SELECTION OF MAIZE GENOTYPES RESISTANT TO GRAY LEAF SPOT (*Cercospora zeae-maydis*)

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ABSTRACT

*Gray leaf spot (GLS), caused by Cercospora zeae-maydis is an important and destructive foliar disease of maize (Zeae mays) mostly in the mid hills of Nepal. Considering its potential threat to maize production, a total of fourty two and thirty different maize genotypes were evaluated in the year 2012 and 2013 respectively with two replications in observation nursery at Kapurkot, Salyan (1480 masl) for resistance to GLS. Disease assessment was made using 1-5 scale at 15 days interval for three times starting from tasseling stage (65 days after sowing). The results showed that no immune and highly resistant genotypes were found in both the year. Response of 27 out of 42 genotypes of maize were identified as moderately resistant (2-2.5 score) and 15 genotypes showed moderately susceptible (3 score) to GLS during 2012. During 2013 out of 30 genotypes of maize 27 were identified as moderately resistant (1.75-2.5 score) and 3 moderately susceptible (2.75-3 score) to GLS. Among the tested genotypes, BGBYPOP, Rampur SO3FO4, Celaya OOHGYA*HGYB, O7SADVI, Manakamana-3, SO1SIYQ and local were found most promising and moderately resistant to GLS disease during 2012 and SYN312-SR and CML-395/CML-444 during 2013.*

Key words: *Cercospora zeae-maydis,* GLS screening, maize genotypes, Mid-hills, resistant

INTRODUCTION

Maize (*Zea mays* L) is third most important cereal crop in the world and second most important crop after rice in terms of area and production in Nepal. Maize contributed 24.93% of total edible cereal grain production and the total area under maize production is 928,761 ha in Nepal where mid hill, terai and high hill occupies 72.85, 17.36 and 9.79 percent respectively (MOAD, 2014). One of the main deterrents to high grain yield in maize is its susceptibility to several diseases among which gray leaf spot (GLS) caused by *Cercospora zeae maydis* Tehon & Daniels is an important and destructive foliage disease mostly in hills of Nepal. It is estimated to be spreading at a rate of 80-160 km each year (Rijal et.al 2015). Grain loss of 80% was estimated in farmer's field due to this disease (Manandhar et al., 2009). The disease was first identified from the state of Illinois, USA in 1925 (Tehon & Daniels, 1925).The occurrence of this disease was recorded for the first time in the Kavrepalanchowk district of Nepal in 2006 (Manandhar, G. 2007, Tiwari and Ferrara, 2007). It affects upper eight or nine leaves which contribute 75—90% of the photosynthesis for grain fill (Ward et al., 1999). The disease is significant since it rapidly destroys foliage when the plant is near at grain maturity. Since, the disease has been observed spreading over the years in 22 districts in the eastern, central and mid-western regions of the country (Manandhar et al., 2009) there is a prompt need of resistant maize varieties to combat the disease. Host resistance for this worldwide important disease of maize has been reported on several hybrids and inbred lines (Hilty et al., 1979; Ward et al., 1999). Choosing a variety/genotype resistant to gray leaf spot is utmost of the day in disease prone hilly areas of Nepal. With an objective to identify the source of resistance for gray leaf spot in maize genotypes for general cultivation for the resource poor farmers in the hills, GLS screening experiment was conducted in the western mid hill environment at Kapurkot, Salyan (1480masl) of Nepal.

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MATERIALS AND METHODS

STUDY SITE DESCRIPTION

A total of 42 and 30 different maize genotypes received from National Maize Research Program (NMRP) in the year 2012 and 2013 respectively with two replications were evaluated in the disease screening nursery at Ginger Research Program (GRP), Kapurkot, Salyan (1480 masl) for resistance to GLS in western mid hills condition of Nepal. The experiment was conducted during summer season and was raised under rainfed condition. Seeds of maize were sown @ 2 seeds/hill on 28th June during 2012 and 5th June during 2013. Thinning of plants was done on 30th day after sowing (DAS) in all the treatments and one plant/hill was maintained. Two times of manual weedings and hoeings were done throughout the maize growing period. First weeding was done at knee high stage followed by earthing up, i.e. 35 DAS, and 2nd before tasseling stage, i.e. 75 DAS.

EXPERIMENTAL DESIGN AND CROP HUSBANDRY

The experiment was laid out in randomized complete block design (RCBD) with two replications in a natural epidemic condition. Plant spacing was maintained 75 cm x 25 cm (row to row and plant to plant respectively) and the plot size was of 2 rows of 3 m length (4.5 m^2). Fertilizer was applied at the rate of 120:60:40 kg NPK/ha with half dose of nitrogen and full dose of phosphorus and potash as basal application and remaining half of nitrogen top dressed at knee height stage. Other agronomic practices to raise the crop were applied as per National Maize Research Program guidelines.

