

Screening of Maize Genotypes against Southern Leaf Blight (*Bipolaris maydis*) during Summer Season in Nepal

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Abstract A study was conducted from 29 March 2014 to 27 July 2014 at the Institute of Agriculture and Animal Science, Paklihawa, Rupandehi with the objective of screening 13 maize genotypes against southern leaf blight caused by *Bipolaris maydis*. Field experiment was laid out in a randomized complete block design with three replications. Disease scoring was done as percentage of leaf area infected on individual plant at 5 days intervals starting from 63 days after sowing, for 3 times, and disease severity and mean AUDPC were calculated and yield was recorded. Among the tested genotypes, disease severity varies significantly. Disease symptoms appeared first in Yellow Popcorn, 64.00 days after sowing (DAS) with the highest severity and at last in RML-32/RML-17 (79.00 DAS) with the least score in field. The 13 genotypes differed significantly in mean AUDPC values. RML-32/RML-17 (AUDPC value 5.90) appeared most resistant, followed by RML-4/RML-17 (AUDPC value 11.50), while Yellow Popcorn (AUDPC value 71.99) was most susceptible among the tested genotypes. Highest maize yield (3.43 metric ton ha⁻¹) was also recorded on RML-32/RML-17 and least (0.75 metric ton ha⁻¹) on Yellow Popcorn. Maximum SPAD value above cob was recorded in RML-4/RML-17 (45.62) followed by S03TLYQ-AB-01 (44.88) while minimum in Yellow popcorn (30.60). So, Yellow popcorn has the highest (3.16) and RML-32/RML-17 (0.08) lowest total AUDPC above cob. Similarly maximum SPAD value below cob was recorded in RML-4/RML-17 (44.37), while minimum in Yellow popcorn (28.82). So, Yellow popcorn has the highest (8.75) and RML-32/RML-17 (0.41) has lowest total AUDPC below cob. The genotypes RML-4/RML-17 and RML-32/RML-17 appeared resistant to SLB with maximum yield. These genotypes could be used as the sources of resistance in breeding program and could be developed to resistant varieties grown under tropical and subtropical climatic conditions during summer season. The genotype Yellow popcorn being highly susceptible to SLB with a maximum mean AUDPC and minimum yield, can be used as susceptible check for breeding purpose and different varietal screening.

Keywords: maize, disease severity, AUDPC, SPAD, susceptible check

Cite This Article: Rishi Ram Bhandari, Laxman Aryal, Suman Sharma, Milan Acharya, Ambika Pokhrel, Apar G.C., Salina Kaphle, Sahadev K.C., Bhagarathi Shahi, Kamal Bhattarai, Arjun Chhetri, and Sunita Panthi, "Screening of Maize Genotypes against Southern Leaf Blight (*Bipolaris maydis*) during Summer Season in Nepal." *World Journal of Agricultural Research*, vol. 5, no. 1 (2017): 31-41. doi: 10.12691/wjar-5-1-5.

1. Introduction

1.1. Background Information

Maize (*Zea mays* L.) is the world leading cereal crop. Maize belongs to tribe maydaca and grass family, Poaceae. It is indigenous to America and was domesticated about 8,000 years ago. Maize does not survive in its wild form probably because of the highly cross pollinated nature [37]. It is a short day plant and monoecious in nature. Maize is the world's most extensively grown crop with an annual worldwide production of 822 and 817 million metric tons in 2008 and 2009 respectively, and contributes to the larger extent in the world agricultural

economy both as food for human beings and feed for animals [11]. It has a very high yield potential than any other cereals and thus is popularly known as the 'queen of cereals' [41]. It is the second most important staple food crop both in terms of area and production after rice in Nepal. It is grown in 278,030 hectares of land with an average yield of 2501 Kg ha⁻¹ [28]. Maize occupies about 28.15% of the total cultivated agricultural land and shares about 23.28 % of the total cereal production in Nepal. It shares about 6.87% to Agricultural Gross Domestic Product [1]. In Nepal, maize is produced in three distinct agro-climatic zones viz. terai and inner terai (below 900m), mid-hills (900-1800) and high-hills (above 1800m). The proportion of maize area consists of about 70% in mid-hills followed by 20% in terai and 10% in high-hills [31].

Productivity of cereal grains including maize is basis for food security as well as means of earning surplus income and better livelihood of farm families [2]. Maize contributes to food security in the hills while in accessible areas it is gradually becoming a commercial commodity due to increasing demand of nutrients in poultry and animal feed. The overall demand for maize has been estimated to grow up by 6-8% per annum for the next two decades because of increasing demand for food in hills and livestock feed in accessible areas of terai and inner-terai [31].

Population growth rate of Nepal is rising by 2.24 % annually [6]. However, food production is inadequate to meet the requirements of people. Maize is one of the five cereal crops meeting food requirements, which contributes approximately 23% of total requirements. However, there is some rise in production of maize in terai, and is not enough to keep up with the increased demand of food and feed industry. Though the yield level of maize seems rising for years, it is at quite slow rate. Maize productivity of 1630 Kg ha⁻¹ during 1990/91 has just reached to 2500 Kg ha⁻¹ in 2011/12 [28] and present yield level is still far behind the 5120 Kg ha⁻¹ of global average [11].

