



IDENTIFICATION OF NEW SOURCES OF RESISTANCE TO TURCICUM LEAF BLIGHT AND MAYDIS LEAF BLIGHT IN MAIZE (*ZEA MAYS* L.)

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SUMMARY

Turcicum leaf blight (TLB) and Maydis leaf blight (MLB) are amongst the important diseases of maize grown in the North Western Himalayas. To identify new resistance sources and establish durability of known resistance sources, 35 short-duration maize inbred lines were evaluated against TLB after artificial inoculation and MLB under natural conditions during *kharif* 2011 and *kharif* 2012. Twelve inbred lines were found resistant against TLB, 19 inbred lines exhibited resistance against MLB and 10 inbred lines found resistant to both TLB and MLB. Principal component analysis (PCA) revealed that grain yield (GY) is negatively correlated with TLB and MLB. Agglomerative hierarchical clustering (AHC) analysis with GY, TLB and MLB revealed the 35 inbred falls into 3 major clusters. In cluster I most of the inbreds were moderately resistant to TLB and resistant to MLB with high yielding potential. In cluster II most of the inbreds are resistant to both TLB and MLB with high GY potential. In cluster III, most of the inbred were highly susceptible to TLB and moderately resistant to MLB with low yielding lines. The AHC classification will be helpful for selection of disease resistance and high yielding inbred for resistance breeding program.

Keywords: Maize inbred, TLB, MLB, Resistance.

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INTRODUCTION

Maize (*Zea mays* L.) is an important staple food crop and provides raw materials for the livestock and many agro-allied industries in the world (Bello *et al.*, 2010; Randjelovic *et al.*, 2011). The area, production and productivity of maize has increased significantly in last few decades. India registered a growth rate of more than 7% in production and more than 6% in productivity in last 5 years. Maize production in India is 21.73 million tones with 8.55 million ha with productivity of 2.6 t/ha (DMR, 2012). Due to moderate low temperature and high humidity

during the maize growing period, TLB and MLB are the 2 major diseases for highland maize farmers in the Himalayan region.

TLB caused by *Exserohilum turcicum* (Pass.) Leonard and Sugs, is known to infect maize from the seedling stage to maturity. The symptoms first start as small elliptical spots on the leaves as grayish green with water soaked lesions parallel to leaf margins, the spots turn greenish with age and increase in size, finally attaining a spindle shape with long elliptical grayish or tan lesions. If the disease starts at an early stage, it causes premature death of blighted leaves. As a result, the crop loses their nutritive

value as fodder (Payak and Renfro, 1968), have reduced germination capacity, vigor, GY and total sugar content (Ferguson *et al.*, 2004), has restricted starch formation, chaffy kernels and infected plants are liable to infection with stalk rots (Cuq *et al.*, 1993; Henry and Kettlewell, 1996). The fungus has a wide host range and a high pathogenic variability with several races already reported in different parts of the world (Pratt R. G., 2003; Agrios, 2005).

The disease has been reported throughout the world wherever maize is cultivated (Atac, 1984; Leonard *et al.*, 1985; Adipala *et al.*, 1993). TLB can be severe in mid-altitude tropical regions where high humidity, low temperature and cloudy weather prevail during the maize growing season (Harlapur *et al.*, 2000; Singh *et al.*, 2004; Harlapur, 2005). Severe losses in GY due to epiphytotic condition have been reported in several parts of world including India and the losses vary from 28 to 91% depending upon the severity of the disease (Jha, 1993; Pandurangegowda *et al.*, 1993; Harlapur *et al.*, 2000). The genetic nature of resistance has been determined to be quantitative and hence can be exploited for the development of resistant cultivar (Kumar *et al.*, 2011).

MLB, caused by *Bipolaris maydis*, is reported from most maize growing regions in the world including India. Under severe conditions depending upon the susceptibility of the variety, MLB may cause significant grain yield losses (Thompson and Bergquest, 1984), upto to 70% (Kumar *et al.*, 2009). The blight spreads from the basal leaves to the developing ear and then flag leaf of maize plant (CIMMYT, 1985). Most of the maize breeding programs aimed at improving disease resistant and grain yield (GY), also consider other parameters *i.e.*, days to 50% tasseling (DFT), days to 50% silking (DFS), plant height (PH), ear height (EH), days to maturity (DTM), since these parameters are also used in management of diseases. Host plant resistance is most effective way to manage diseases in general; TLB and MLB in particular because chemical measures are expensive, often ineffective and sanitation practices in maize are difficult to apply. The aim of this study was to identify sources of resistance against TLB and MLB for use in a hybrid breeding program.