DISEASE SCORING

GLS severity was measured as percentage of leaf area infected on average plant of the plot visually at 15 days intervals. A total of 3 scorings were done from July to August in both the years.

Disease assessment was done by using 1-5 rating scales (Maroof et al., 1993) in all genotypes at 15 days interval for three times starting from the occurrence of lesions on leaves as,

1. (Resistant) = Plants with one or two to few scattered lesions on lower leaves,
2. (Moderately resistant) = Moderate number of lesions on leaves, affecting less than 25 per cent of the area,
3. (Moderately susceptible) = Abundant lesions on lower leaves, few on other leaves affecting 26-50% leaf area,
4. (Susceptible) = Lesions abundant on lower and mid leaves, extending to upper leaves affecting 51-75% leaf area and
5. (Highly susceptible) = Lesions abundant on almost all leaves, plant prematurely dried or killed with 76-100% of the leaf area affected.

HARVESTING, THRESHING AND YIELD

Harvesting was done manually from net plot area, i.e. 4.5 m^2 on 9th November during 2012 and 6th October during 2013. Yield/plot was taken by weighing all dehusked cobs and randomly taken cobs were shelled to calculate shelling percentage and the grains were used for moisture recording by a moisture meter, and grain yield (t/ha) was adjusted to 15% moisture level using the following formula,

$$\text{Yield (mt/ha)} = \frac{\text{FW (kg)} \times (100 - \text{MC \%}) \times \text{SP} \times 10}{\text{Net harvested area (m2)} \times 85}$$

Where, FW = Fresh Weight; MC = Moisture Content and SP= Shelling Percentage

The shelled grains were cleaned by winnowing and sun dried so as to maintain 15% and grain yield per hectare was calculated by converting yield per plot into grain yield per hectare.

DATA ANALYSIS

The data on disease scoring and agronomic characters such as days to tasseling, silking, plant height and ear height along with the weight of ears and moisture content of kernels were recorded and analysis of variance for grain yield and other ancillary characters of maize were analyzed using the statistical package MSTAT-C (Russel and Eisensmith, 1983).

RESULTS

During 2012, response of 27 out of 42 genotypes of maize were identified as moderately resistant (2-2.5 score) and 15 genotypes showed moderately susceptible (3 score) to GLS. Among the tested genotypes BGBYPOP, Rampur SO3FO4, Celaya OOHGYA*HGYB, O7SADVI, Manakamana-3, SO1SIYQ and local were found most promising and moderately resistant to GLS disease (2-2.5 score). Highly significant difference on genotypes for the plant population, plant height and grain yield and only significant differences for ear height and GLS were observed, where as rest of the evaluated traits did not differ significantly. The highest plant height (219.90 cm) and ear height (106.00 cm) was recorded in Deuti variety followed by genotypes 07 SADVI (plant height 199.70 cm, ear height 88.10 cm) and Obatanpa (plant height 193.10 cm, ear height 102.80 cm). The genotypes Poshilo makai-1 and Celaya 00HGYA*HGYB were found to be GLS tolerant (2.50 and 2.00 GLS score respectively) with the highest yield potentiality of 4.39 and 4.00 mt/ha, respectively. The yield of the rest genotypes was found to be lower than the local genotypes (3.62 mt/ha).

Table 1: Evaluation of different maize genotypes against GLS at GRP, Kapurkot, Salyan (1480 masl), during summer season of 2012 and 2013