Yield of maize in Nepal is more or less static at around 2500 Kg ha⁻¹ [1], but there is increased demand, estimated to grow by 6-8% per annum [29]. The rapidly increasing demand of maize is driven by increased demand for direct human consumption in the hills as a staple food crop [15] and for livestock feeds in terai and inner terai areas [30]. It is used for food, feed, fodder and fuel. In Nepal, 86.70% of the total maize production is used for food, 12.80% for poultry, 0.40% for cattle and 1% for other purposes [27].

Annually 86,166 metric ton maize worth of Nepalese Rupees 1,200 million is imported from India which accounts for 40-45% of Nepal's demand. Rest of the demand is fulfilled by the production within the country. As the rate of increase of consumption of livestock products like meat, milk, and egg is 3.50% per annum, the number of feed industries are being increased as a result of which maize and soybean like raw materials demand will inevitably increase [10].

The major constraints for maize production in South Asian region including Nepal are several biotic and abiotic factors. Among them, diseases are one of the major biotic constraints. The most important diseases of maize in Nepal are leaf blights (turicum and maydis leaf blight), ear rot, stalk rot, rust, downy mildews, etc. ([3,20,26,33,40,44]). Disease reduces quality and yield of maize. During summer season, southern leaf blight of maize, caused by *Bipolaris maydis* syn. *Helminthosporium maydis* (Teliorph: *Cochliobolus heterostrophus*) is considered as the most important disease of maize, creating a threat to its successful cultivation in Nepal. The disease has been occurring in terai, inner terai and mid-hills (lower elevation) since many years in summer and winter maize [34]. This disease causes significant yield loss in maize in Nepal especially in the tropical and subtropical region, and its severity is increasing in terai region. Due to the appearing of new pathogen races, resistant varieties may no longer remain resistant. Management becomes more complex due to development of resistance by the pathogen to chemicals. In future, using fungicides may become less favorable, as a result of restrictions in their use and

increased cost of discovery and registering of new active compounds. Hybrid genotypes of maize have also been affected by this disease. Disease data in experimental trail and disease situation in farmer's field support the need for screening of genotypes against SLB [35].

SLB of maize caused by *Bipolaris maydis* is a multiple cycle disease, i.e. new repeated inoculations are needed for disease development making it highly dependent upon sporulation from other lesions. It occurs worldwide and is important in regions of warm and humid climate with 20-30°C temperature. In the United States, in 1970, this disease reached an epidemic level, resulting in estimated losses of one billion dollars [46]. The epidemic was caused by the "race T" attacking corn with Texas male sterile cytoplasm (T-cms), which comprised 85% of corn at that time. "Race O" occurs mainly in subtropical and tropical areas, where it causes minor losses. Yield reduction up to 50% was recorded when "race O" inoculated into susceptible line ([12,17]). Resistance against "race O" of *Bipolaris maydis* has been described as quantitative, with a predominance of additive gene action as well as significant dominance effects present in some populations ([4,22,25,45]) as well as qualitative, with resistance inherited as a single recessive gene, designated *rhml* [43]. [8] hypothesized that the *rhml* locus is actually composed of two linked recessive genes controlling resistance. The presence of the dominant allele at either locus confers susceptibility [8]. [9] reported that the *rhml* gene provides adequate resistance during early stages of growth, but after silking, quantitative resistance is needed. Maize hybrids with Normal (N) cytoplasm are resistance to "race T", which is virulent to Texas male sterile cytoplasm (T-cms) hybrids and produces a patho-toxin. Hybrids with T-cms are no longer in commercial use in the US. Other male sterile cytoplasm hybrids such as C-cms and S-cms are resistant to "race T". Hybrids with N cytoplasm differ in their susceptibility to a biotype similar to "race T" which was observed in the Philippines. Partially dominant genetic factors which interact with T-cms to provide partial resistance were reported by [23]. The male sterile cytoplasm 'pool' released by Illinois agriculture experimental station and the United States department of agriculture (USDA) contains resistance to "race T" of *H. maydis* including cms B,C, CA, D, EK, F, G, H, I, IA, J, K, L, M, ME, ML, MY, PS, R, RB, S, SD, TA, TC, VG, and W [18]. When *H. maydis* avirulent race "O" and virulent race "T" on T-cms were crossed, the recombination showed monogenic segregation for virulence and pathotoxin production [24].

Emergence of new races is a constant threat. As use of fungicides is costly and environment unfriendly, the major thrust of disease control has been through breeding resistant cultivars. Moreover, fungicide use becomes even less favorable in the future as a result of restrictions in their use [19]. Use of resistant varieties is a simple, effective, safe and economical means of controlling diseases. Resistant genotypes helps to stabilize yield. Resistant levels should be updated each year for each variety. In such contest, identification of resistant genotypes/varieties would be good alternatives to manage SLB.

In this study, an attempt was made to assess the level of resistance on different maize genotypes against SLB of

maize. The study will be helpful to evaluate the level of resistance against this disease in different maize genotypes.

2. Materials and Methods

2.1. Site Selection

The field experiment was conducted at Paklihawa Campus, Rupandehi, Nepal during summer (29 March to 27 July, 2014) under irrigated conditions. The site is situated between 27° 32' N latitude and 83° 25' E longitudes and 105 m above the sea level. Climatically, Rupandehi lies in humid sub-tropical region with average annual rainfall of 2000 mm (mainly during mid to late summer).