MATERIALS AND METHODS

Thirty-five short duration maize inbred lines studied under the present investigation comprised of 32 lines of normal maize and 3 lines of quality protein maize (QPM). The pedigree of all the lines developed at Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora are presented in Table 1. Field trials were conducted during *kharif* 2011 and *kharif* 2012 in randomized complete block design (RCBD) with 2 replications at Hawalbagh Research farm (29° 38' 3" N and 79° 37' 49" E) of VPKAS, Almora located in Uttarakhand (India) at an altitude of 1250 m to identify new sources of resistance against TLB and MLB and also to test the durability of known TLB resistant lines developed by VPKAS, Almora. All the 35 inbred lines were screened against TLB under artificial epiphytotic condition. Each test line was sown in 2 rows of 3 m and rows were spaced at 60 cm. Spreader rows of highly susceptible local inbred V 351 were planted at the border as a source of secondary inoculum for disease development. The recommended package of practices was followed during crop growth. Since the MLB occurrence has become an endemic at Hawalbagh farm, Almora in recent years, hence the natural incidence of MLB was recorded from all the 35 inbred lines in both the years. The data on days to 50% tasseling (DFT), days to 50% silking (DFS), plant height (PH), ear height (EH) and days to maturity (DTM) were recorded as per Stickler *et al.* (1961).

Inoculum preparation and inoculation

The inoculum of *E. turcicum* prevalent in North Western Himalayas was prepared by growing the fungal mycelium on sorghum grains. After proper fungal growth (7 days after inoculation), the grains were dried under the shade at room temperature and powdered with the help of a mixer-grinder.

For the creation of appropriate disease pressure (Epiphytosis), 0.5 gram powder was put in the leaf whorl of each plant at 4-5 leaf stage, followed by two more inoculations at 7-8 day

Table 1. List of maize inbred and their pedigree used in the TLB and MLB screening studies.

Inbred name	Pedigree details
V 25	Riveirao preto 8233 (Alm)-1-4-f-#-15-#-⊗-bulk-B-B-B
V 334	TZI-9-F-#-#-B-⊗-1-B-B-B-B-#-#-B-#-#-#-B-B-B-B
V 335	TZI-25-F-##-B-4-1-B-B-14-#-#-#-B-#-B-#-#-B-B
V 336	CML145, P ₆₃ CDH-C-181-3-2-1-4-#-2-B-B-B-B-#-#-F-B-B-B-B-B-#-#-2-#-#-B-#-#-B-#-B-#-B-B
V 338	BIO-45010-OP-⊗-2-1-5-3-B-#-B-#-#-#-B-#-#-#-B-B-B
V 340	(CM128 X CM129)-⊗-1-8-6-1-B-#-B-#-B-#-B-B
V 341	MEXICO ACC. No. 3136-F-⊗ -3-2-3-8-1-B-#-B-#-B-#-B-#-B
V 345	BIO-45010 OP, F⊗ -2-1-8-5-5-B-#-B-#-#-B-B
V 346	BIO-45010 OP, F⊗ -2-1-8-5-6-#-B-#-B-#-#-B-B
V 351	(Shakthi (SO) HE 25#CCB50%F-#-⊗-1-F-⊗-4-#-B-#-#-#-B-B
V 372	PRO-337 OP⊗ 6-4-1-3-4-1-#-B-#-#-#-#-B
V 373	JKMH-175-4(OP) ⊗ -16-7-12-1-B-#-#-B-#-B-B
V 383	(CM212 X CM145) BC ₃ P ₂ ⊗ -B-B-B-#-B-B-B-B-B-B
V 390	CML-470-F-⊗ 4-B-#-B-#-#-B-B-B
V 398	JKMH-178-4, OP, BC ₃ P ₁ ⊗ -2-4-1-4-10⊗ -B-2-5-B-1-B
V 400	P ₄₅ C ₈ -164-1-1-2-8-B-B-B-B-F-B-B-#-B-2-B-#-B-B
V 401	HKI-193-1-F-B-B-B-B-#-B
V 402	X-102, OP, ⊗-6-1-2-6-10-#-B-#-#-B
V 403	(V190 X V338)- ⊗-4-2-3-3-10-9-7-#-#-#-#-B
V 404	ZAURI2050, OP, ⊗-13-5-2-2-2-1-3-B-B-#-#-B
V 405	KH-581, OP, ⊗-1-1-9-1-6-7-4-B-B-#-#-B
V 406	CML32⊗-4-3-B-#-6-#-B
V 407	(V373 X CML173) BC ₂ P ₁ ⊗ -7-B-B-#-B-#-#-B
V 409	CYMMYT Introduction Nursery No.7250-HYD-2007-RABI-17-F ⊗-2-B-B-#-#-B
V 410	CYMMYT Introduction Nursery No.7248-HYD-2007-RABI-3-F- ⊗-7-B-B-#-#-B
V 414	CYMMYT Introduction Nursery No.7250-HYD-2007-RABI-24-3-F-13-B-B-#-#-B
V 418	WSKHOTHAI-1-WAXY-1-1-1-5-2-B-1
VQL 1	(CM212 x CML180) BC ₃ P ₁ ⊗ -B-B-B-B-#-B-B
VQL 2	(CM145 X CML170) BC ₃ P ₁ ⊗ -B-B-B-#-#-B-B
VQL 17	(V341 X CML189) BC ₂ P ₁ ⊗ -B-B-#-B-B-#-B-#-B-B
CM 141	Pool 33 (Alm) ⊗-198-2-2-2-2-#-#-B-B-B
CM 145	Pop31-C4-HS-bulk(Alm) -⊗-70-2-7-7-#-#-#-B-#-#-B-B
CM 152	U15-1 (Pop 31- C4-HS-bulk(Alm)) -#-#-#-⊗-B-#-#-#-⊗-B-#, U3-1)-f-f-#-#-#
CM 153	Syn I (Inter crosses of three inbreds derived from Pop31-C4-HS-bulk(Alm) viz., V198 V270and V273)f-f-#-#-#
CM 212	USA/ACC. No.2132 (Alm.)-3-2-f-13-#-B-#-#-#-B-B)

