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SCREENING OF MAIZE GENOTYPES AGAINST NORTHERN CORN LEAF BLIGHT

RAJESH SINGH* R. P. SRIVASTAVA, V. P. MANI, R. S. KHANDELWAL AND LEKHA RAM
Department of Genetics and Plant Breeding,
Institute of Agricultural Sciences, BHU, Varanasi - 221 005
e-mail: rsingh6361@gmail.com

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***Corresponding author**

ABSTRACT
A study was carried out, involving 118 maize genotypes to identify, the new sources of resistance to 'Northern Corn Leaf Blight' under artificial epiphytotic condition at three locations viz, Almora, Nagenahalli and Varanasi for 12 years (1999-2011) with the help of three experiments. All the three Experiments included separates set of genotypes except 13 inbreds, (CM 145, CM 141, V336, V341, V348, CM145, V273, V342, V346, V338, V335, CM126 and CM212) which were common in II nd and IIIrd Experiments. The present study has helped in the identification of 26 resistant, 56 moderately resistant, 26 susceptible and 10 highly susceptible maize genotypes. Thirteen lines viz. V53, V334, V335, V336, V338, V339, V 341, V 345, V346, V 350, CM 104, CM118, CM 145 showed high level of resistance, where as inbred lines viz, V 25,V128, CM 126, CM 127, CM 212 and CM 202 showed high level of susceptibility as they scored about 3.5 disease score across the environment. The maize inbred lines CM 145, V 338 and V 336 expressed high level resistance in all three environments. It was also observed that average disease incidence was high in Mandya then Almora and Varanasi thus indicating that isolates of *Exserohilum turcicum* was more virulent at Mandya than rest of two environments.

INTRODUCTION

Maize (Zea mays L.) occupies an important place in world agriculture due to its high yield potential and great demand. In India, it is the third important cereal after wheat and rice in terms of area and plays an important role in the overall progress of the national economy. As per the latest reports by USDA; the area, production and productivity of maize in India is 8.6mha, 20.5mt and 2.4t/ha, respectively (DMR annual report 2012). Despite its high yield potential, one of the major limiting factors to maize grain yield is its sensitivity to several diseases (Shah, 2006). Approximately 65 pathogens infect maize (Rahul and Singh, 2002). Northern Corn Leaf Blight also commonly known as *Turcicum Leaf Blight (TLB)* is one of most important foliar disease of maize and caused by *Exserohilum turcicum*, the residue borne fungus. This disease occurs sporadically in most temperate, humid areas where maize is grown (Lim *et al.*, 1974).The disease causes leaf necrosis and premature death of foliage which reduces the fodder and grain value of the crop (Payak and Renfro, 1968 and Payak and Sharma, 1985).In India, this disease is prevalent in the states of Karnataka, Himachal Pradesh, Uttar Pradesh Uttarakhand, Orissa, Andhra Pradesh and North Eastern Hill states. It also affects the *Rabi* maize in the plains of India. Yield losses can easily exceed 50 percent, if the disease appears before flowering (Raymundo *et al.*, 1981; Tefferi *et al.*, 1996). Although the losses due to NCLB can be minimized by the foliar application of fungicides, the most appropriate and economical strategy to manage is to use host plant resistance Harlapur *et al.*, 2008; Singh *et al.*, 2004 Singh *et al.*, 2012). The genetics of TLB resistance have been extensively studied (Welz and Geiger, 2000 and in Wisser *et al.*, 2006). Northern Corn Leaf Blight is unusual among necrotrophic diseases as several dominant or partially dominant qualitative genes have been described that confer race-specific resistance to it, including *Ht1* (Hooker, 1963), *Ht*2 (Hooker, 1977), *Ht*3 (Hooker, 1981), *Htn*1 (also known as HtN; Gevers, 1975) and *Ht*P (Ogliari *et al.*, 2005). In Indian germplasm, resistance to disease is polygenically governed (Sharma and Payak, 1990). Earlier effort were made to identify addinitional sources in maize for *Exserohilum turcicum* (Gowda *et al.*, 1994) and ideal maize breeding programme with high level of NCLB resistance requires to be supported by addinitional new sources of resistance at regular intervals. New and stable additional sources of resistance are obtained by continuous screening of germplasm across the year and environment. (Gowda *et al.*, 2002; Chandrashekara *et al.*, 2012). The aim of this study was to identify new sources of resistance against Northern Corn Leaf Blight for use in maize breeding program.