2.2. Experimental Setup

The experiment was conducted in a randomized complete block design with 3 replications. Individual plot size was 3 m² (2×1.5 m²) and net area of cultivation was 117 m² while the gross area of the research field was 215 m². There were 2 rows of 2 m length per plot spaced 75 cm apart. Plant to plant spacing was 25 cm. The susceptible check variety, "Yellow popcorn" was sown in 2 rows around the whole field to provide a uniform source of inoculum.

2.3. Collection of seeds

Seeds of thirteen maize genotypes (POSILO-1, S03TLYQ-AB-02, S03TLYQ-AB-01, RAMPUR COMPOSITE, RAMPUR S03F04, ARUN-2, ACROSS 9942/ACROSS 9944, ZM-401, ZM-627, YELLOW POPCORN, RAMPUR HYBRID-2, RML-4/RML-17, RML-32/RML-17), originated from diverse sources were obtained from the National Maize Research Centre, Rampur as these were the promising maize lines for hybrid production. Yellow popcorn was taken as a susceptible check, being highly susceptible to SLB of maize in Nepal.

2.4. Land Preparation and Sowing

The field was prepared by plowing with harrow 2 weeks prior to sowing. Farm yard manure (FYM) were applied at the rate of 10 tons ha⁻¹ during the time of field preparation and chemical fertilizers were applied at the rate of 120:60:40 NPK Kg ha⁻¹. During planting time half dose of Nitrogen and full dose of P and K was applied as basal dressing alongside the maize rows. Remaining half dose of Nitrogen was applied in two split doses

during first and second weeding. Seeds were sown during March 29, 2014 at the rate of 1 seed hill⁻¹ on 16 hills.

2.5. Plant Protection

Cutworms and other polyphagous pests were severe problems in the field during the early stage of growth. Cypermethrin 25% EC (Emulsifiable Concentrate) at the rate of 1.5ml litre⁻¹ per plot was sprayed at 15 DAS during April 12, 2014.

2.6. Weeding and Irrigation

Manual removal of weeds was done throughout the maize growing season. Weeds were removed at knee high stage, i.e. 35 DAS following earthing up as second weeding. Due to the hot season, two irrigations were given per week up to 35 days of sowing to enhance the germination and early plant establishment. One irrigation per week was given after 35 days.

2.7. Harvesting, Threshing and Yield

Crop was harvested manually from net plot area, i.e. 3 m², when the plants turned yellow, ear husk turned brown and a black layer appeared at the base of each kernel when scratched. Grain yield plot⁻¹ was taken by weighing all dehusked cobs (shelling percentage considering to be 80%). Randomly selected cobs were shelled and the grains were used for moisture recording by a moisture meter, and grain yield (Kg ha⁻¹) was adjusted to 15% moisture level using the following formula.

$$\text{Yield} = \frac{\text{FW (Kg)} \times (100 - \text{MC}\%) \times 0.8 \times 10}{\text{NHA} \times 85}$$

Where, FW = Fresh Weight, NHA= Net harvested Area (m²) and MC = Moisture Content.

The shelled grains were cleaned and sun dried so as to maintain 15% moisture level and thousand kernel weight (g) was taken.

2.8. Disease Assessment

2.8.1. Disease Scoring

Disease incidence was recorded immediately after appearance of disease, and disease scoring was started 10 days after disease appearance. The disease estimation was based on a modified 0 to 5 scale reported by [42] and was used to estimate percent leaf area diseased (PLAD) on individual plant visually at 5 days intervals. Disease scoring was done on 1 – 5 scale as below.

Table 1. Disease severity and scoring scale of SLB

Scale	Disease Severity
1	Plants with one or two to few scattered lesions on lower leaves (Resistant)
2	Moderate number of lesions on leaves, affecting <25% of the leaf area (Moderately Resistant)
3	Abundant lesions on lower leaves, few on other leaves affecting 26-50% leaf area (Moderately Susceptible)
4	Lesions abundant on lower and mid leaves, extending to upper leaves affecting 75% leaf area (Susceptible)
5	Lesions abundant on almost all leaves, plants prematurely dried or killed with 76-100% of the leaf area affected (Highly Susceptible)

Disease intensity was calculated/plant and mean intensity was computed/plot.

2.8.2. Confirmation of SLB Disease

Infected leaf was stained with lacto phenol and observed under microscope. Several branched conidiophores and elliptical conidia and few septa and pseudosepta were observed which confirmed the disease.

2.8.3. Estimation of AUDPC

AUDPC gives a quantitative measure of disease development and intensity of disease [38], and the variety having the lowest AUDPC value were categorized as the most resistant, while susceptible one has higher values. The average data of each score at five days interval was converted to percent leaf area for computation of Area under disease progress curve (AUDPC) according to the formula suggested by [7] and [14].

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left[\left\{ \frac{Y_i + Y_{i+1}}{2} \right\} \times (t_{i+1} - t_i) \right]$$

Where, Y_i = disease severity on the i^{th} date,
 t_i = time on which Y_i was recorded and
 n = number of times observations were taken.
 AUDPC values were also used to identify disease resistance in maize [21].

