of stalk rating for autumn and spring under artificial conditions, LL^{a} = mean of lesion length % for autumn and spring under artificial conditions, w = genotype ranking using SR, x= genotype ranking using LL, y = rank-sum for (w + x) each genotype, z=deviation from grand mean of rank-sums ([z=(y-G)/ standard deviation]x2), G= grand mean of rank-sums, $classes^{a}$ = (HR= highly resistance, R=resistant,MR=moderately resistant, MS=moderately susceptible, S= susceptible, HS= highly susceptible),Max=maximum value, Min=minimum value.

Effect of stalk rot under artificial inoculation

Fusarium stalk rot was studied on hundred genotypes along with two varietal checks (sahiwal-2002, agatti-2002) following the artificial inoculation during autumn (2009) and spring (2010). In autumn SR ranged from 1.2 to 8.7 with the mean of 5.08 while 1.1 to 8.3 with the mean of 4.48 in spring. Disease severity recorded on the basis of LL fluctuated from 3.8 to 20.8% with the mean of 13.94% and 2.9 to 19.6% with mean of 12.06% in autumn and spring respectively (table 4). SR and LL was recorded more in autumn than spring and both (SR and LL) showed highly significant results ($P < 0.01$) in two growing seasons under artificial inoculations. Genotype-season interaction showed highly significant results ($P < 0.01$) for SR and LL in both growing seasons. Mean of SR and LL showed highly significant results ($P < 0.01$) when studied under artificial inoculation. Maximum value of SR was observed in DR57 (8.9) while minimum value of SR was observed in EL17 (1.2) in autumn. Similarly maximum value of LL was observed in DR60 (20.8) while minimum value was noted in EL17 (3.8). In spring season, maximum SR and LL was observed in DR69 (8.3) and DR33 (19.6) while minimum value of SR and LL was found 1.1(EL17) and 2.9(Y11) respectively table (4). Varietal checks including agatti-2002 and sahiwal-2002 showed SR 8 and 7.9 while LL 14.3 and 14.5 in autumn season respectively. In spring SR 7.2 and 7 while LL 17.6 and 19 were recorded in agatti-2002 and sahiwal-2002 respectively. Significant positive association ($r = 0.82$ to 0.90; $P < 0.01$) was studied between SR and LL under autumn and spring season respectively (table 2). Similarly Coefficient of determination (R^2) also showed association between SR and LL (0.67 to 0.82) under autumn and spring season respectively (Fig 4, 5). Classification of genotypes on the basis of rank-sum analysis (table 5) elaborated that 3 genotypes were highly resistant, 19 were resistant, 29 were moderately resistant, 29 were moderately susceptible, 17 were susceptible (including agatti-2002 and sahiwal-2002) and 5 were highly susceptible (Fig 3). Frequency distribution of all genotypes ranked on the basis of their resistance level in artificial infection appeared to be continuous and Anderson-Darling test ($P < 0.05$) showed the normality of data.

Weather informations

Data about weather was collected from MMRI that

was maintained on daily as well as monthly basis in the registers. Mean of Minimum and maximum temperatures recorded monthly in the registers were used to elaborate the study. Mean temperature varied from 29.77 to 33.67 C° and 17.19 to 34.11 C° during autumn and spring respectively. Similarly mean of relative humidity ranged from 53.19 to 66.53 and 26.14 to 47.97 in autumn and spring respectively. No rainfall was recorded in October while maximum rainfall was in July (79.5mm) in autumn. Minimum rainfall was recorded in February (5mm) while maximum rainfall was recorded in June (11.4mm) (Fig 6).

Table 5. Coefficient of correlation and Coefficient of determination values for severity scale under two seasons.