MATERIALS AND METHODS

This experiment was conducted at three locations *viz.*, Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi; Zonal Agricultural Research Station, V.C.Farm Mandya, Karnataka and VPKAS Research Station at Hawalbagh (ICAR), Almora during 1999-2011. The Ist and 2nd Experiment was laid out for the evaluations of NCLB at VPKAS (ICAR), Almora during the *Kharif* season of 1999-2001. The IIIrd Experiment was conducted at two locations *viz.,* Banaras Hindu University, Varanasi and Nagenahalli Mandya, Karnataka during the *Kharif* season of

2010-2011.The details of materials used and method employed in the all experiments were as follows;

Ist Experiment

In this experiment 27 entries involving 15 Open Pollinated varieties and composites, four narrow based synthetics and eight Pool and Population were included for screening of NCLB. The experiment was conducted at VPKAS Research Station at Hawalbagh during *Kharif* 1999 and *Kharif* 2000 in RBD with two replications with plot size of 3.0 m x 1.8 m each involving 27 entries and a local check Dhiari Local.

IInd Experiment

The experiment evaluated and screened 38 inbred lines at VPKAS research station at Hawalbagh during *Kharif* 2000 and 2001 under artificial epiphytotic field condition as well as in glass house condition. The 38 maize genotypes were screened for 2 years at field condition and for 1 year in the glass house *(Kharif 2001)*. In field conditions the entries were evaluated in 3-row plot of 3.0 m x 1.8 m each with a local susceptible variety Dhiari local was planted at regular interval as infector row in RBD with two replications. In addition to it, all the 38 inbreds were planted in glass house with five plants of each in pots during *Kharif* 2001. The data recorded is the average of the 3 trials.

IIIrd Experiment

Another experiment involving 53 inbreds were evaluated and screened for identification of resistance sources in two environments at Agriculture Farm, Institute Agricultural Science, BHU and Zonal Agriculture Research Station, Mandya, Karnataka during 2010 and 2011. In field condition the entries were evaluated in 3-row plot of 3.0 m x 1.8 m each with a local susceptible variety which was planted at regular interval as infector row in RBD with two replications.

Inoculation

Seedlings were inoculated at the 5-6 leaf stage. To prepare the inoculum, lesions were cut from infested leaves, placed on moist paper towels in Petri dishes for 48 hours to allow sporulation. Single spores were picked from lesions with the aid of a sterile microscope and placed on Potato Dextrose Agar (PDA) plate and incubated at room temperature. Individual colonies of *E. turcicum* were subsequently sub-cultured to fresh PDA plates and used to inoculate autoclaved sorghum kernels and allowed to colonize the grains for about 10 days. The colonized sorghum kernels were air-dried prior to field inoculation. Inoculation was done at the four- to six-leaf stage by placing 20 to 30 seeds of colonized sorghum kernels into the leaf whorls. Inoculation was done in the evening to allow successful infection when dew and ambient temperature is optimal and followed by two additional sprays of inoculums at 7-8 days intervals (Carson, 1995). Disease symptoms developed within 1-2 weeks of inoculum and by the time of flowering, disease were severe in the infector rows and susceptible genotypes. The same procedure was followed in all the 3 experiments.

Disease Scoring

The scale consists of five broad categories designated by numerals from 1 to 5 (Payak and Sharma, 1985). Intermediate ratings between two numerals (1.5, 2.5, 3.5 etc.) have also been given, thereby providing for a total of nine classes or categories. Wherever possible, observations on lesion types can also be made, such as large sporulating wilt type or small chlorotic, non-sporulating type. Data was recorded 30-35 days after inoculation, then on flowering and finally just before dough stage. The disease scoring was done as per symptoms mentioned below.

Very slight to slight infection, one or two to few scattered lesions on lower leaves.

Disease score 1: Light infection, moderate number of lesions on lower leaves only.

Disease score 2: Moderate infection, abundant lesions are on lower leaves, few on middle leaves.

Disease score 3: Heavy infection, lesions are abundant on lower and middle leaves, extending to upper leaves.

Disease score 4: Very heavy infection, lesions abundant on almost all leaves plants prematurely dry or killed by the disease.

RESULTS AND DISCUSSION

About 118 maize genotypes involving 91 maize inbreds, 27 pool, population, synthetics and open pollinated varieties were screened to identify additional source of resistance for NCLB (Fig.1). *T*hese maize genotypes were collected from Directorate of Maize Research New Delhi; VPKAS Almora, CIMMYT Mexico, Private Companies ,BHU Varanasi and Indian public sectors etc.

Ist Experiment

This experiment evaluated all the non-inbred genotypes involving 15 open pollinated varieties, 4 narrow based synthetics and 8 pool and population as mentioned in the Table 1. In this screening, 27 genotypes were evaluated consecutively for two years during 1999 and 2000 at Agriculture Research Farm, Hawalbagh VPKAS (ICAR), Almora. The genotypes VL Sankul Makka11, U15-1, CM502, Pop31 were classified as highly resistance genotype as they scored 1-2 disease score during screening while Dhiari Local, VL pool 1, VL Makka 90 were classified as most susceptible maize verities. Out of 27 maize cultivars, 4 were screened as highly resistance, 10 were screened as moderately resistant whereas 10 cultivars were susceptible and 3 cultivars were highly susceptible.