2.9. Observation of Agronomical Characters

Five plants per plot, selected earlier for disease scoring were used to assess yield attributing characters. Yield was obtained by hand harvesting from all plants/plot. Cobs were shelled and 1000 kernel weight was taken at 15% moisture level.

2.10. Yield Attributing Characters

2.10.1. Number of Harvested Ears

Total number of ears harvested from net harvestable area was recorded as harvested ears per plot and it was converted to hectare basis.

2.10.2. Thousand Kernel Weight (TKW) or Test Weight

One thousand shelled maize grains from each plot was randomly taken, weighed and recorded as test weight and expressed in gram (g). The kernels used for test weight was adjusted to 15% moisture content.

2.10.3. Shelling Percentage

It is the ratio of grain to ear (grain: ear) and expressed in percentage. Five randomly selected ears were weighed with grains. All grains were shelled out and the weights of grain were taken and the shelling percentage was calculated as:

$$\text{Shelling percentage} = \frac{\text{Grain yield (kg)}}{\text{Cob yield (kg)}} \times 100.$$

2.10.4. Grain Moisture Content (%)

Ten ears were selected randomly and central two kernel rows were shelled out and the kernels were bulked from all ears and moisture was measured by multigrain moisture meter.

2.10.5. Grain Yield

Grain yield was calculated on hectare basis by using following formulae:

$$\text{Grain Yield (Kg/ha)} = \frac{\text{FEW} \times \text{SP} \times (100 - \text{GMC})}{\text{NHA} \times 85 \times 10}$$

Where, FEW = filled ears weight (Kg)
 SP = shelling percentage (%)
 GMC = grain moisture content at harvest (%)
 NHA = net harvested area (m^2).

2.11. Phenological Study-SPAD (Soil Plant Analysis Development)

SPAD is used to measure the chlorophyll content of leaf. 15 SPAD readings of 5 randomly selected plants from each plot were recorded and average SPAD above/below cob was calculated.

2.12. Statistical Analysis

The collected data was compiled and entered treatment wise under 3 replications. The data was processed to fit into MSTAT-C [13] software for analysis. MS-excel program (2007) was used for data tabulation, and DMRT was done at 5% level of significance for mean comparison from the reference of [16]. Data entry was carried out to develop ANOVA table, and DMRT was applied to identify the most resistant genotypes. Correlation and regression analysis were done for group comparison [16].

Table 2. Appearance of SLB disease symptom on 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

S.N	Treatments	Appearance of symptoms (DAS)
1	Yellow popcorn	63.67 ^J
2	Rampur S03F04	65.33 ^I
3	Rampur Hybrid-2	66.00 ^{HI}
4	Rampur Composite	66.67 ^{GH}
5	Posilo-1	67.33 ^{FG}
6	ZM-401	68.00 ^F
7	Arun-2	69.33 ^E
8	ZM-627	71.00 ^D
9	Across 9942/Across9944	71.00 ^D
10	S03TLYQ-AB-02	74.67 ^C
11	S03TLYQ-AB-01	75.33 ^{BC}
12	RML-4/RML-17	76.00 ^B
13	RML-32/RML-17	79.00 ^A
SEM (\pm)		0.4159
LSD (0.05)		1.214
CV (%)		1.03
Probability		0.0000**

DAS: Days after sowing, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEM (\pm) indicates standard error of mean.

3. Result and Discussion

3.1. Disease Incidence

Symptoms of southern leaf blight in all maize genotypes appeared on 63.67 to 79 days after sowing (DAS) i.e. on 1st and 16th June, 2014 respectively and differed significantly from each other at 5% level of significance. Symptoms were observed first on Yellow popcorn (63.67 DAS) on 1st June 2014 followed by Rampur S03F04 (65.33 DAS), Rampur Hybrid-2 (66.00 DAS) and so on while longest duration was taken by RML-32/RML-17 (79.00 DAS) (Table 2).

3.2. Area under Disease Progress Curve (AUDPC)

The genotypes varied significantly in area under disease

progress curve (AUDPC) values in all 3 observation dates. AUDPC values increased with time of observation in all the genotypes. The genotype RML-32/RML-17 had the lowest and Yellow popcorn had the highest AUDPC values in all observation dates, with a mean value of 5.90 and 71.99 respectively (Table 3).

The genotypes RML-4/RML-17 (11.50) and RML-32/RML-17 (5.90) also did not differ significantly. The variation in disease increment might be due to variation in susceptibility of genotypes to the pathogen. Yellow popcorn (71.99) showed drastically highest mean AUDPC value as compared to genotype Rampur S03F04 (47.13), with the 2nd highest mean AUDPC value (Table 3). [32] and [36] also reported that Yellow popcorn was the most susceptible genotype against SLB in terai and inner terai of Nepal.

Based on the mean AUDPC values of the 13 maize genotypes, a scale of mean AUDPC value was proposed to categorize the genotypes into 4 resistance levels (Table 4).