intervals and higher relative humidity was maintained by spraying water with knapsack sprayer.

Disease severity estimation

Each inbred line was phenotyped for TLB incidence at dough stage using standard 1-5 scale, 1 being complete resistant and 5 being the complete susceptible (Payak and Sharma, 1982). Based on this rating scale over the years, the maize lines were classified into four groups namely, resistant (R) genotypes with a score < 2.0; moderately resistant (MR) 2.1-3.0; moderately susceptible (MS) 3.1-3.5 and highly susceptible (S) > 3.5. For MLB, a scale recommended by Shekhar and Kumar (2012) was used and maize genotypes were classified into four groups as described for TLB.

Statistical analysis

Combined ANOVA and simple correlation analyses of two year data were analyzed using SAS JMP 9.0 version software. Multifactorial comparisons i.e. PCA and AHC analysis were carried out using XLSTAT 2013 version software to display the correlation between the various parameters i.e. TLB, MLB, GY and their relationships with the different maize genotypes. Varimax rotation was performed to produce orthogonal transformations to the reduced factors to identify the high and low correlations better and the data normalization was done according to Kaiser's rule.

RESULTS

The combined/pooled analysis of variance showed that mean squares for GY were significantly different for $Y \times R$ ($P < 0.05$), G ($P < 0.000$) and $G \times Y$ ($P < 0.000$). Genotypic (G) mean squares were found significant ($P < 0.000$) for all the traits, whereas $G \times Y$ mean squares was significant for DTM ($P < 0.05$), PH ($P < 0.000$), EH ($P < 0.000$), TLB ($P < 0.05$), MLB ($P < 0.01$), GY ($P < 0.000$) but non-significance were observed for DFT and DFS (Table 2). However, significant differences were not observed for year mean squares in all the studied

traits, while $Y \times R$ mean squares showed significant differences for DFT ($P < 0.05$), PH ($P < 0.01$), EH ($P < 0.01$), Yield ($P < 0.05$). However, $Y \times R$ mean squares were non-significant in case of DFS, DTM, TLB and MLB. The lowest DFT (48.5 days) and DFS (50 days) were observed in CM 153, while higher DFT (58.3 days) and DFS (60 days) were recorded in V 414. The mean DFT and DFS across the 35 lines were 54.4 days and 55.8 days respectively along with average tasseling-silking interval of 1.4 days. The DTM was found to vary from 92.3 days (CM 152) to 106 days (V 414) with mean of 98.41 days. The PH and EH varied from 130.5 cm (V 338) to 185.3 cm (V 400) and 49.5 cm (V 404) to 95 cm (V 410) with overall mean of 153.92 and 74.24 cm respectively (Table 2).

TLB severity was noted on 1-5 scale at dough stage after inoculation in both the years and mean score over the years was used to group the genotypes (Table 3). Two year pooled average score for TLB was found to vary from minimum of 1.3 in V 373 to highest score of 4.6 in CM 212 with a mean of 2.55. Out of 35, 12 inbred lines namely V 335, V 336, V 341, V 346, V 373, V 398, V 400, V 401, V 407, V 418, VQL 2, VQL 17 and CM 145 exhibited score of less than 2.0 and therefore grouped as resistant (Table 3). The another 13 inbred lines namely V 334, V 338, V 340, V 345, V 383, V 390, V 404, V 405, V 409, V 410, CM 141, CM 152 and CM 153 possessing scores between 2.1 to 3.0 were categorized as MR. Two inbred lines namely V 372 and V 403 possessed TLB scores between 3.1-3.5 were categorized as MS. Whereas the remaining 7 inbred lines (V 25, V 351, V 402, V 406, V 414, VQL 1 and CM 212) had score of more than 3.6 score and therefore categorized as HS to TLB.