IInd Experiment

This experiment was conducted at Agriculture Research Farm, Hawalbagh, VPKAS (ICAR), Almora involving 38 inbred lines from Directorate of Maize Research New Delhi; VPKAS Almora; CIMMYT Mexico and Private Companies as mentioned in Table 2. This experiment was conducted consecutively for two years during 2000 and 2001 in the field under artificial epiphytotic condition whereas for one year during *Kharif* 2001 it was also screened under glass house conditions. Thirteen resistant sources *viz*.V53, V334, V335, V336, V338, V339, V 341, V 345, V346, V 350, CM 104, CM118 and CM 145 were identified as they scored between 1-2 disease score whereas the inbred line viz V 25, V128, CM 126, CM 127, CM 212 and CM 202 were classified as susceptible lines as they scored above 3.5 disease score. The results of glass house as well as in field condition indicated similar trends. The result

Table 1: Screening of Pool, Population and other genotypes for identification of Additional Sources of NCLB at VPKAS, Almora during 1999 and 2000.

Pool, population and other genotypes
Resistant: VL Sankul Makka 11, U 15-1, CM 502, Population 31 Moderately Resistant: VL 78, VL 15, VL 87, VL Makka-16, Pool 39, HEY Pool, Syn-1, Syn-2, U19, VL Pool-3 Susceptible: VL Amber pop Corn, VL Makka 41, VL Makka 88, VL89, Kiran, Surya, Navjot, VL Pool 2, VL Heterotic Pool 1, VL Heterotic Pool 2 Highly Susceptible: Dhiari Local, VL Pool 1,VL Makka-90

Table 2: Screening of maize inbred for identification of Additional Sources of NCLB at VPKAS, Almora during 2000 and 2001

Inbred lines
Resistant: V53, V334, V335, V336, V338, V339, V 341, V 345, V346, V 350, CM 104, CM118, CM 145 Moderately resistant: V 17, V 26,V 49, V 178, V 190, V 198, V 241, V 273, V 324, V 340, V 342, V348, CM 105, CM 119, CM 129, CM 141. Susceptible: V 12, V13, CM 128 Highly Susceptible: V 25,V128, CM 126, CM 127, CM 212 and CM 202

presented in Table 2 is the average of field as well as glass house experiment. With this experiment, 38 maize inbred lines were classified as having 13 resistance lines scoring below 2 disease score, whereas 16 moderately resistant inbred were identify by scoring disease score between 2-2.5 whereas 3 cultivar indicated in table 2 were classified as susceptible and 6 highly susceptible with disease score 4.0.

IIIrd Experiment

Fifty three maize inbred lines were evaluated for two years and in two environments. The results of screening against NCLB have been presented in Table 3. The IIIrd experiment included 13 inbreds (CM 145, CM 141, V336, V341, V348, CM145, V273, V342, V346, V338, V335, CM126 and CM212) which were also tested in the IInd experiment at VPKAS, Almora. The screening led to the identification of 9 sources of resistance viz, CM 145, V 336, CML 192, V 338, CML 172, HKI 586, HUZM 47, HUZM 211-1, HUZM 53. The lines such as CM-145, V-338 and V-336, which were resistant in IInd Experiment, were also classified as resistant in this experiment, thus indicating wide adaptability for resistance to NCLB. The lines V53, V334, V335, , V339, V 341, V 345, V346, V 350,

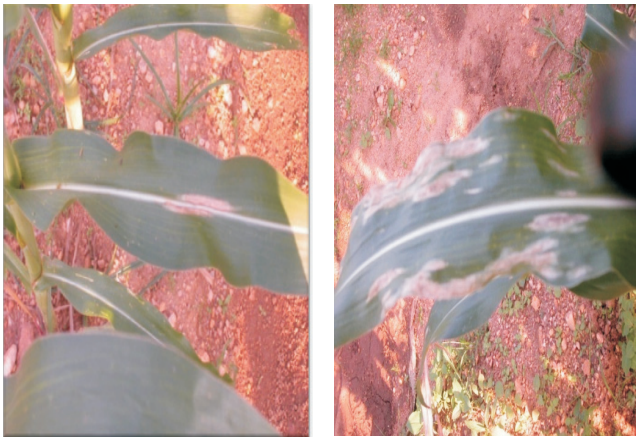
Table 3: Screening of maize inbreds for identification of Additional Sources of NCLB in two environments during 2010 and 2011