Table 3. Resistance category of 13 maize genotypes on the basis of mean AUDPC values in field during March to July, 2014 at Paklihawa, Rupandehi

S.N	Treatments	Mean AUDPC value	Nos. of genotypes	Resistance category
1	Yellow popcorn	71.99 ^A	1	HS
2	Rampur S03F04	47.13 ^B	3	S
3	Rampur Hybrid-2	44.72 ^B		
4	Rampur Composite	42.67 ^B		
5	Posilo-1	35.12 ^C	7	MR
6	ZM-401	34.93 ^C		
7	Arun-2	34.64 ^C		
8	ZM-627	32.10 ^{CD}		
9	Across 9942/Across9944	30.99 ^{CD}		
10	S03TLYQ-AB-02	27.84 ^D		
11	S03TLYQ-AB-01	27.13 ^D		
12	RML-4/RML-17	11.50 ^E	2	R
13	RML-32/RML-17	5.905 ^F		
SEm (±)		1.744		
LSD (0.05)		5.091		
CV (%)		8.79		
Probability		0.0000**		

R: Resistant, MR: Moderately Resistant, S: Susceptible, HS: Highly Susceptible, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEm (±) indicates standard error of mean.

Among them, only two genotypes RML-32/RML-17 (5.90) and RML-4/RML-17(11.50) were observed resistant while only the genotype Yellow popcorn (71.99) fell into highly susceptible category (Table 4). Seven genotypes fell under moderately resistant category and three under susceptible category.

3.3. AUDPC and Appearance of Symptoms Days after Sowing (DAS)

AUDPC and DAS varied significantly among the genotypes. Yellow popcorn having minimum appearance of disease DAS showed the highest mean AUDPC value (71.99). So this genotype appeared as maximum susceptible genotype to SLB among all tested genotypes

in the field under natural infection condition. Similarly genotype RML-32/RML-17 having maximum appearance of disease (79 DAS) showed lowest mean AUDPC (5.905). Thus, the genotypes RML-32/RML-17 was the most resistant to SLB than other screened genotypes (Table 5).

Table 4. Proposed resistance category of different maize genotypes

Mean AUDPC value	Resistance category	Code
1-20	Resistant	R
20-40	Moderately Resistant	MR
40-60	Susceptible	S
>60	Highly susceptible	HS

Table 5. Mean AUDPC value of 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

Treatments	Appearance of symptoms DAS	Mean AUDPC value
Yellow popcorn	63.67 ^J	71.99 ^A
Rampur S03F04	65.33 ^I	47.13 ^B
Rampur Hybrid-2	66.00 ^{HI}	44.72 ^B
Rampur Composite	66.67 ^{GH}	42.67 ^B
Posilo-1	67.33 ^{FG}	35.12 ^C
ZM-401	68.00 ^F	34.93 ^C
Arun-2	69.33 ^E	34.64 ^C
ZM-627	71.00 ^D	32.10 ^{CD}
Across 9942/Across9944	71.00 ^D	30.99 ^{CD}
S03TLYQ-AB-02	74.67 ^C	27.84 ^D
S03TLYQ-AB-01	75.33 ^{BC}	27.13 ^D
RML-4/RML-17	76.00 ^B	11.50 ^E
RML-32/RML-17	79.00 ^A	5.905 ^F
SEm (±)	0.4159	1.744
LSD (0.05)	1.214	5.091
CV (%)	1.03	8.79
Probability	0.0000**	0.0000**

DAS: Days after sowing, AUDPC: Area Under Disease Progress Curve, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEm (±) indicates standard error of mean.

3.4. SPAD Value (above and below Cob) and Total AUDPC

SPAD value and AUDPC varied significantly among all the genotypes. Maximum SPAD value above cob (45.62) was recorded in RML-4/RML-17, while minimum (30.60) was recorded in Yellow popcorn (Table 6). Similarly maximum SPAD value below cob (44.37) was recorded in RML-4/RML-17, while minimum (28.82) was recorded in Yellow popcorn. RML-32/RML-17 scored lowest SPAD than that of RML-4/RML-17, this might be because of the larger leaf size and presence of some interfering substance on the surface of leaf to the SPAD meter which should be verified through several trails.

Table 6. SPAD above cob and total AUDPC of 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

Treatments	SPAD above cob	Total AUDPC above cob
Yellow popcorn	30.60 ^D	3.167 ^A
Rampur S03F04	39.80 ^{ABC}	1.167 ^{BCD}
Rampur Hybrid-2	44.55 ^A	0.8333 ^{BCD}
Rampur Composite	36.90 ^C	1.583 ^{BC}
Posilo-1	39.63 ^{ABC}	1.833 ^B
ZM-401	35.53 ^{CD}	0.8333 ^{BCD}
Arun-2	34.42 ^{CD}	0.6667 ^{BCD}
ZM-627	39.88 ^{ABC}	0.8333 ^{BCD}
Across 9942/Across9944	36.57 ^{CD}	0.4167 ^{CD}
S03TLYQ-AB-02	43.45 ^{AB}	0.4167 ^{CD}
S03TLYQ-AB-01	44.88 ^A	0.7500 ^{BCD}
RML-4/RML-17	45.62 ^A	0.6667 ^{BCD}
RML-32/RML-17	37.83 ^{BC}	0.08333 ^D
SEm (±)	1.916	0.3517
LSD (0.05)	5.591	1.026
CV (%)	8.46	59.79
probability	0.0002**	0.0003**

SPAD: Soil Plant Analysis Development, AUDPC: Area Under Disease Progress Curve, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance; SEm (±) indicates standard error of mean.