Severity Scale (Season)	Coefficient of Correlation (r)		Coefficient of Determination (R^2)	
	Natural	Inoculated	Natural	Inoculated
SR and LL (Autumn)	0.90**	0.82**	0.82	0.67
SR and LL (Spring)	0.93**	0.90**	0.87	0.82

**= highly significant value (P<0.01).

Discussion

Fusarium species are major source of stalk rot in maize and the disease has caused serious losses all over the world (Neish *et al.*, 1983). So evaluation of genotypes for resistance is an effective way to overcome these losses (Munkvold, 1996). For this purpose, study was carried out in two different seasons to identify resistant genotypes. Findings of the study clearly elaborated the more severity in autumn than spring. Our results confirmed the findings of Ahmad *et al.,* (1997) and Dodd (1980) while against the reports of Chambers (1987) that seasonal variations did not produce any significant effect on the disease. Significant differences in disease development in two seasons may be due to influence of environmental conditions. Low rainfall was recorded in both season except July and August of autumn season. More average temperature and humidity was observed in September and October.

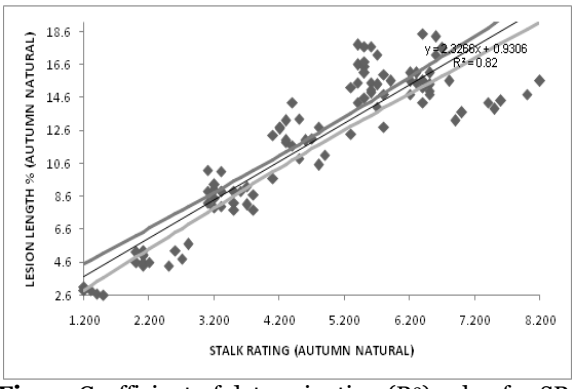

Fig. 1. Coefficient of determination (R^2) value for SR and LL in autumn under natural conditions.

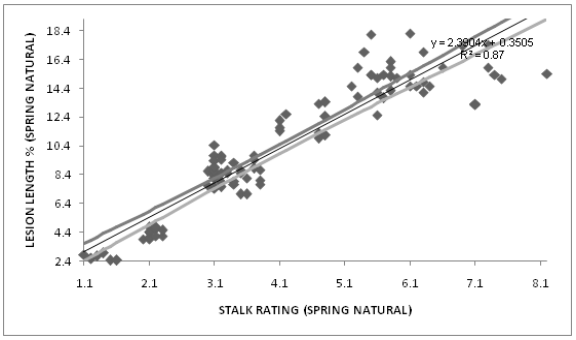

Fig. 2. Coefficient of determination (R^2) value for SR and LL in spring under natural conditions.

The average temperature was also high in April, May and June but humidity is less. Ahmad *et al.*, (1997) reported that hot and humid environment was more favorable for disease development. Hooker and Draganic (1980) emphasized on the disease assessment procedure in order to identify the resistant genotypes. They preferred artificial inoculation to evaluate maize genotypes against stalk rot. Colakoglu (2002) reported that stalk rot infection proceeds faster after maturity of maize plant. That is why inoculation in both seasons was performed after flowering. Afolabi *et al.*, (2008) and Ledencan *et al.*, (2003) followed natural infection as well as artificial inoculation to get resistant lines. Considering the worth of their contributions, we followed natural infection as well as artificial inoculation in autumn and spring season separately. Eight genotypes showed highly resistant response in natural infection while only three genotypes exhibited highly resistant

response in artificial inoculation (table 3, 5). This showed that artificial inoculation gives more variation in results than natural infection and is helpful in identifying and separation of resistant genotypes among the population (Ledencan *et al.*, 2003).

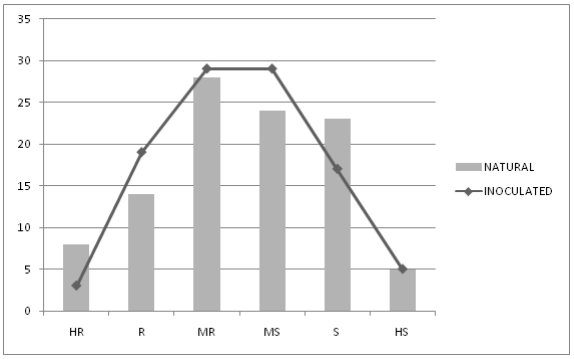

Fig. 3. Classification of genotypes by rank-sum analysis under natural and inoculated conditions.

