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RESEARCH ARTICLE

Phenotypic Diversity Analysis and Screening for Northern Corn Leaf Blight Resistance in Maize (*Zea mays* L.) Landraces Grown in North Eastern Hill Region of India

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A total of 139 diverse maize landraces from seven hill states of North East India were evaluated for genetic diversity and resistance to Northern Corn Leaf Blight (NCLB) in an augmented design. ANOVA for cob and flowering characteristics were highly significant. PCA revealed cob traits contributed to PC 1 while flowering traits loaded on PC 2. Agglomerative Hierarchical Clustering (Ward's method) grouped the landraces into four distinct classes on cob weight variation. For NCLB resistance, qualitative disease assessment under natural field conditions followed by controlled quantitative assessment based on AUDPC scores were consistent for the landraces studied implying genetic basis of inheritance. SSR markers which map close to resistant *Ht* genes when run on a subset of the landraces showed distinct polymorphism specifically between the most susceptible T(9)8) and resistant landrace M9(4) collected from Tripura and Meghalaya, respectively for markers close to resistant *Ht2* gene. Diversity and disease screening studies hereby establish presence of genetic variability for initiating or fortifying resistance breeding programmes.

Key Words: Cluster analysis, Maize landraces, Morphological diversity, NCLB, *Turcicum* blight resistance

Introduction

Maize possesses highest levels of diversity because of selective breeding over time in different regions of the world with most of the variation present in landraces (Mangelsdorf, 1986). Very high genetic variation for maize landraces also exists in the North Eastern Hill (NEH) Region of India (Sharma *et al.,* 2010; Singode and Prasanna, 2010). One of the secondary centers of diversity, thirteen highly prolific primitive maize landraces are distributed across NEH region. These landraces christened "Sikkim Primitives" were believed to have originated from a single popcorn variety and subsequently domesticated by different ethnic groups of the region (Prasanna and Sharma, 2005).

Although modern farming techniques have enhanced yield, greater crop uniformity has led to extensive loss of germplasm diversity. However, landraces till date represent one category of locally adapted germplasm which lack formal crop improvement, are genetically diverse, associated with traditional farming systems (Villa *et al*., 2005) and provide a ready source of alleles for improving disease and pest resistance, nutritional quality and other traits of interest (Yao *et al.,* 2007). Germplasm characterization in conserving genetic variation is critical to crop improvement and diversity studies to generate basic information about variability (Lucchin *et al.,* 2001) leading to better understanding of traits that have shaped quantitative variation in maize (Carvalho *et al.,* 2004).

Plant breeders are known to use variation found in landraces especially with respect to resistance to counter continuously evolving pests and diseases (Smale *et al.,* 2001). Prominent among foliar disease of maize is *Turcicum* blight also known as Northern Corn Leaf Blight (NCLB) caused by *Setosphaeria turcica* (Leonard & Suggs) with its conidial state *Exserohilum turcicum.* Damaging in both the tropics and the Himalayan Region with yield losses exceeding 70% during a severe attack, annual yield losses in maize growing nations from NCLB range from 15 to 30 % (Welz and Geiger, 2000; Wisser *et al.,* 2011; Haasbroek *et al.,* 2014). Genetic control of NCLB can be achieved qualitatively with dominant

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or partially dominant *Ht* genes or quantitatively, used separately or together (Ogliari *et al.,* 2005; Wisser *et al.,* 2006; Zwoniter *et al.,* 2009).

The present study was taken up for characterization of 139 landraces collected from NEH region for nine quantitative DUS descriptors and screening for NCLB disease resistance at the field as well as in controlled greenhouse environmental conditions based on AUDPC (Area Under Disease Progress Curve) values. Additionally, a subset of the landraces was also screened with SSR markers known to map close to *Ht* genes.

Materials and Methods

The study site was College of Post Graduate Studies, Umiam, Meghalaya India located 25º34'32"N latitude and 91º52'23"E longitude, set at an elevation of 950 m above mean sea level. Temperatures during the maize planting season range from 28º to 32ºC with a relative humidity of 85-95% which makes it a hot spot for occurrence of NCLB (Biswar *et al.,* 2007).

