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Influence of growth stage on sensitivity to helminthosporol toxin of *Bipolaris sorokiniana* of barley (*Hordeum vulgare*)*

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Bipolaris sorokiniana (Sacc.) Shoemaker (syn. *Helminthosporium sativum* teleomorph: *Cochliobolus sativus*), a hemibiotrophic phytopathogenic fungus, is a well-known for inciting spot blotch disease in barley and wheat. Among a variety of symptoms induced by this pathogen on all parts of the plant, foliar Spot blotch caused by *B. sorokiniana* has emerged as one the major biotic stresses hampering the commercial production of barley (Ghazvini and Tekauz 2007). The disease is found all over the world wherever the barley is grown. It is a serious pathogen of wheat and barley in North America (Mathre 1997), South America and several countries of Asia (Joshi *et al.* 2007).

Ludwig (1957) demonstrated that *B. sorokiniana* (*Helminthosporium sativum*) produces a toxin that is essential for the development of the diseases in the attacked plants. Numerous compounds have been isolated from culture filtrates of *B. sorokiniana* and highest phytotoxic activity has been associated with the metabolites of sesquiterpene nature (De Mayo *et al.* 1961; Carlson *et al.* 1991). The nonselective phytotoxin produced by *B. sorokiniana* is capable of membrane disruption, inhibition of mitochondrial electron transport and oxidative phosphorylation, general degeneration of nuclei (Aggarwal *et al.* 2011) and induce chlorosis (Ludwig 1957). Among the various toxins produced by *B. sorokiniana* helminthosporol is the major sesquiterpene metabolite when grown in broth culture and is responsible for the disease (Carlson *et al.* 1991). The helminthosporol toxin produces similar symptoms as it was obtained with non- purified filtered fungus culture on test plant (Stoessl 1981). Toxin has been utilized for screening of barley (Bashyal *et al.* 2009, Carlson *et al.* 1991). However, effect of helminthosporol at different growth stages of barley is not known yet.

Fourteen diverse genotypes reported for variable disease severity were obtained from the Directorate of Wheat Research Karnal, Haryana (Chand *et al.* 2008). The experiment was carried out during 2008–09 in the Agricultural Research Farm, Banaras Hindu University,Varanasi. The crop was raised in November with row to row and plant to plant distance of 25 cm and 5 cm respectively. Agronomic practices recommended for normal fertility (60N:40P:30K) under irrigated conditions were followed.

Crude toxin was obtained by following the method of De Mayo *et al.* 1961. Isolate of *B. sorokiniana* (29 B) was grown in Minimal Medium (Leach *et al.* 1982) for eight days. Mycelium of *B. sorokiniana* was filtered with Whatman paper 36 to obtain the cell free culture filtrate. Culture filtrate was adjusted to *p*H 2 and activated charcoal was added and stirred and kept for 30 min. The charcoal with the absorbed toxin was separated from the liquid in a centrifuge and suspended in ethanol. After filtering on a Buchner Funnel the charcoal cake was suspended in chloroform, stirred and filtered. Process was repeated three times. The chloroform extracts were combined and the solvent removed in *vaccuo* to yield crude extract.

Barley plants of growth stage 33 (third node detectable), 60 (beginning of flowering) and 75 (medium milk) of (Zadoks *et al.* 1974) were selected for the infiltration of toxin. Individual penultimate leaf of each plant was infiltered with 100 µl (1: 10 dilution) of crude toxin using a 1 ml plastic syringe (Mercado *et al.* 2006). Each penultimate leaf was infiltered in the centre and water soaked area was marked with non-toxic felt pen at each end of water soaking point. Observations were taken for appearance of necrotic symptom

*Short note

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Table 1 Analysis of variance for the effects of genotypes, growth stages and infiltration area for the appearance of necrotic symptom in barley

Source of variation	df	SS	MSS	F value
Replication	2	0.13	0.06	0.35
growth stages	2	420.52	210.26	1109.42**
Error (A)	4	0.76	0.19	
Genotypes (G)	13	862.39	66.34	1368.73**
Genotypes (G) × Growth stages (GS)	26	22.78	0.87	18.08**
Error (B)	78	3.78	0.05	
Infiltration (I)	1	2204.25	2204.25	152.46**
Infiltration × Growth stages	2	286833.59	43416.80	3003.08**
Infiltration × Genotype	13	2306.25	177.40	12.27**
Infiltration × Genotypes × Growth stages	26	1552.55	59.71	4.13*
Error (C)	52	751.78	14.46	
Total	251			

** highly significant, I, infiltration area

and infiltered area of leaves. Total 10 randomly selected plants were tested in each genotype. Split-plot design was used for conducting experiment and data was analyzed for variance.