Inbred lines
Resistant: CM 145, V 336, CML 192, V 338, CML 172, HKI 586, HUZM 47, HUZM 211-1, HUZM 53 Moderately Resistant: HKI-164-4-(1- 3)-2, HKI-PC-8, HKI-1352-5-8-9, V-348, HUZM-356, CML-451, CML-161, HKI-209, HKI 323, HKI 193, HUZM 457, CML 150, HKI 335, CML 152, HUZM 69, HUZM 60, V342, HKI 287, CML 140, V273, V386, HUZM 478, V 341, V346, V388, HUZM 509, HKI 536, HUZM 97-1-2, HUZM-81-1, CM141 Susceptible: HUZM-80-1, HUZM 88, HUZM 185, HUZM 36, HKI 162, HKI 1105, CML395, V351, CM126, V335, 219-J, HUZM 121, V 25 Highly Susceptible: CM 212

CM 104, CM118 exhibiting resistant were classified as partial resistance in IInd Experiment. These are the valuable material as it expressed resistance across the environment. Interestingly the 3rd Experiment has revealed that in general the germplasm from BHU has exhibit moderate degree of resistance in both the environment. The inbred lines HUZM-185 which scored 2.1 moderate resistances in Varanasi expressed susceptibility in Nagenhalli by scoring 4.0. Similar trends were also observed by HUZM 121, HKI 536, HUZM 81-1 while reverse trend were observed with HKI 1105, HKI 162 and CM126 where they expressed susceptible reaction in Varanasi and expressed resistance reaction in Nagenhalli .This indicate that strain of *Exserohilum turcicum* are more virulent in Mandya than Varanasi while vice versa results were obtained by different set of inbred. In a similar study Abebe *et al*. (2008) conducted an experiment with 30 maize cultivars to study the reaction of the northern corn leaf blight at 3 locations. They found that significant differences among genotypes based on lesion number, size, AUDPC and severity rating scale at all locations. Susceptible varieties Gussau, Aboboko and Local- M had high AUDPC, large lesion size, fast onset of disease and many lesions in numbers. They further observed that the host entries used in this study indicated that Kuleni was resistant to northern leaf blight across three locations, with low rating score. Meena *et al*. (2009) also conducted an experiment for evaluation of 60 indigenous and exotic inbred lines under artificial epiphytotic conditions at two locations, Almora and Nagenahalli for eavaluation of Turcicum Leaf Blight (TLB). After two years continuous screening, a total of 20 inbred lines were identified as a sources of resistance at both the locations for NCLB, whereas 10 inbred lines were identified as resistant

Table 4: Reaction of important maize gene pools and populations available at Almora and BHU against NCLB (*Exserohilum turcicum*)

S. No.	Sources	No. of genotypes Resistant	Moderate Resistant	Susceptible	Highly Susceptible
1	Vivekananda Populations	10	24	7	7
2	Indian Public Sector	1	9	3	1
3	CIMMYT Mexico	2	5	1	0
4	Population 31	7	7	6	0
5	Exotic	2	3	3	2
6	Private Sector	0	0	1	0
7	BHU, Varanasi	4	8	5	0
8	Total	26	56	26	10



A - Resistant lesion to TLB **B- Susceptible lesion to TLB**

Figure 1: Different Plant stages to Turcicum Leaf Blight

against *Polysora rust* at Nagenahalli. A clear cut variation in virulence pattern in case of *E. turcicum* was observed. Some of the inbred lines, *i.e.* CM 138, CM 212, IML 235, NAI 135, showed resistant reaction in both the locations in the year 2005, whereas they acted as susceptible in 2006 at both locations. It was also observed that the Nagenahalli isolates of *E. turcicum* was more virulent than the Almora isolates. Chandrashekara *et al.* (2014) also evaluated 35 short-duration maize inbred lines against *TLB* after artificial inoculation and *MLB* under natural conditions during *Kharif* 2011 and *Kharif* 2012 to identify new resistance sources and establish durability of known resistance sources in maize. They found 12 inbred lines resistant against *TLB*, 19 inbred lines exhibited resistant against *MLB* whereas, 10 inbred lines were found resistant to both *TLB* and *MLB*.

Based on susceptible and resistant reactions of maize genotypes, an attempt was made to identify the resistant gene pool sources for this disease. It was observed that Vivekananda population and population 31 were very good source for breeding resistant cultivars. The other important sources were exotic materials obtained from CIMMYT and USA (Table 4). It may be mentioned here that the BHU Varanasi and Indian Public Sector germplasm, majority coming from Directorate of Maize Research, New Delhi or its network have been generated out of CIMMYT and USA materials. Thus, the CIMMYT and USA have contributed tremendously for the development of resistant cultivars for NCLB in Indian Maize Breeding Programme.

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