The experimental material comprised a collection of landraces from the seven hill states of North East India *viz*. Arunachal Pradesh (A), Manipur (Ma), Meghalaya (M), Mizoram (Mi), Nagaland (N), Sikkim(S) and Tripura (T). Local composities *viz.* RCM-1-1 and RCM-1-2 obtained from ICAR, NEH Region, Umiam Meghalaya and inbred lines *viz.* CM-145 (resistant) and V 334 (susceptible) from ICAR-VPKAS, Almora, Uttrakhand were used as standard checks for NCLB evaluation studies. These checks were assigned codes C1, C2, C5 and C6, respectively. While a total of 152 landraces were collected from various maize growing districts, 139 survived and were used for genetic diversity studies of which forty three landraces were collected from Meghalaya, twenty seven from Nagaland, twenty one from Manipur, eleven from Arunachal Pradesh, sixteen from Sikkim and five from Mizoram. Care was taken to collect only such landraces which have been traditionally conserved by farmers at their homes. The experiment was conducted over a span of two years from 2012-13 and 2013-14. The landraces were assigned alphanumerical codes according to their respective states of origin followed by the collection number assigned to a particular landrace.

An augmented design of field trial was laid out for the 139 maize landraces. Replicated checks were also planted in four blocks. Sowing was done in single rows of ten plants per landrace. Plant to plant spacing was maintained at 30 cm while row to row distance was fixed at 60 cm. Routine intercultural operations were carried out. Individual cobs from each plant within and between the landraces were always harvested separately. While data on 28 morphological descriptors both qualitative and quantitative were studied, data pertaining to nine economically important agronomic traits defined by DUS (Distinct, Uniform and Stable) requirements were used for further analysis. Tasselling (TA) and Silking (SI) data were calculated when 50 % of the plants of a particular landrace came into flowering based on which the Anthesis Silking Interval (ASI) was calculated. Data on cob weight with husk (WwH) in grams, cob weight without husk (WwoH) in grams, cob length without husk (CL) in centimeters, number of row grains (NRG), 100 grain weight (100GW) in grams and plant height (PH) in meters were the other observations used for analysis.

Qualitative scoring of the landraces for NCLB was carried out based on the method and disease ratings of 1 to 5 as per Payak and Sharma (1983). A score of 1 was used to characterize the resistant and a score of 5 to characterize the susceptible plant. Cobs of individual plants within and between landrace were harvested separately. Three plants which were treated as three replications from individual cobs with highest and lowest field disease scores were subsequently grown in the greenhouse to verify results of field screening.

Seedlings grown in the greenhouse, when at knee height stage were inoculated with *E. turcicum* spores under controlled environmental conditions of high humidity and temperature. All plants were inoculated with 0.5 ml spore suspension (3×10^4 conidia spore/ml, with 0.02% Tween 20) in leaf whorls and relative humidity levels were maintained at 80%. To measure disease progress, AUDPC was calculated based on area of disease spread on the leaf surface and, the progress recorded every three days from the onset of the disease as per Madden *et al.* (2000) till flowering. The disease progress of three lesions per plant were recorded and subsequently averaged over the three replications for final calculations of AUDPC.

Plant genomic DNA was extracted from leaves of individual plants at the three leaf emergence stage using SDS method of extraction. Based on high and low AUDPC scores recorded, ten landraces from either group were selected for bulk DNA studies as per Shen *et al*. (2003). DNA extraction was first done on individual basis. The individual genomic DNA was then bulked in

equal proportion in five separate bulks with each bulk containing DNA from landraces with similar AUDPC scores (Table 1). For each bulk, the same DNA samples were pooled twice to produce replicated samples. Twenty reported SSR primers that are known to tag close to resistant *Ht* genes from bin 8.06 and 2.08 were used to screen for polymorphism. The SSR sequences were obtained from Maize Genetics and Genomics Database (http://www.maizegdb.org/data_center/ssr#). PCR for bulked as well as individual DNA was carried out in a reaction volume of 10 µl. The amplification was carried out in thermal cycler using the PCR profile of 5 minutes initial denaturation at 95 ºC, 30 seconds at 55 ºC for annealing and 45 seconds at 72 ºC for extension and a final extension at 72 ºC for 5 minutes. Scoring was done using 3% agarose gel (Sigma, Ultrapure Agarose 1000) and the bands were scored based on length of the amplified products using the molecular ladder as reference. The number of polymorphic and monomorphic fragments was determined by observing the amplified fragments for each pair of primer.