Two year pooled average of MLB was found to vary from minimum of 1.3 in V 345 to highest score of 3.1 in V 402 with a mean of 2.05. Out of 35, 19 genotypes (V 334, V 336, V 340, V 341, V 345, V 372, V 373, V 383, V 398, V 400, V 404, V 407, V 410, V 414, V 418, VQL 2, VQL 17, CM 145 and CM 153) had disease severity score of less than 2.0 and therefore classified as resistant (Table 3).

Table 2. Combined analysis of variance for GY, TLB, MLB and other traits evaluated during 2011 and 2012.

Source of variation	df	Mean Squares							
		¹ DFT	DFS	DTM	PH	EH	TLB	MLB	GY
Year (Y)	1	1.21 ^{ns2}	2.86 ^{ns}	6.86 ^{ns}	318.01 ^{ns}	102.86 ^{ns}	0.20 ^{ns}	0.15 ^{ns}	14.15 ^{ns}
Year × Rep (Y × R)	2	1.06 [*]	0.51 ^{ns}	1.41 ^{ns}	35.18 ^{**}	9.16 ^{**}	8.74 ^{ns}	6.17 ^{ns}	12.35 [*]
Genotype (G)	34	25.27 ^{***}	25.85 ^{***}	58.97 ^{***}	863.06 ^{***}	382.18 ^{***}	576.35 ^{***}	150.54 ^{***}	109.74 ^{***}
Genotype × Year (G × Y)	34	0.35 ^{ns}	0.28 ^{ns}	0.81 [*]	18.55 ^{***}	7.55 ^{***}	5.32 [*]	4.86 ^{**}	12.41 ^{***}
Pooled error	68	0.26	0.24	0.50	5.99	1.44	3.19	2.16	3.69
Total	139	6.42	6.54	14.93	221.37	96.91	143.97	39.16	31.96
Treatment mean		54.35	55.8	98.41	153.92	74.24	2.55	2.05	2.82
Treatment range		9.75	10	13.75	54.75	45.5	3.3	1.7	1.8

¹ DFT = days to 50% tasseling; DFS = days to 50% silking; DTM= days to maturity; PH = plant height; EH = ear height; TLB = Turcicum leaf blight; MLB = Maydis leaf blight

² ns = non-significant; *** = significant at $P < 0.0000$; ** = significant at $P < 0.01$,
* = significant at $P < 0.05$.

Table 3. Pooled mean of promising maize inbred showed resistance to TLB and MLB during 2011 and 2012.

Inbred line	TLB Score (1-5)		MLB Score (1-5)		Grain yield (t/ha)
	TLB	Reaction type	MLB	Reaction type	
V 334	2.4	MR	1.9	R	2.69
V 335	1.9	R	2.6	MR	2.50
V 336	1.4	R	2	R	2.88
V 338	2.4	MR	2.9	MR	3.45
V 341	1.6	R	1.5	R	3.43
V 345	2.5	MR	1.3	R	3.21
V 346	2	R	2.1	MR	3.12
V 373	1.3	R	1.6	R	3.73
V 383	2.3	MR	1.5	R	2.75
V 398	1.4	R	1.5	R	3.33
V 400	1.9	R	1.5	R	3.48
V 401	1.4	R	2.5	MR	2.64
V 405	2.6	MR	2.1	MR	3.21
V 407	1.4	R	1.5	R	3.62
V 418	1.5	R	2	R	2.32
VQL 2	1.6	R	1.6	R	2.47
VQL 17	1.8	R	1.5	R	3.29
CM 145	1.8	R	1.5	R	2.61
CM 152	2.4	MR	2.3	MR	3.82
CM 153	2.6	MR	2	R	3.69

R=Resistant, MR=Moderately Resistant

Table 4. Two year combined correlation analysis among the different quantitative traits of maize inbreds.