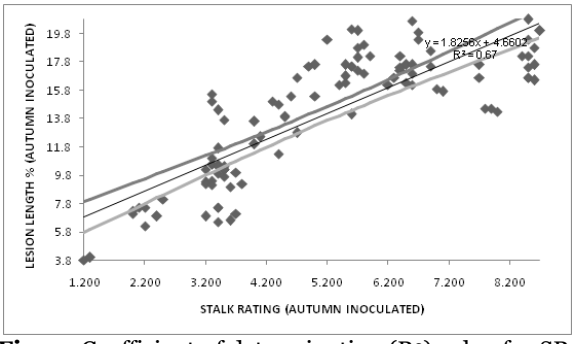

Fig. 4. Coefficient of determination (R^2) value for SR and LL in autumn under inoculated conditions.

Genotypes EL7, EL17 and Y11 showed highly resistant response while DR59 and DR69 exhibited highly susceptible response in both season and disease assessment methods while other genotypes responded differently. Our results confirmed the results of Rheeder *et al.*, (1990) who found that incidence of stalk rot varied from cultivar to cutivar. Bohra *et al.*, (2001) and Afolabi *et al.*, (2008) used severity scale to assess the stalk rot in maize. Severity rating is easy and less time consuming as compare to quantitative measurement. But rating of qualitative character varies from human to human. Considering this view point, lesion length was noted to elaborate quantitative presentation of the results. Forbes and Korva (1994) emphasized the importance of direct estimation of disease than rating scale. Our positive correlation results (table 2) in autumn and spring between SR and LL with two disease assessment methods and coefficient of determination values validated the quantitative measurement (LL). Moreover, direct estimation of lesion length provides more continuous variation that helps breeder to elaborate more precisely the genetic basis of resistance (Afolabi *et al.*, 2008). In the study, we utilized two checks varieties (Sahiwal-2002, Agatti-2002) that exhibited susceptible response in both growing season with two disease assessment methods. Comparisons of genotypes with the checks are widely used for the validity of results against resistance (Happstadius *et al.*, 2003).

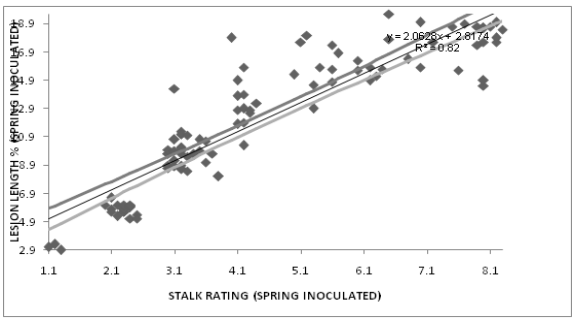

Fig. 5. Coefficient of determination (R^2) value for SR and LL in spring under inoculated conditions.

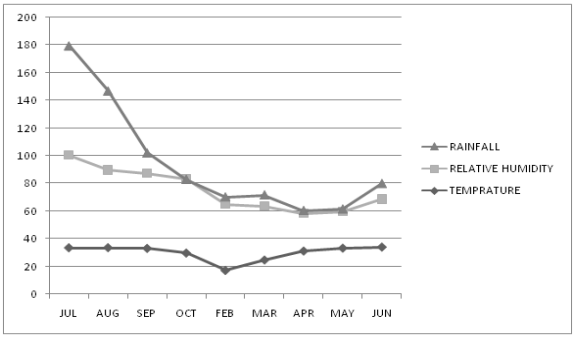

Fig. 6. Average temperature, relative humidity and rainfall under autumn and spring seasons.

Classification of genotypes into different groups was performed by Rank-sum method as utilized by Afolabi *et al.*, (2008). Importance of this method was elaborated by Onyeka *et al.*, (2005) for the separation genotypes into distinct resistant groups. On the basis of this method, highly resistant genotypes (EL7,EL17,Y11) can be utilized in further breeding programs to transfer the resistant gene or genes in local high yielding varieties and highly susceptible genotypes (DR59, DR69) can be exploited for the study of their mode of inheritance.

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