Analysis of variance (ANOVA) for augmented design based on the morphological traits was done using adjusted treatment means. Data for the nine traits studied were further subjected to Principal Component Analysis using XLSTAT. Genetic diversity and cluster analysis for the quantitative traits were studied using Agglomerative Hierarchical Clustering (AHC) with Euclidean Distances and Ward's Method as measures of dissimilarity among populations using XLSTAT. The cluster diagram was generated using DARwin Version 6. For the molecular data analysis, data of polymorphic and monomorphic amplification was extracted to Excel table for further interpretation.

Result and Discussion

The maximum and minimum values for the nine different quantitative data studied under field conditions exhibited a range of variation around the mean data for the 139 landraces under study. ANOVA for the morphological descriptors based on quantitative data similarly confirmed presence of highly significant variation for the traits under study (Table 2). Anthesis Silking Interval was highly variable with differences between days to Tasseling and Silking being as low as 1 day in M25 to as high as 32 days in landrace T7. Similarly, cob weight was highly variable and was highest for landraces with higher Anthesis Silking Interval values. Landrace M31 with the highest average cob weight of 270 g without husk recorded an average ASI of 10 days. Cobs of landraces from Nagaland recorded the highest plant height, longer Anthesis Silking Interval, days to maturity and cob length and landrace N9 recorded the highest average 100 grain weight at 74.25g among all the landraces studied.

Landraces which do not serve as sources for improved maize germplasm contain untapped allelic variation in the form of resistance to biotic and abiotic stresses which can be effectively utilized in modern maize breeding (Warburton *et al.,* 2008).The goal of plant breeders has long been to produce broadly adapted, improved varieties by exploiting the available germplasm for variation (Mercer *et al.,* 2008). A substantial fraction of maize germplasm is maize landraces conserved in various pockets of NEHR by famers with small landholdings (Prasanna, 2010) but limited efforts have been made to characterize and explore the vast maize genetic variability available in the NEHR with only 2% of the available maize germplasm utilized in breeding programmes (Devi *et al.,* 2013). In the present study too, considerable variation for different yield contributing traits was present in the 139 landraces studied which can be effectively exploited in future breeding programmes.

Association studies (Table 3) using Pearson's correlation revealed highly significant correlation for days to 50% Silking with days to 50% Tasseling and Anthesis Silking Interval. Cob weight with husk was highly and significantly correlated with other cob traits *viz.* cob weight without husk, 100 grain weight and cob length. Number of row grains was also highly significantly correlated with cob length, 100 grain weight and plant height. Plant height was highly significantly correlated with cob weight with husk, cob weight without husk, cob length and number of row grains. The first four components of Principal Component Analysis could explain 83.80 % of the variation in the data. While PC1 accounted for 34.22 % of variability in the data, PC 2 could explain 22.48 % of the variability. PC 3 and PC 4 explained 14.76 % and 12.32 % variation in the data respectively. Cob traits cumulatively were found to have the highest loadings on PC1 accounting for 88.12 % of the total variation while plant height which also loaded on PC1contributed to around 10% to the total variation. Flowering traits days to 50% Silking and Anthesis Silking Interval had the highest loadings on PC2 contributing to 86.02 % of the total variation in PC2 of which, 44.30% was contributed by days to 50%

Silking alone. Traits, number of row grains and100 grain weight had high loadings on both PC1 and PC3 while days to 50% Tasseling had almost equal loadings on PC3 and PC4. Squared cosines of the variables studied to further confirm the contribution of the individual traits showed that cob length, cob weight with husk, cob weight without husk with values higher than 0.5 contributed to maximum variation in PC1, while Days to 50% Silking and Anthesis Silking Interval with cosine values higher than 0.5 in PC2 were the main contributors to variation.