Character	DFT	DFS	PH	EH	DTM	TLB	MLB	GY
DFT	1.00	0.976**	0.168	0.080	0.806**	-0.254	-0.244	-0.040
DFS		1.00	0.120	0.007	0.844**	-0.251	-0.301	-0.086
PH			1.00	0.686**	0.262	0.386*	0.155	-0.362*
EH				1.00	-0.067	0.352*	0.148	-0.315
DTM					1.00	-0.166	-0.156	-0.076
TLB						1.00	0.499**	-0.520**
MLB							1.00	-0.249
GY								1.00

Note: * Significant ($P = 0.05$), ** Significant ($P = 0.01$)

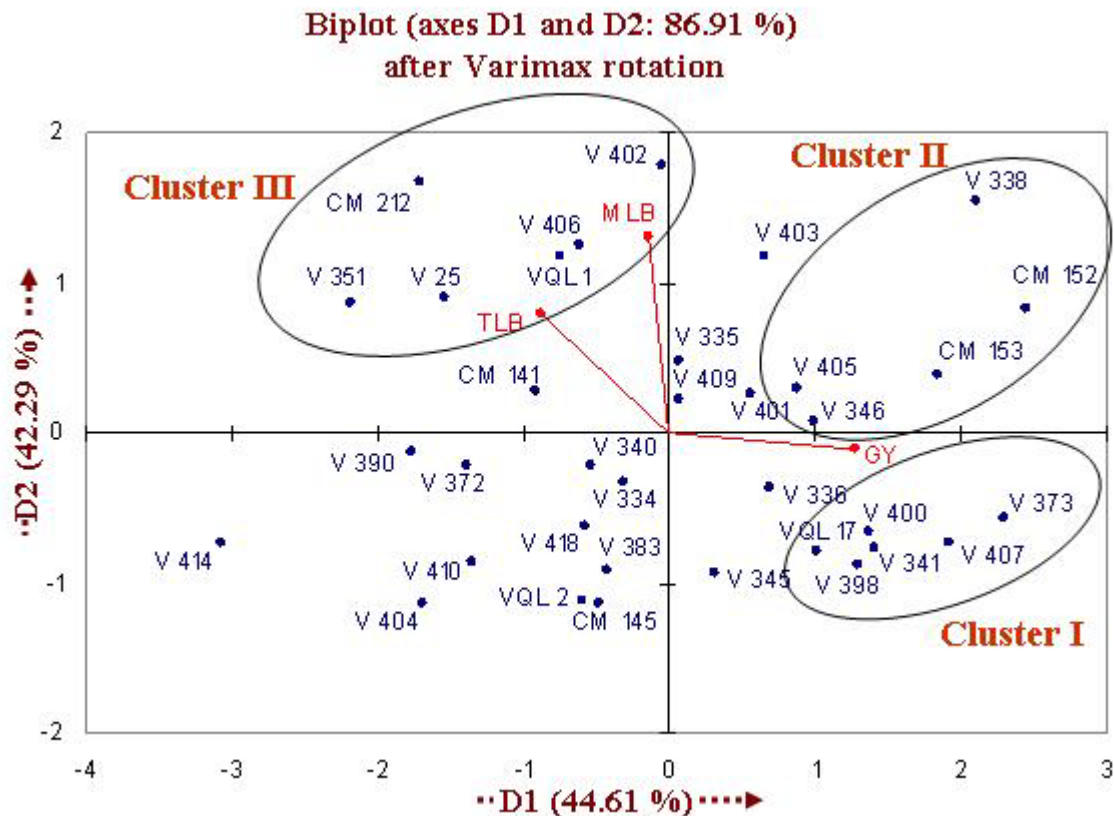


Figure 1. Multifactorial comparison of different maize inbred and other parameters using principal component analysis.