SN	Genotypes	Plant #/m^2	Tasseling days	Plant height, cm	Ear height,cm	GLS	ET	BLSB	Adjusted Grain yield mt/ha
1	Across 9942/ Across 9944	5.3	71.00	146.90	80.90	2.00	2.00	2.00	2.39
2	P501SRCO/ P502 SRCO	5.2	71.00	157.50	70.10	3.00	2.00	2.00	2.08
3	Rampur S03 F02	5.3	70.00	150.40	79.40	2.50	2.00	2.00	2.39
4	BGBYPOP	5.3	68.00	150.00	74.40	2.00	2.00	2.00	3.36
5	S99TLY-GH-B	5.3	72.00	158.70	87.90	3.00	2.00	2.00	3.40
6	S01SIWQ-3	5.3	66.00	165.20	80.90	3.00	2.50	2.00	2.65
7	S99TLYQ-B	5.3	70.00	159.30	88.30	2.50	2.00	2.00	2.12
8	S99TLYQ-A	5.2	67.00	150.50	79.70	3.00	2.50	2.00	2.66
9	S00TLY-1AB	5.3	66.50	173.50	85.60	3.00	2.50	2.00	2.92
10	Rampur S03 F08	5.3	69.00	166.50	90.00	3.00	2.00	1.50	1.90
11	Rampur S03 F04	5.2	68.00	163.00	82.90	2.00	2.50	2.00	3.23
12	Celaya00HGYA*HGYB	5.2	67.50	177.50	87.90	2.00	2.50	1.50	4.00
13	Poshilo makai-1	5.1	68.50	172.40	92.40	2.50	2.00	2.00	4.39
14	Cotaxla0024	5.3	70.00	148.30	76.50	3.00	2.50	2.50	1.51
15	TLBRS07F16	5.1	68.00	158.80	82.20	3.00	2.00	2.00	1.98
16	BLSBRS07F12	5.3	70.00	166.30	87.70	3.00	2.00	2.00	3.12
17	ZM-401	5.3	66.00	153.20	76.40	2.50	2.00	2.00	3.11
18	ZM-627	5.2	66.50	147.20	75.70	2.50	2.50	2.00	2.00
19	Heterotic Group A	4.3	66.00	146.80	68.20	3.00	2.00	2.00	1.33
20	Heterotic Group B	4.1	68.50	125.40	68.90	3.00	2.00	2.50	1.46
21	R Pop-2	4.8	68.00	156.70	83.40	3.00	2.00	2.00	1.66
22	BLSBRS07F10	5.2	66.00	141.60	63.30	3.00	2.00	2.00	1.90
23	TLBRS07F14	5.3	65.50	143.70	64.25	2.50	2.00	2.00	2.13
24	Rampur S10F18(A)	7.7	66.50	126.05	74.50	2.50	2.00	2.00	0.37
25	05SADVI	5.3	71.00	158.10	88.80	2.00	2.00	2.50	2.12
26	07SADVI	5.3	70.00	199.7	88.10	2.00	2.00	2.00	3.17
27	Manakamana-3	4.6	70.50	183.50	97.90	2.00	2.00	2.00	3.07

28	GLSYW	4.8	70.50	167.50	92.10	2.00	2.00	2.00	2.99
29	Obatanpa	5.1	67.50	193.10	102.80	2.50	2.00	2.00	2.74
30	S99TLYQ-HG-AB	5.1	68.50	151.00	80.80	2.50	2.00	2.00	1.83
31	S01SIWQ-2	5.3	70.50	146.30	69.00	2.50	2.00	2.00	2.63
32	S00TLYQ-B	1.1	70.00	134.15	59.45	2.00	2.00	2.00	0.22
33	S01SIYQ	5.3.	73.50	160.00	83.60	2.00	2.00	2.00	3.08
34	Rampur S03FQ-02	5.3	66.00	147.50	74.50	2.00	2.00	2.00	2.35
35	S99TLWQ-B	5.3	65.00	141.70	69.00	3.00	2.00	2.50	2.14
36	S00TLWQ-B	5.2	69.50	152.50	74.20	2.50	2.00	2.00	2.03
37	R Pop-3	5.3	69.00	159.20	94.20	3.00	2.00	2.00	2.77
38	Deuti	4.7	73.00	219.90	106.00	2.00	2.00	2.00	2.29
39	Manakamana-5	5.3	69.00	182.50	102.40	2.00	2.00	2.00	1.92
40	Manakamana-6	5.3	68.00	175.50	84.20	2.00	2.00	2.00	1.56
41	OEHPW	5.1	69.00	148.20	75.70	2.50	2.00	2.00	1.74
42	Local	5.3	70.00	169.10	91.00	2.00	2.00	2.00	3.62
CV %		5.07	3.66	10.06	14.05	17.46	14.82	13.26	33.70
P value		**	NS	**	*	*	NS	NS	**
LSD (p=0.01 and 0.05)		0.51		32.38	23.20	0.088			0.37

NS – Not significant * Significant ** – Highly significant

During 2013, Highly significant difference on genotypes for the tasseling days, silking days, ear height, grain yield was recorded and only significant differences for plant height was found where as plant stand and GLS score did not differ significantly. All the plants bear tassel between 73 to 86 days. The highest plant height (199.9 cm) and ear height (117.2 cm) was recorded in genotype JH-1203 followed by CZH 0923 (plant height 199 cm, ear height 113.6 cm). During 2013 out of 30 genotypes of maize 27 were identified as moderately resistant (1.75-2.5 score) and 3 moderately susceptible (2.75-3 score) to GLS. Genotype CZ 1108 produced highest grain yield (7.38 mt/ha) with lowest disease severity (2.25 score) followed by JH1203 (yield 7.01 mt/ha, GLS- 2 score). DTM # -38 was found inferior from GLS (3 score) and yield (2.26 mt/ha) point of view. Among the tested genotypes SYN312-SR and CML-395/CML-444 were found most promising having moderately resistant (1.75 GLS score) reaction to GLS with higher production of 5.9 mt/ha and 6.6 mt/ha respectively.