Agglomerative Hierarchical Clustering (AHC) with Euclidean Distances using Wards Distance for the 139 landraces and the four reference checks studied generated four major classes (Figure 1). Cob weight with husk and cob weight without husk were the most variable parameters and appeared to influence grouping of the landraces in the different clusters. Cluster 1 comprised of 45 landraces, Cluster 2 of 29 landraces, Cluster 3 of 63 landraces and Cluster 4 of 6 landraces. Class 3 was markedly dissimilar from Classes 1, 2 and 4 and recorded the lowest cob weight and length. Classes 1 and 2 had very low levels of dissimilarity. In Class 4 clustered landraces were recorded with the highest cob weight and also the highest 100 grain weight. For all the nine quantitative traits studied, the maximum distance for the

Landraces:

A1	M18	M4	Ma3	N20	S3
A10	M19	M40	Ma4	N21	S4
A11	M2	M41	Ma5	N22	S5
A2	M20	M42	Ma6	N23	S6
A3	M21	M43	Ma7	N24	S7
A4	M22	M5	Ma8	N25	S8
A5	M23	M6	Ma9	N26	S9
A6	M24	M7	Mi1	N27	T1
A7	M25	M8	Mi2	N3	T10
A8	M26	M9	Mi3	N4	T11
A9	M27	Ma1	Mi4	N5	T12
C1	M28	Ma10	Mi5	N6	T13
C2	M29	Ma11	N1	N7	T14
C5	M3	Ma12	N10	N8	T15
C6	M30	Ma13	N11	N9	T16
M1	M31	Ma14	N12	S1	T2
M10	M32	Ma15	N13	S10	T3
M11	M33	Ma16	N14	S11	T4
M12	M34	Ma17	N15	S12	T5
M13	M35	Ma18	N16	S13	T6
M14	M36	Ma19	N17	S14	T7
M15	M37	Ma2	N18	S15	T8
M16	M38	Ma20	N19	S16	T9
M17	M39	Ma21	N2	S2	