Table 7. SPAD below cob and total AUDPC of 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

Treatments	SPAD below cob	Total AUDPC below cob
Yellow popcorn	28.82 ^C	8.750 ^A
Rampur S03F04	40.82 ^{AB}	3.250 ^{BC}
Rampur Hybrid-2	41.37 ^{AB}	2.250 ^{BCD}
Rampur Composite	36.00 ^B	3.167 ^{BC}
Posilo-1	35.88 ^B	4.417 ^B
ZM-401	36.65 ^B	2.167 ^{BCD}
Arun-2	37.65 ^{AB}	1.500 ^{CD}
ZM-627	38.37 ^{AB}	1.583 ^{CD}
Across 9942/Across9944	34.20 ^{BC}	1.000 ^{CD}
S03TLYQ-AB-02	37.52 ^{AB}	1.500 ^{CD}
S03TLYQ-AB-01	40.12 ^{AB}	3.250 ^{BC}
RML-4/RML-17	44.37 ^A	1.417 ^{CD}
RML-32/RML-17	37.25 ^B	0.4167 ^D
SEm (±)	2.110	0.7452
LSD (0.05)	6.157	2.175
CV (%)	9.71	48.40
probability	0.0070**	0.0000**

SPAD: Soil Plant Analysis Development, AUDPC: Area Under Disease Progress Curve, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEm (±) indicates standard error of mean.

So total AUDPC above cob of Yellow popcorn (3.16) was recorded highest and that of RML-32/RML-17 (0.08) was lowest respectively (Table 6).

Similarly total AUDPC below cob of Yellow popcorn (8.75) was recorded highest and that of RML-32/RML-17 (0.41) was lowest (Table 7).

The negative correlation between SPAD value and AUDPC was also suggested by [39] in different wheat genotypes.

Table 8. SPAD above cob, below cob and yield (metric ton ha⁻¹) of 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

Treatments	SPAD above cob	SPAD below cob	Yield (metric ton ha ⁻¹)
Yellow popcorn	30.60 ^D	28.82 ^C	0.7533 ^E
Rampur S03F04	39.80 ^{ABC}	40.82 ^{AB}	2.780 ^B
Rampur Hybrid-2	44.55 ^A	41.37 ^{AB}	1.713 ^{CD}
Rampur Composite	36.90 ^C	36.00 ^B	1.413 ^D
Posilo-1	39.63 ^{ABC}	35.88 ^B	2.780 ^B
ZM-401	35.53 ^{CD}	36.65 ^B	2.030 ^{CD}
Arun-2	34.42 ^{CD}	37.65 ^{AB}	2.240 ^{BC}
ZM-627	39.88 ^{ABC}	38.37 ^{AB}	2.290 ^{BC}
Across 9942/Across9944	36.57 ^{CD}	34.20 ^{BC}	1.387 ^D
S03TLYQ-AB-02	43.45 ^{AB}	37.52 ^{AB}	2.233 ^{BC}
S03TLYQ-AB-01	44.88 ^A	40.12 ^{AB}	1.870 ^{CD}
RML-4/RML-17	45.62 ^A	44.37 ^A	2.850 ^{AB}
RML-32/RML-17	37.83 ^{BC}	37.25 ^B	3.437 ^A
SEm (±)	1.916	2.110	0.2025
LSD (0.05)	5.591	6.157	0.5910
CV (%)	8.46	9.71	16.39
probability	0.0002**	0.0070**	0.0000**

SPAD: Soil Plant Analysis Development, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEm (±) indicates standard error of mean.

3.5. SPAD Value (above and below Cob) and Yield

SPAD value and yield varied significantly among all the genotypes. Maximum SPAD value above cob (45.62) was recorded in RML-4/RML-17, while minimum (30.60) was recorded in Yellow popcorn. Similarly maximum SPAD value below cob (44.37) was recorded in RML-4/RML-17, while minimum (28.82) in Yellow popcorn. So yield of Yellow popcorn (0.7533 metric ton ha⁻¹) and RML-32/RML-17 (3.43 metric ton ha⁻¹) was lowest and highest respectively (Table 8).

3.6. Yield (metric ton ha⁻¹) and Total AUDPC above/below Cob

Grain yield varies significantly among the genotypes. Maximum yield was recorded in RML-32/RML-17 (3.43 metric ton ha⁻¹) while minimum yield was recorded in Yellow Popcorn (0.75 metric ton ha⁻¹). However, the yield of RML-32/RML-17 (3.43 metric ton ha⁻¹) and RML-4/RML-17 (2.85 metric ton ha⁻¹) does not differ significantly.

Total AUDPC below cob differs significantly among thirteen genotypes. The highest total AUDPC below cob was recorded in Yellow popcorn (8.75) and lowest total AUDPC below cob was recorded in RML-32/RML-17 (0.41) (Table 9).

From the above result it was found that the Yellow popcorn showing highest total AUDPC below cob (8.75) showed lowest yield (0.75 metric ton ha⁻¹). Similarly RML-32/RML-17 showing lowest total AUDPC below cobs (0.41) showed highest yield (3.43 metric ton ha⁻¹) (Table 9). The genotypes RML-32/RML-17 was found most resistant to SLB and produce highest grain yield than other genotypes.