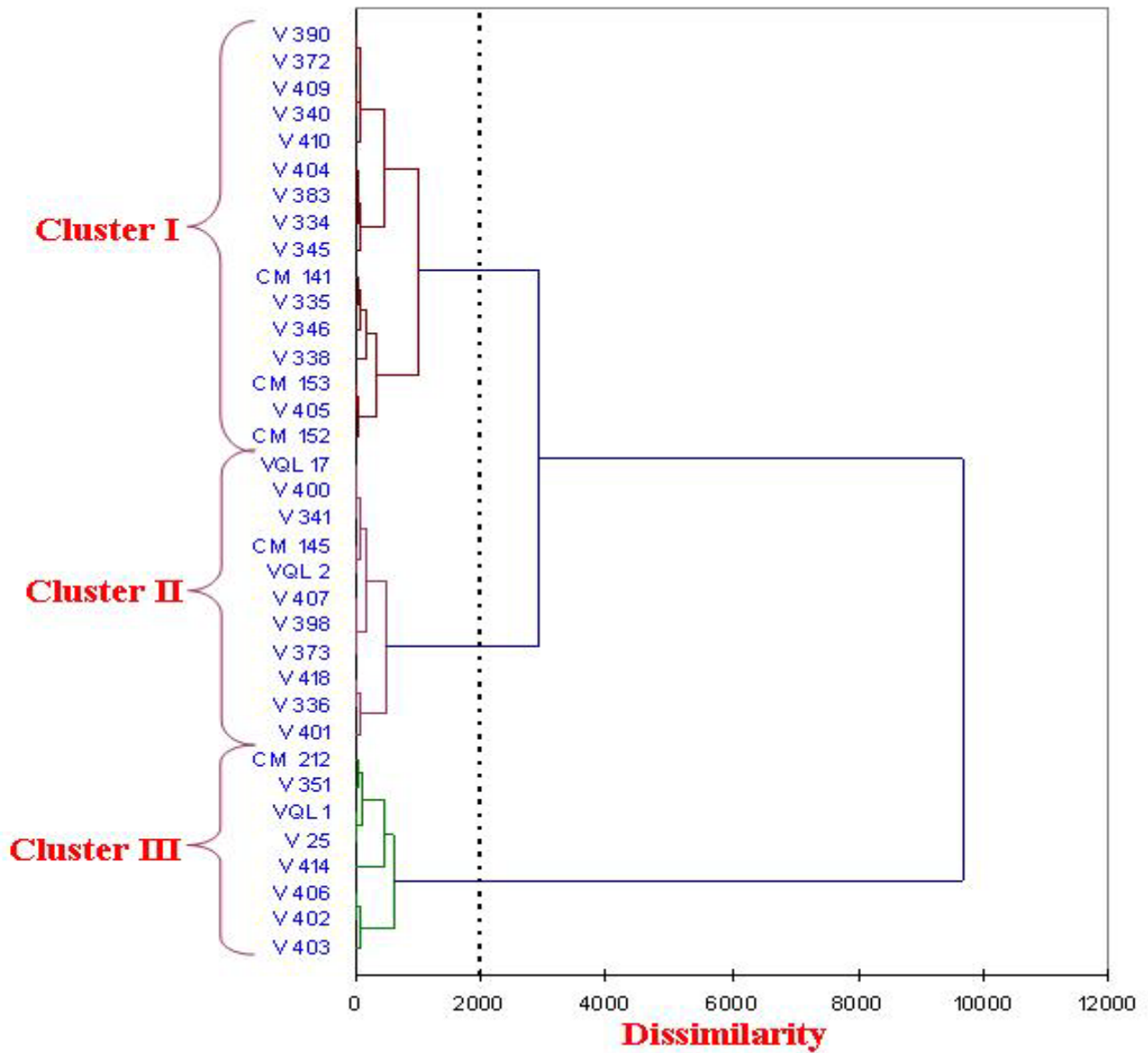


Figure 2. Agglomerative hierarchical clustering among 35 maize inbred based on GY, TLB and MLB severity under field conditions

Fifteen inbred lines (V 25, V 335, V 338, V 346, V 351, V 390, V 401, V 403, V 405, V 406, V 409, VQL1, CM 141, CM 152, CM 212) with score between 2.0 to 3.0 and one inbred (V 402) with score of more than 3.1 was classified as MR and MS to MLB, respectively.

The GY data pooled over 2 years varied from 2.01 t/ha (V 414) to 3.82 t/ha (CM 152) with a mean of 2.82 t/h. Four inbred *i.e.*, V 373, V 407, CM 152 and CM 153 recorded maximum GY and showed resistance to both TLB and MLB (Table 3). Inbred namely CM 212, V 414, V 351, and V 25 found highly susceptible to TLB and were lowest grain yielding lines under epiphytotic condition.

Simple correlation analysis revealed significant positive correlation ($P < 0.01$) between DFT and DFS (Table 4). Both DFT and DFS were significantly and positively correlated with DTM ($P < 0.01$), exhibited positive non-significant correlation with PH and EH but negative non-significant association with TLB, MLB and GY. The PH showed a significant positive correlation with EH ($P < 0.01$) and TLB ($P < 0.05$), non-significant positive correlation with DTM and MLB, but significant negative correlation with GY ($P < 0.05$). Likewise, TLB showed significant positive correlation with MLB ($P < 0.01$) and both are negative correlated with GY.

Multivariate analysis

The PCA biplot and their correlation with different variables (TLB, MLB and GY) and exploration (maize genotypes, $n = 35$) are shown in Figure 1. The first factor F1 represents 44.61% of variability, while the second factor F2 represents 42.29% of variability among the data.