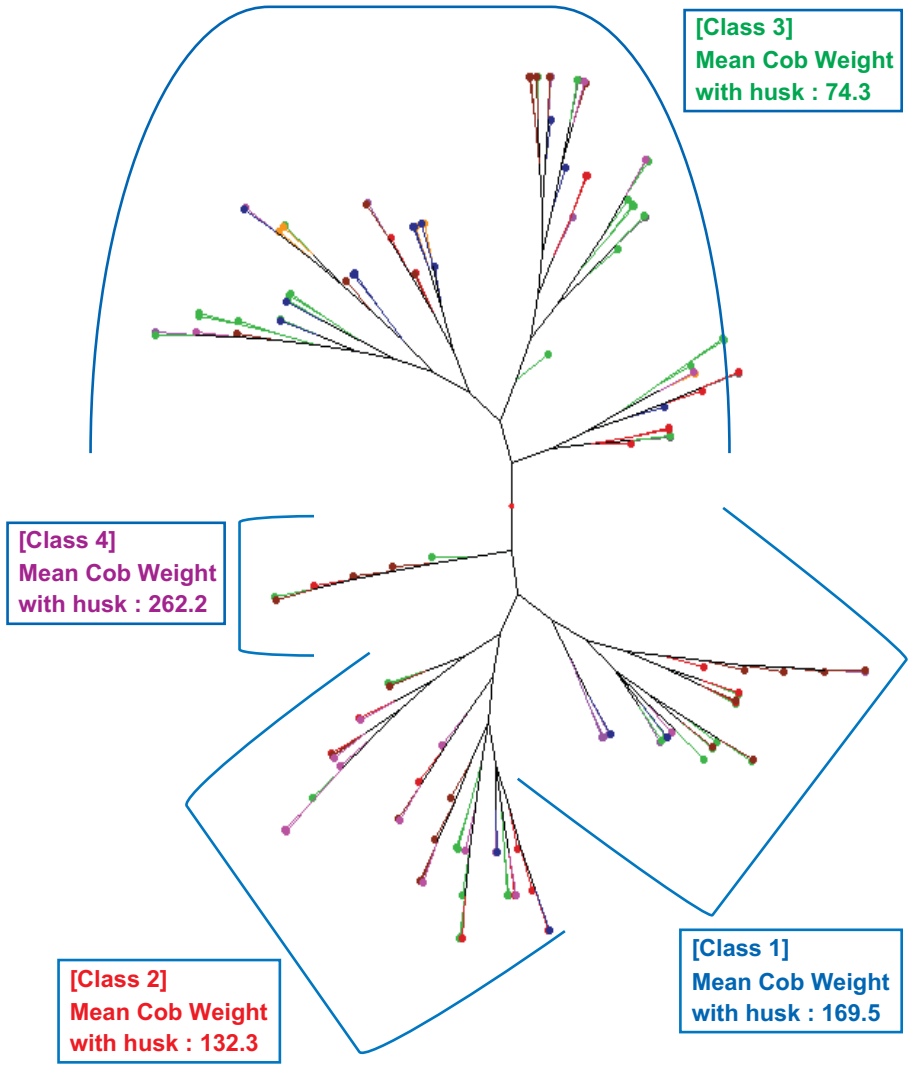

Fig. 1. A radial phylogenetic tree illustrating clustering pattern of the landraces under study using Agglomerative Hierarchical Clustering with Euclidean Distances and Ward's method. Clustering of the landraces appeared to be on the basis of cob weight with landraces from similar regions grouping into different classes without any geographical bias. Class 3 with the highest number of landraces clustering together had the lowest average cob weight without husk while in Class 4 were clustered landraces with highest mean cob weight.

class centroids was recorded between clusters 3 and 4. Landraces N24, T2, M30 and Ma7 were identified as central objects in clusters 1, 2, 3 and 4 respectively. The greatest distance between central objects was recorded between landraces of Classes 3 and 4.

Spread in the foothills of the Eastern Himalayan region at altitudes varying from around 280 m to more than 7000 m above the mean sea level (Mao *et al.*, 2009), North East India is an established secondary centre of diversity for maize. Locally grown maize which can be grouped into primitive, advanced or derived, recent introductions and hybrid races as a result of crosses between the Sikkim primitives and advanced races within the region (Medhi *et al.*, 2010) are found at varying altitudinal and climatic variations of the NEH region, where farmers grow maize to suit their diverse food and cultural habits. In the present study despite being representatives of diverse altitudes and climatic adaptations, the clustering of these 139 landraces was not based on geographical origin. A possible explanation is that because like elsewhere, landraces in the NEH region are also subjected to open pollination resulting in highly heterozygous cobs which as a result are distinct from each other even in neighboring fields of the same area. Also, selection for cobs in landraces at the individual farmer's level is always phenotypic, favouring the most vigorous plants/cobs leading to high heterogeneity within the collections of landraces. Heterogenity is also advantageous for plant breeders from evolution point of view as it promotes genetic variability and helps to stem genetic drift while promoting new genetic recombinations in the farmers' fields for selection. Qi-Lun *et al.* (2008) while studying the diversity of 124 landraces of maize had reported presence of considerable genetic variation within landraces because farmers select from the most heterozygous plants contributing to the high genetic variation.

For the 139 landraces studied, ANOVA, PCA and AHC studies established the significant contribution of cob weight as the highest contributor to variability in the dataset. Contribution of individual landraces to the PCs also revealed that the six landraces in Cluster 4 (N9, M31, N11, Ma7, M32 and N20) with highest mean cob weight had very high loadings on PC1. Since traits with higher loadings on PC1 are the highest contributors to variation in a dataset and serve as valuable indicators for selection in a crop improvement programme, selection based on high cob weight for the 139 landraces under study is expected to be effective.