Table 9. Total AUDPC below and above cob and yield (metric ton ha⁻¹) of 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

Treatments	Total AUDPC below cob	Total AUDPC above cob	Yield (metric ton ha ⁻¹)
Yellow popcorn	8.750 ^A	3.167 ^A	0.7533 ^E
Rampur S03F04	3.250 ^{BC}	1.167 ^{BCD}	2.780 ^B
Rampur Hybrid-2	2.250 ^{BCD}	0.8333 ^{BCD}	1.713 ^{CD}
Rampur Composite	3.167 ^{BC}	1.583 ^{BC}	1.413 ^D
Posilo-1	4.417 ^B	1.833 ^B	2.780 ^B
ZM-401	2.167 ^{BCD}	0.8333 ^{BCD}	2.030 ^{CD}
Arun-2	1.500 ^{CD}	0.6667 ^{BCD}	2.240 ^{BC}
ZM-627	1.583 ^{CD}	0.8333 ^{BCD}	2.290 ^{BC}
Across 9942/Across9944	1.000 ^{CD}	0.4167 ^{CD}	1.387 ^D
S03TLYQ-AB-02	1.500 ^{CD}	0.4167 ^{CD}	2.233 ^{BC}
S03TLYQ-AB-01	3.250 ^{BC}	0.7500 ^{BCD}	1.870 ^{CD}
RML-4/RML-17	1.417 ^{CD}	0.6667 ^{BCD}	2.850 ^{AB}
RML-32/RML-17	0.4167 ^D	0.08333 ^D	3.437 ^A
SEm (±)	0.7452	0.3517	0.2025
LSD (0.05)	2.175	1.026	0.5910
CV (%)	48.40	59.79	16.39
Probability	0.0000**	0.0003**	0.0000**

AUDPC: Area Under Disease Progress Curve, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEm (±) indicates standard error of mean.

Total AUDPC above cob differs significantly among 13 genotypes. The highest total AUDPC above cob was recorded in Yellow popcorn (3.16) and lowest total AUDPC above cob was recorded in RML-32/RML-17 (0.08) (Table 9).

From the above result it was found that the Yellow popcorn showing highest total AUDPC above cob (3.16) showed lowest grain yield (0.75 metric ton ha⁻¹). So this genotype appeared as highly susceptible genotype to the southern leaf blight among all tested genotypes in the field under natural infection condition. Similarly RML-32/RML-17 showing lowest total AUDPC above cob (0.08) showed highest grain yield (3.43 metric ton ha⁻¹). Thus, the genotype RML-32/RML-17 was found most resistant to SLB and produced highest grain yield during summer than other tested genotypes.

3.7. Mean AUDPC and Yield (metric ton ha⁻¹)

Yield and mean AUDPC varied significantly among the genotypes. Maximum yield (3.43 metric ton ha⁻¹) was recorded in RML-32/RML-17, while minimum (0.75 metric ton ha⁻¹) in Yellow popcorn (Table 10).

Mean AUDPC differed significantly among the thirteen maize genotypes in the field. Maximum mean AUDPC (71.99) was recorded in Yellow popcorn while minimum mean AUDPC (5.90) was recorded in RML-32/RML-17. From the result it was found that the genotype Yellow popcorn, showing the maximum mean AUDPC value (71.99) also showed the lowest yield (0.75 metric ton ha⁻¹). Similarly genotype RML-32/RML-17 showing the minimum mean AUDPC value (5.90) also showed the highest yield (3.43 metric ton ha⁻¹) (Table 10). So, Yellow popcorn appeared as highly susceptible genotype and RML-32/RML-17 as highly resistant to the SLB among all tested genotypes in the field under natural infection condition.

Table 10. Mean AUDPC value and yield of 13 maize genotypes in field during March to July, 2014 at Paklihawa, Rupandehi

Treatments	Mean AUDPC value	Yield (metric ton ha ⁻¹)
Yellow popcorn	71.99 ^A	0.7533 ^E
Rampur S03F04	47.13 ^B	2.780 ^B
Rampur Hybrid-2	44.72 ^B	1.713 ^{CD}
Rampur Composite	42.67 ^B	1.413 ^D
Posilo-1	35.12 ^C	2.780 ^B
ZM-401	34.93 ^C	2.030 ^{CD}
Arun-2	34.64 ^C	2.240 ^{BC}
ZM-627	32.10 ^{CD}	2.290 ^{BC}
Across 9942/Across9944	30.99 ^{CD}	1.387 ^D
S03TLYQ-AB-02	27.84 ^D	2.233 ^{BC}
S03TLYQ-AB-01	27.13 ^D	1.870 ^{CD}
RML-4/RML-17	11.50 ^E	2.850 ^{AB}
RML-32/RML-17	5.905 ^F	3.437 ^A
SEm (±)	1.744	0.2025
LSD (0.05)	5.091	0.5910
CV (%)	8.79	16.39
Probability	0.0000**	0.0000**

AUDPC: Area Under Disease Progress Curve, CV: Coefficient of Variation, LSD: Least Significant Difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, SEm (±) indicates standard error of mean.

3.8. Regression Analysis

There was a significant ($P \leq 0.01$), negative, linear relationship between appearance of symptoms DAS and mean AUDPC (Figure 1).