Both the disease parameters TLB and MLB intersected on the left upper side of the biplot and GY was observed on the right middle side of the biplot. This suggests that TLB and MLB have a positive correlation among themselves; however they showed negative correlation with GY. The cosine of 180° (*i.e.*, the angle between TLB and GY on the PC1–PC2 plot) is -1; therefore, they are negatively correlated. Based on this mathematical rule, uncorrelated variables occur at right angles to one another because the cosine of the angle

between them is $\cosine\ 90^\circ = 0$, or not correlated. Similarly, the cosine of 0 is 1, which denotes a positive correlation between the variables (Kaiser, 1970; Lopez *et al.* 2006). The biplot generated by PCA revealed into 3 clusters. Cluster I- genotypes indicated a high positive correlation with GY and a negative association or resistance with TLB and MLB. Similarly, cluster II- genotypes indicated a high positive association with GY and moderately resistance with TLB and MLB. Cluster III- genotypes indicated a negative association with GY and positive association or susceptible with TLB and moderate resistant with MLB. In general, genotypes other than the clusters showed relatively moderate resistant / susceptible to TLB and MLB along with moderate to lower GY.

Agglomerative hierarchical clustering (AHC) analysis with GY, TLB and MLB revealed that 35 maize inbred fall into three major clusters (Figure 2). Cluster I included 16 inbred (V 390, V 372, V 409, V 340, V 410, V 404, V 383, V 334, V 345, CM 141, V 335, V 346, V 338, CM 153, V 405 and CM 152), while cluster II included 11 inbred (VQL 17, V 400, V 341, CM 145, VQL 2, V 407, V 398, V 373, V 418, V 336, V 401) and cluster III included 8 inbred (CM 212, V 351, VQL 1, V 25, V 414, V 406, V 402 and V 403). In cluster I, most of the inbreds that included were moderately resistant to the TLB, while cluster II included inbred lines were TLB resistant and high GY potential and cluster III included were susceptible to TLB and had low GY inbreds. Similarly, in cluster I and cluster II, most of the inbreds were resistant to the MLB, while cluster III inbred were moderately resistant to MLB.

DISCUSSION

Genotype (G), $G \times Y$ and $Y \times R$ were significant for GY. This suggests differences in yield performance of different inbreds in both years. The G and $G \times Y$ were significant for TLB, MLB, DFS and DTM, while Y and $Y \times R$ showed non-significant differences for TLB, MLB, DFS and DTM. In multiple cropping systems and for regions with short growing seasons like north western Himalayas, early and

extra early maturing varieties are desirable. Days required to tasseling along with other maturity traits are commonly used by plant breeders as basis of determining maturity of maize. Lesser the gap between tasseling to silking in a cross, greater will be the probability of grain setting. The present investigation showed that the average anthesis silking interval was 1-2 days, which is ideal for better fertilization, good cob filling and higher GY. Ibikunle *et al.* (2009) and Ahmad *et al.* (2011) noted similar observations earlier. The results on PH and EH also corroborate with results of Esechie *et al.* (2004), Prakash *et al.* (2006), Salami *et al.* (2007), Bello *et al.* (2010), Ali *et al.* (2011b) who reported that lower plant height and central or near central placement of ear on plant is desired, because such plants are more resistant to lodging. In addition, Nazir *et al.* (2010) reported that plant height was positively correlated with days to flowering morphologically, as internodes formation stops at floral initiation and that early flowering maize varieties are usually shorter in height. Yao *et al.* (2011) also concluded that selection for plant height and its components would be effective in early generation and improvement in these traits will be promising to develop new varieties with desirable traits, most importantly lodging resistance.

Resistance to *E. turcicum* in maize germplasm was previously reported (Muriithi and Mutinda, 2001; Pandurangegowda *et al.*, 2002; Dharanendraswamy, 2003; Singh *et al.*, 2004; Harlapur, 2005). Kumar *et al.* (2011) identified 20 inbred lines as sources of resistance against TLB; Shikari and Zafar (2009) reported that inbred NAI-147 and composite Girija expressed resistance to the TLB; Adipala *et al.* (1993) reported that Babungo 3, population 42 and KWCA were significantly more resistant to TLB than the susceptible check B73. Similarly, resistance to MLB was reported previously. Rai *et al.* (2009) described that out of the 51 genotypes, 26 genotypes recorded resistant, 8 moderately resistant, 13 moderately susceptible, 2 susceptible and 2 were highly susceptible to MLB; Aziz *et al.* (1992) reported that early maturity genotype Ehsan and Ehsan x SW49-2 showed resistant to both TLB and MLB and also recorded highest yield.