Under natural field conditions, NCLB development was recorded in all 143 genotypes approximately 60 DAS (days after sowing), varying from highly resistant to moderately resistant to highly susceptible indicating presence of variability for NCLB resistance in the collection (Table 1). The standard inbred susceptible check C6 was also susceptible to NCLB in our field conditions. Under controlled green house conditions disease lesions appeared 47 days after planting and significant differences in the area of necrotized leaf tissue for the different landraces were observed. The susceptible landraces began by producing small lesions which enlarged and coalesced to form large necrotic patches expanding to cover the entire leaf blade whereas, in case of resistant plants, the necrotic lesions remained localized. The rate of sporulation as perceived by blackening of the necrotized surface was also lower in case of landraces with low AUDPC scores. With the exception of M9 (4) (where 4 indicates the 4^{th} of ten M9 plants grown in the field), every other plant inoculated artificially under green house conditions developed the disease in varying degrees and AUDPC scores ranged from a minimum of 0 in M9 (4) to a maximum of 273.4 in T9 (8) (where 8 indicates the 8^{th} of ten T9 plants grown in the field). The field scores based on qualitative scoring for M9 had also ranged for individual plants from 1(highly resistant) to 3 (moderately resistant) while field scores of T9 ranged from 3 (moderately resistant) to 5 (highly susceptible). Certain landraces like A7 in which were recorded plants with moderate resistance scores in field conditions were exceptions which recorded very high AUDPC values in green house conditions (Table 1). Given that AUDPC scores are more reliable in assessing disease resistance, the moderately resistant field scores for A7 can be attributed to environmental reasons. In general, the susceptible landraces recorded higher AUDPC values as a result of larger lesion size, faster onset of disease and higher number of lesions post artificial inoculation. Resistance to NCLB was not specific to landraces from a particular geographical region but was found in different landraces representing all the seven hill states.

The AUDPC studies were followed by bulked DNA analysis in a subset of individuals within landraces with the highest/lowest AUDPC scores using twenty SSR markers which map close to *Ht* genes in bins 8.06 and 2.08. Seven SSR markers screened *viz. umc2005*, *umc1947*, *umc1864*, *umc1149*, *umc1997*, *umc2361* and

Table 1. Panel of twenty selected plants used for molecular studies based on their AUDPC scores under controlled greenhouse conditions

DNA Bulk No.	Selected Individual Plants	Greenhouse Audpc Score	Qualitative Disease Scores
	M9(4)	0	1
1	M24(8)	11.2	1
	N25(4)	12.2	2
	M23(2)	12.8	2
2	S2(10)	15.2	3
	M23(9)	15.6	2
	N21(5)	15.6	2
3	S9(1)	18.2	3
	Mi4(1)	18.4	3
	N25(5)	18.6	2
4	T9(8)	273.4	5
	M25(1)	202.6	5
	Ma7(7)	200.4	5
	Ma6(7)	198.7	5
	T16(3)	193	5
5	T13(1)	185.2	5
	M3(3)	181.4	5
	A11(4)	180.8	5
	A7(1)	179	3
	M11(3)	178.6	5
	C1	25.8	5
	C2	5.6	5
	C5 (Resistant Inbred)	85.9	1
	C6 (Susceptible Inbred)	105.8	5

*Figures in parenthesis for selected individual plants, indicate the plant tag number for a particular landrace.

umc2119 were found to be polymorphic. For bulk DNA studies, M9(4) which did not show any disease incidence under greenhouse conditions was excluded from the bulks and used as reference along with the standard resistant (C5) and susceptible (C6) checks (Table 1). Of the seven polymorphic SSR markers, when run on individual plant DNA, markers *umc1149*, *umc2199* and *umc2361* exhibited distinct polymorphism (Figure 2) between the resistant M9 (4) and the highly susceptible T9 (8). A7 (1) behaved similarly as the susceptible T9 (8) for all the three SSR markers. Landraces M23 (9), S9 (1), Mi4 (1) were also polymorphic like M9 (4) for *umc1149* while, M23 (2) and S9 (1) showed similar polymorphism as M9 (4) for *umc2361*. In landraces M23, S9 and Mi4 were present individual plants with high to moderate resistance disease scores that were consistent with the low AUDPC values under greenhouse conditions.