Table 2: Evaluation of different maize genotypes against GLS at GRP, Kapurkot, Salyan (1480 masl), during summer season of 2013 and 2014

SN	Genotypes	Plant #/m^2	Tasseling days	Silking Days	Plant height, cm	Ear height, cm	GLS	Grain yield mt/ha
1	J613-6	5.33	81.0	84.5	128	68.3	2.5	3.7
2	J586	4.77	73.5	77	163.7	77.4	2.5	4.4
3	ITS6C1F238	5.33	73.0	76	161	68.9	2.25	5.2
4	SYN312-SR-	5.11	80.50	83.5	171.7	94	2.5	5.1
5	CML-444/CML-489	5	80.50	84	173.5	92.1	2.25	4.8
6	SYN312-SR	4	77.50	81	192.7	91.5	1.75	5.9
7	CML-312/CML-444	5.12	80.50	84	196.4	105.4	2	6.1
8	CML-395/CML-444	5.11	84.50	87.5	191.6	100.8	1.75	6.6
9	JH-1203	5.33	86.50	90	199.9	117.2	2	7.0
10	CML144/SNSYN	5	77.50	82	189	105	2.5	4.24
11	CZH0837	5.11	73.0	76.5	159.4	69	2.5	3.52
12	CZH06623	5.22	76.0	80	189.5	86	2.5	5.65
13	CZH0923	5.33	81.0	84.5	199	113.6	2.25	6.75
14	CZH1012	5.22	74.50	78	185.6	90	2.25	6.52
15	CZH111	5.33	78	82	198.5	107.8	2.25	6.28
16	CZH0838	5.33	80	82.5	189.5	101.1	2.25	5.79
17	CZH1108	5.33	75.50	79	174.5	94.8	2.25	7.38
18	CZH1120	4.88	70.50	74	157.1	85	2.75	3.74
19	JH-1104	5.11	75.0	79.5	142.6	62.7	2.5	3.34

20	JH-1204	5.33	81	84.5	172.9	90.4	2.5	4.51
21	CZH0935	3.11	75	78	156	71.1	2.75	2.21
22	CZH0928	5.11	77	80.5	153.8	58.7	2.5	3.20
23	CML-305/CML-444/(CML-)	5.11	86	91	178.5	97	2	4.01
24	CML-444/CML-445	5.22	83	87	191	107.1	2	4.90
25	DMT#-35	4.77	82	85.5	135.7	67.7	2	2.47
26	DTM#-38	4.55	82.5	86	159.2	67.8	3	2.26
27	TLBRS07F16	5.33	80	82.5	158.6	80.5	2.25	4.32
28	BLSBRS07F12	5.11	79	81.5	148.1	75.8	2	2.89
29	TLBRS07F14	5.11	76	79	150.9	79.2	2.5	2.78
30	Local	5.11	84.5	88	163.1	77.3	2	2.88
CV %		10.12	4.01	4.09	11.21	14.02	15.50	27.14
P value		NS	**	**	*	**	NS	**
LSD (p=0.01 and 0.05)			6.46	6.88	39.21	24.98		2.56

NS – Not significant * Significant ** – Highly significant

The twenty maize genotypes could be categorized into four resistance levels, i.e. resistant, moderately resistant, susceptible and highly susceptible on the basis of disease severity. Among them, none of the genotypes were observed resistant, susceptible and highly susceptible reaction. Only 27 maize genotypes were observed moderately resistant during both the year 2012 and 2013 while 15 and 3 genotypes fell under moderately susceptible during 2012 and 2013 respectively.