In maize resistance to TLB is conditioned by quantitative and qualitative mechanisms (Hooker, 1981; Ogliari *et al.*, 2007). Quantitative resistance is characterized by low lesion number, small lesion area with typical necrotic lesion types, as well as reduced severity and area under disease progress curve (AUDPC) values; whilst qualitative resistance is characterized by small lesions surrounded by chlorotic halo also referred to as Ht (*Helminthosporium turcicum*)-lesions type. In this study, the resistant plants had typical of quantitative resistance to TLB. Quantitative resistance in general, supports mild levels of epidemics, invariably allowing the existence of a variable population of pathogens (McDonald and Linde, 2002). Hence, the mechanism of quantitative resistance do not impose extreme selection pressure and accelerate patho-evolution (Dangle and Jones, 2001; McDonald and Linde, 2002). Some of the known TLB resistant inbred lines reported in the earlier studies at our center were also included in the present study. In nature a continuous change/buildup of a new pathogen races/strain develops intermittently, hence in order to reconfirm durability of resistance to prevailing *E. turcicum* isolate and to further evaluating for MLB, FTD, FSD, PH, EH, DTM and GY, they were included in the this study.

In the present investigation, the GY of resistant and moderately resistant inbreds were in general high in comparison to susceptible inbred. The *H. maydis* conidia causing MLB may germinate and penetrate the host epidermal cells and stomata of both susceptible and resistant hybrids of both young and mature plants. After entering the chlorenchyma tissue of the susceptible plants, the hyphae caused chloroplast destruction and cell wall collapse (Hesseltine *et al.*, 1971). However, the *H. turcicum* (*E. turcicum*) causing TLB mycelium rapidly grew into the xylem elements so extensively that eventually it plugged the water-transporting vessels until there was no water movement, resulting in localized wilting. Evidently the resistant factor(s) in maize to *H. maydis* is present in the chlorenchyma and the resistant factor to *E. turcicum* is present in the xylem elements (Hilu and Hooker, 1965; Hesseltine *et al.*, 1971). This indicates that GY of resistant or moderate resistant lines are not

affected by the pressure of TLB / and MLB (Shivankar and Shivankar 2000; Harlapur 2005). However, Byrnes *et al.*, (1989) found that MLB with each 1% increase between 0-25% infection, decreased the yield of FR27 x Pa91 and B73 x Mo 17 by 0.6-0.7%. But the grain yield of Pioneer 3183 hybrid decreased by an additional 23% when the disease severity increased from 23-40%. The negative association of GY with TLB and MLB incidence noted in the present investigation also indicates that yield losses are directly proportional to the disease severity as well as degree susceptibility to both the diseases.

Principal Component Analysis (PCA) is a useful statistical technique which has found application in reduction of the original variables (TLB, MLB and GY) in a smaller number of underlying variables (Principal Component) in order to reveal the interrelationships between the different variables. The principal Component 1 (PC 1) describes higher sample variation and the following PC 2 successively explains smaller parts of the original variance. In the earlier studies by using PCA analysis Kaiser (1970) and Lopez *et al.* (2006) reported similar results. Positive correlation was observed between TLB and MLB and both were negatively correlated with GY. The genotypes in the cluster-I showed resistant to TLB and MLB and also recorded higher GY, while the genotypes in the cluster-II showed moderate resistant to TLB and MLB along with higher GY. But the genotypes in cluster III were found highly susceptible to TLB, moderate resistant to MLB along with poor GY. The present results are in agreement with the Pataky *et al.* (1998) and Byrnes *et al.*, (1989). While evaluating the yield loss assessment trial, they reported sever yield losses ranged from 0-60% and 23% due to TLB and MLB, respectively. Similarly, Frank *et al.* (2013) and Presello *et al.* (2004) used the PCA analysis for distinguishing dent and flint heterotic maize genotypes and for distinguishing resistant and susceptible genotypes against *Fusarium* ear rot of maize, respectively.

The AHC classification will be helpful for selection of disease resistance, high yielding inbreds for resistance breeding program. In cluster I most of the inbreds are moderately resistant to TLB, resistant to MLB with high GY. In cluster II most of the inbreds are resistant

to TLB, MLB along with higher GY. In cluster III, most of the inbreds are highly susceptible to TLB, moderately resistant to MLB and low GY. Similar results were reported by Biabani *et al.* (2004) while evaluating nuclear polyhedrosis virus against silk worm *Bombyx mori*, they reported that AHC analysis categorized different hybrids into 2 classes based on their degree of resistance. The AHC classification also revealed that, genotypes in same cluster were found to be of different pedigree.

Based on the TLB and MLB incidence scores, GY and other related parameters, new promising inbred lines were identified and the durability of known resistance lines were established. These inbred lines are V334, V338, V 341, V 345, V 346, V 373, V 398, V 403, V 405, V 407, VQL 17, CM 152 and CM 153 having a lower interval between pollen shed with silking, medium plant stature and suitable ear placement. They will serve as potential donors/ parental lines in hybrid development program in general and for the North Western Himalayas in particular. The identified resistance sources may also be potential parents in hybrid development program across the different zones of the country or even in other maize growing countries however, resistance with challenged inoculation under target ecosystem requires to be validated prior to induction in hybrid breeding program.

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