Our studies of the 139 landraces based on field screening under natural disease conditions revealed variability for disease ratings not only between but also within the landraces. This variability also did not have a geographical basis and can be similarly explained by the fact that maize landraces are subjected to high degree of cross pollination at the farmer's level as a result of which landraces collected from individual farmers tend to be heterogenous seed mixtures. Consistency between the field scores of the individual plants within and between landraces and AUDPC scores recorded under controlled conditions for majority of the landraces establish a genetic basis for nature of inheritance of the disease. Distinct polymorphism between the resistant M9 (4) and susceptible T9 (8) for markers, *umc1149*, *umc2199* and *umc2361* known to tag close to *Ht2* gene was observed. Various workers (Yin *et al.,* 2003; Chung *et al.,* 2010) had reported the association of SSR markers *umc1149*, *umc2199* and *umc2361* and the *Ht2* major gene with genetic distance between SSR marker *umc1149* and the *Ht2* gene on chromosome 8 being as little as 7.2 centimorgan. Going by these reports, polymorphism in landraces M9 (4) and T9 (8) for *umc1149 umc2199* and *umc2361* indicate presence of resistance governed by the monogenic dominant resistant *Ht2* gene. Also, since quantitative disease resistance in plants is known to be frequently associated with basal resistance (Poland, 2011) these landraces can further be explored for presence of durable resistance.

Screening for NCLB resistant germplasm is critical to check destruction by an NCLB epidemic because the pathogen is fast evolving with several reported races. Dong *et al.* (2008) while studying the race distribution in northern China had reported variation in physiological races of *S. turcica* between neighbouring provinces while Human *et al.* (2016) have reported high diversity among *E. turcicum* isolates as a result of mixed modes of reproduction adopted by the pathogen for survival in South Africa. Successful gene pyramiding through seven different backcross populations and four resistant donors into elite lines for major and minor resistant genes to NCLB have been reported in India (Prasanna *et al.,* 2010) which makes disease resistant germplasm invaluable for crop improvement (Kraja *et al*., 2000).

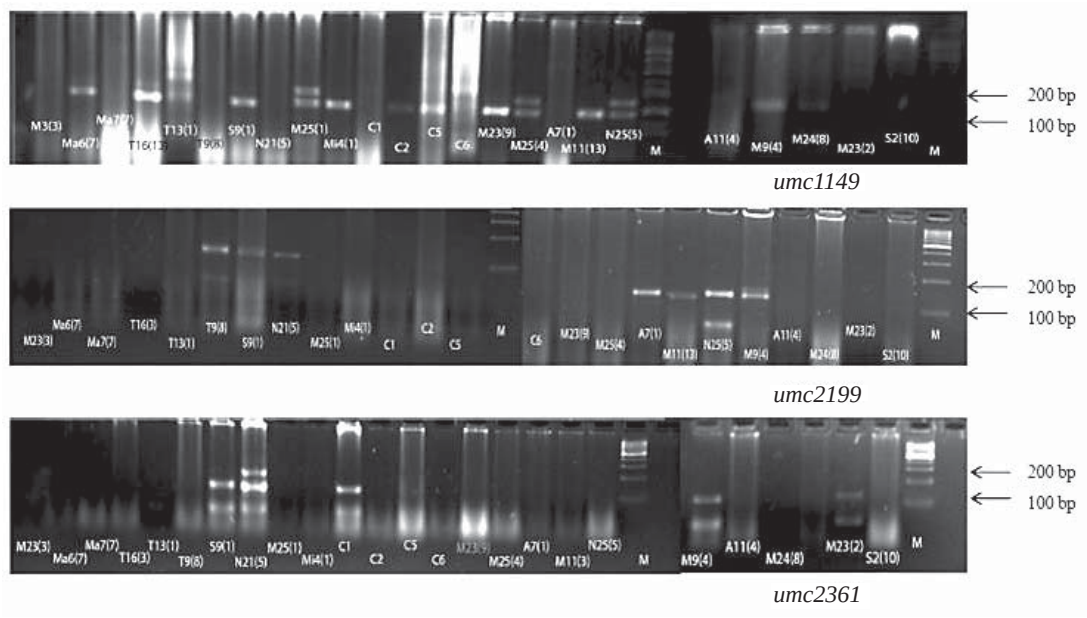

Fig. 2. Amplification patterns of SSR markers *umc1149*, *umc2199* and *umc2361* reported to map close to the *Ht2* gene for *Turcicum* blight resistance when run on a subset of individuals from the different landraces identified as resistant/susceptible based on their field and AUDPC scores. The individual lanes for the landraces are labeled accordingly where figures in parenthesis indicate individual plant tag number and M = molecular size ladder (100 base pair). C1, C2, C5 and C6 were the standard checks used in the study. The resistant M9 (4) and susceptible T9 (8) are polymorphic for all the three SSR markers.