Table 3: Classification of maize genotypes based on disease reaction and severity scale

Reaction	Maize Genotype	
	During 2012	**During 2013**
Resistant (1-1.5)	None	None
Moderately Resistant (1.75-2.5)	BGBYPOP, Rampur S03 F02 Across 9942/ Across 9944, S99TLYQ-B Rampur S03 F04, Celaya00HGYA*HGYB Poshilo makai-1, ZM-401, ZM-627, Rampur , TLBRS07F14, Rampur S03FQ-02, S01SIYQ, S00TLYQ-B, S01SIWQ-2 S99TLYQ-HG-AB, Obatanpa, GLSYW Manakamana-3, 05SADVI, 07SADVI S10F18(A), S00TLWQ-B, Deuti, Manakamana-5,Manakamana-6, OEHPW, local	SYN312-SR CML-395/CML-444, Local, TLBRS07F14 BLSBRS07F12, TLBRS07F16 DMT#-35, CML-444/CML-445 CML-305/CML-444/(CML-) CZH0928, JH-1204 JH-1104, CZH1108 CZH0838, CZH111, CZH1012 CZH0923, CZH06623, CZH0837, CML144/SNSYN, JH-1203 CML-312/CML-444, CML-444/CML-489, SYN312-SR, ITS6C1F238, J586, J613-6
Moderately Susceptible (2.75-3.5)	P501SRCO/ P502 SRCO, S99TLY-GH-B, S01SIWQ-3, S99TLYQ-A, S00TLY-1AB, Rampur S03 F08, Cotaxla0024, TLBRS07F16, BLSBRS07F12, Heterotic Group A, Heterotic Group B, R Pop-2, BLSBRS07F10, R Pop-3, S99TLWQ-B	DTM#-38, CZH1120, CZH0935
Susceptible (3.75-4.5)	None	None
Highly Susceptible (4.75-5)	None	None

DISCUSSION

In this research work, 42 and 30 maize genotypes were screened against GLS disease in naturally infested conditions during 2012 and 2013 respectively to identify the sources of resistance in mid hills condition of Nepal. Among the screened maize genotypes only 27 maize genotypes were observed moderately resistant during both the year while 15 and 3 genotypes showed moderately susceptible reaction during 2012 and 2013 respectively. The difference of genotypes in disease severity may be due to diversity in their genetic makeup. Among the moderately resistant genotypes, BGBYPOP, Rampur SO3FO4, Celaya OOHGYA*HGYB, O7 SADVI, Manakamana-3, SO1SIYQ and local were found most promising during 2012 while genotypes SYN312-SR and CML-395/CML-444 were found promising during 2013. Similar results were obtained in the GLS trial conducted at Pakhribas, Nepal which revealed that one genotype 07 SADVI was found highly resistant against GLS and produced 9.12 t/ha grain yield and other moderately resistant genotypes were S00TLWQ-B, Rampur S03FQ-02, Manakamana-6 and S01SIYQ (NMRP, 2013). Similar findings were also reported by Rijal et al. (2015) in the GLS disease screening trial conducted at Suping, Makwanpur and Dhungkharka, Kabre. The result of NMRP, 2014 also showed that at Rampur conditions the maize genotype 07 SADVI was found resistant against BLSB and CML-395/CML-444 was found moderately resistant to southern leaf blight on the disease screening trials.

During the year 2012 highly significant difference on genotypes for the plant population, plant height and grain yield and only significant differences for ear height and GLS were observed; whereas rest of the evaluated traits did not differ significantly however during 2013, highly significant difference on genotypes for the tasseling days, silking days, ear height, grain yield was recorded and only significant differences for plant height was found where as plant stand and GLS score did not differ significantly. The result is supported by Ali et al. (2011) that plant height is an important trait which affects the overall grain yield of the crop. These results showed differences in disease reaction along with other yield attributing character indicating that genetic variations exist among maize genotypes. There seems to be a good basis for introducing resistance against Grey leaf spot in breeding programs.

CONCLUSION

Since the maize genotypes varied highly in grey leaf spot severity, screening of genotypes seemed to be one of the important techniques for finding the sources of resistant and susceptible genotypes. In this respect use of resistance is an important part of the control strategy where different measures are combined to give an overall good disease control. Genotypes of maize resistant to moderately resistant to GLS have been identified. The open pollinated varieties like Manakamana-3 and Deuti which are already released and recommended for mid hills are still tolerant to GLS which can reduce yield loss in GLS prone environments of the hills. Several resistant genotypes of maize can be useful for source of disease resistance in the national maize breeding program. Genotypes of maize including BGBYPOP, Rampur SO3FO4, Celaya OOHGYA*HGYB, O7SADVI, SO1SIYQ, SYN312-SR and CML-395/CML-444 were identified as promising and resistant/moderately resistant to GLS and these genotypes should be used in breeding program of national maize research for further verification in agronomical and other yield attributing traits in the hills of Nepal.

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