Significant differences were observed in barley genotypes (F value 1368.73) and growth stages of genotypes (F value 1109.42) and further interaction between genotypes and growth stages (F value 18.08) for the sensitivity to the toxin (Table 1). Further significant differences were observed for the area of infiltration (F value 152.46), area of infiltration and growth stages (F value 3003.08), area of infiltration and genotypes (F value 12.27), area of infiltration genotypes and growth stages (F value 4.13). Time for the appearance of necrosis was maximum at the growth stage 33 and it was minimum for growth stage 75. Necrosis appeared after 7.20 days in RD2503 at growth stage 33. It took 5 days at growth stage 60 and symptom appeared after 3 days at growth stage 75. Maximum days for the appearance of necrotic symptom

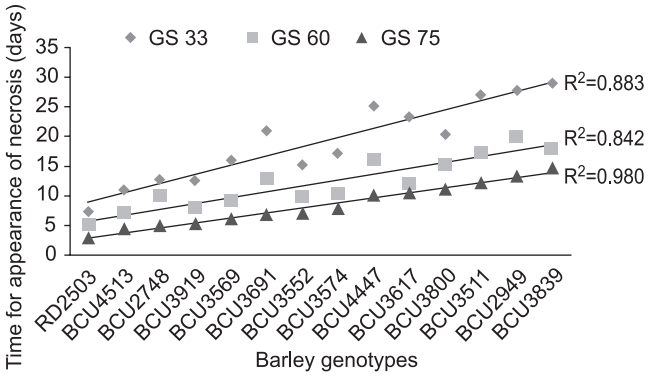


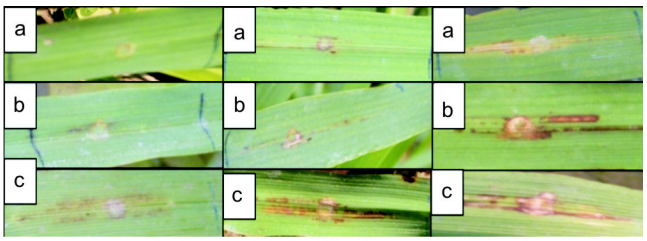
Fig 1 Effect of growth stages on different barley genotypes

Table 2 Field and laboratory performance of barley genotypes for the appearance of necrotic symptom at growth stage 75

Genotype	Time for appearance of necrotic symptom (days)		Mean	Rank
	Field condition	Laboratory condition		
RD2503	3.00	2.00	2.50	1
BCU4513	4.60	3.00	3.80	2
BCU2748	5.00	3.50	4.25	3
BCU3919	5.40	4.00	4.70	4
BCU3569	6.20	4.50	5.35	5
BCU3691	7.00	5.00	6.00	6
BCU3552	7.20	5.20	6.20	7
BCU3574	8.00	6.00	7.00	8
BCU4447	10.20	7.00	8.60	9
BCU3617	10.60	8.00	9.30	10
BCU3800	11.20	8.20	9.70	11
BCU3511	12.20	8.50	10.35	12
BCU2949	13.40	9.00	11.20	13
BCU3839	14.80	10.00	12.40	14
LSD (*P*=0.01)	0.85	0.51		

was taken by BCU3839, which was 29, 18 and 14.8 days at the growth stages 33, 60 and 75 respectively (Figs 1, 2). However, growth stage 75 of different genotypes gave maximum variation as compared to 33 and 60. The coefficient of determination ($R^{2)}$ value was minimum at growth stage 60 (0.84), followed by growth stage 33 (0.88) and growth stage 75 (0.98) (Fig 1).

Helminthosporol toxin effect varied between genotypes and growth stages of genotypes, and area of infiltration of the plants. Hodges and Campbell, (1999) reported that more resistant varieties produced more ethylene than less resistant variety in *Poa pratensis* from leaf blades after *Bipolaris sorokiniana* infection. It has also been observed that ethylene production also increased after anthesis in barley, which could be responsible for the susceptibility to the pathogen. Chand *et al.* 2010 also reported variation in symptoms type



Growth stage 33 Growth stage 60 Growth stage 75

Fig 2 Symptom appeared after eight days of toxin infiltration at different growth stages in selected genotypes of barley where, a: BCU3839; b: BCU3691; c; RD2503

with advancing growth stages. It has been observed that in young barley leaves mesophyll area and vessel area is less as compared to older barley leaves. Further mesophyll cells in growing region are closely packed and immature vessel have membrane that prevents water transport (Tang and Boyer 2002) in maize, it might have been prevented to cover more area by toxin in young barley plants as compared to the older plants.

Growth stage 75 could be the best stage for the toxin infiltration as it could be taken for field screening as well as laboratory conditions. As it gave maximum variation and similar rank in both (laboratory and field) the conditions (Table 2). Further longer time for the appearance of necrotic symptom at growth stage 33 and growth stage 60 makes these stages not suitable for *in vitro* screening.

Present findings signify that toxin produced by *B. sorokiniana* of barley is influenced by the barley genotypes and its growth stages. In this study growth stage GS75 identified as a most suitable for toxin infiltration. Lines with consistently longer incubation period and least spread of toxin symptoms could be utilized in crossing programme for the pyramiding toxin resistance with other resistance traits.

SUMMARY

Fourteen barley genotypes were tested in field condition for their reaction to helminthosporol toxin of *Bipolaris sorokiniana* of barley at third node detectable, beginning of flowering and medium milk stage. Appearance of necrotic symptom was highly influenced by growth stage of genotypes. Symptom appeared early in medium milk stage (GS 75) and it took maximum time at third node detectable stage (GS 33). RD2503 has taken three days and BCU3839 has taken 14.8 days for the necrotic symptom at growth stage 75. Further percentage of infiltered area also increased at growth stage 75 compared to other stages. Growth stage 75 identified as most appropriate growth stage for the screening of barley genotypes to helminthosporol.

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