To summarize, the current study was aimed at genetic diversity studies and characterization for NCLB resistance qualitatively and quantitatively in a collection of locally adapted and unexploited maize germplasm. ANOVA and genetic diversity studies established presence of variation for cob traits while field disease scores and AUDPC values established presence of variation for NCLB in the material studied. Both NCLB resistance and cob characteristics being critically important agronomic traits, the identified landraces can serve as raw material for exploitation in crop improvement. Also, important is that neither crop diversity nor resistance is localized to a specific geographical area within NEH region but is present across the representative germplasm studied. These landraces can therefore be further tapped to introduce variability

Table 2. Range, mean and MS (Mean Square) values of adjusted treatments and error estimated from ANOVA for the different quantitative parameters studied

Variables	Minimum value	Maximum value	Mean	Standard deviation	MS treatments (Adjusted)	MS error
SI	62.10	95.80	75.47	7.58	57.4**	10.6
TA	47.00	74.00	64.72	4.99	56.1**	3.0
ASI	1.04	32.00	10.75	6.03	36.4**	1.1
CL	3.00	7.00	4.18	1.00	1.0**	0.2
WwH	19.50	300.00	115.95	52.61	2747.4**	1409.8
WwoH	14.50	270.00	96.84	44.27	1786.6**	1264.4
NRG	3.00	22.62	11.04	4.19	1786.6**	0.4
100GW	3.50	74.25	21.50	10.02	55.5**	24.5
PH	3.20	9.00	6.43	1.48	1.7**	0.3

Days to 50% Tasseling (TA); Days to 50% Silking (SI); Anthesis Silking Interval (ASI);Cob length (CL); Cob weight with husk (WwH); Cob weight without husk (WwoH); 100 Grain weight (100GW); Number of row grains (NRG); Plant height (PH)
*- Significant at 5% level of significance; **-Significant at 1% level of significance

Table 3. Association studies based on Pearson's Correlation coefficients and squared cosines of Principal Component Analysis, for the nine quantitative parameters under study

	Pearson's Correlation coefficients:									Squared cosines of the variables			
Variables	SI	TA	ASI	CL	WwH	WwoH	NGR	100GW	PH	PC1	PC2	PC3	PC4
SI	1									0.014	**0.897**	0.085	0.004
TA	0.608**	1								0.046	0.291	0.012	0.57
ASI	0.753**	-0.063	1							0.001	**0.553**	0.076	0.301
CL	0.031	0.062	-0.012	1						**0.649**	0.003	0.002	0.008
WwH	-0.012	0.06	-0.065	0.558**	1					**0.642**	0.066	0.247	0.006
WwoH	-0.056	0.03	-0.095	0.516**	0.979**	1				**0.595**	0.091	0.256	0.006
NRG	0.127	0.153	0.032	0.491**	0.214*	0.182*	1			**0.449**	0.057	0.348	0.006
100GW	0.129	0.102	0.078	0.415**	0.232**	0.208*	0.719**	1		0.38	0.061	0.283	0.06
PH	-0.002	0.155	-0.131	0.414**	0.295**	0.274**	0.355**	0.154	1	0.306	0.005	0.019	0.148

Days to 50% Tasseling (TA); Days to 50% Silking (SI); Anthesis Silking Interval (ASI);Cob length (CL); Cob weight with husk (WwH); Cob weight without husk (WwoH); 100 Grain weight (100GW); Number of row grains (NRG); Plant height (PH)

*- Significant at 5% level of significance; **-Significant at 1% level of significance

For squared cosines of variables, figures in bold indicate high contribution of the trait in PC 1 and 2 respectively.

in maize germplasm for developing new hybrids and fortifying existing programmes via introgression.

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