If there is a unit increase in appearance of symptoms DAS, mean AUDPC on the plant could decrease by 3.11 times. According to coefficient of determination, about 81.64% variation in mean AUDPC on plant was due to the appearance of symptoms DAS and the remaining portion due to other factors.

There was a significant ($P \leq 0.01$), negative, linear relationship between mean AUDPC and mean SPAD above/below cob. According to linear regression equation, it can be said that if there is a unit increase in mean AUDPC, mean SPAD above/below cob would have been decreased by 0.14 and 0.13 times respectively. According to the coefficient of determination, about 26% and 31.66% variation in mean SPAD above/below cob was due to mean AUDPC and remaining portion due to other factors as shown in Figure 2.

There was a significant ($P \leq 0.01$), negative, linear relationship between total AUDPC above/below cob and mean yield (metric ton ha^{-1}). According to linear regression, it can be said that if there is unit increase in total AUDPC above/below cob, mean yield (metric ton ha^{-1}) would have been decreased by 0.48 and 0.18 times respectively. According to the coefficient of determination, about 28.75% and 29.84% variation in mean yield (metric ton ha^{-1}), was due to total AUDPC above and below cob

respectively and remaining portion due to other factors as shown in Figure 3.

There was a significant ($P \leq 0.01$), positive, linear relationship between mean SPAD above/below cob and mean yield (metric ton ha^{-1}). According to linear regression equation, it can be said that if there is a unit increase in mean SPAD above/below cob, mean yield (metric ton ha^{-1}) would have been increased by 0.06 and 0.11 times respectively. According to the coefficient of determination, about 15.7% and 33.53% variation in mean yield (metric ton ha^{-1}) was due to mean SPAD above/below cob and remaining portion due to other factors as shown in Figure 4.

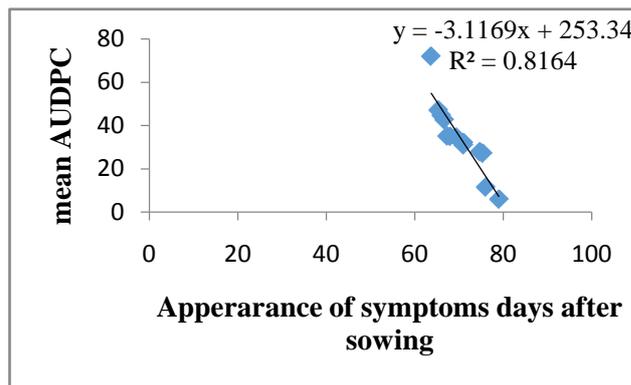


Figure 1. Estimated linear relationship between appearance of symptoms DAS and mean AUDPC in 13 maize genotypes at Paklihawa, Rupandehi, 2014

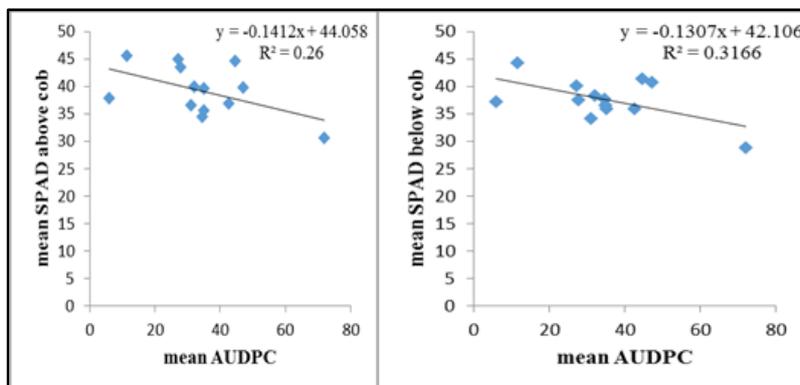


Figure 2. Estimated linear relationship between mean AUDPC and mean SPAD below/above cob of 13 maize genotypes in Paklihawa, Rupandehi, 2014

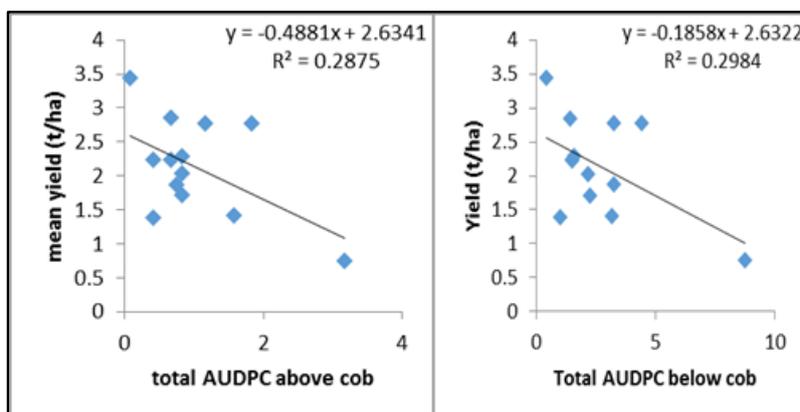


Figure 3. Estimated linear relationship between total AUDPC above/below cob and mean yield (metric ton ha^{-1}) of 13 maize genotypes in Paklihawa, Rupandehi, 2014

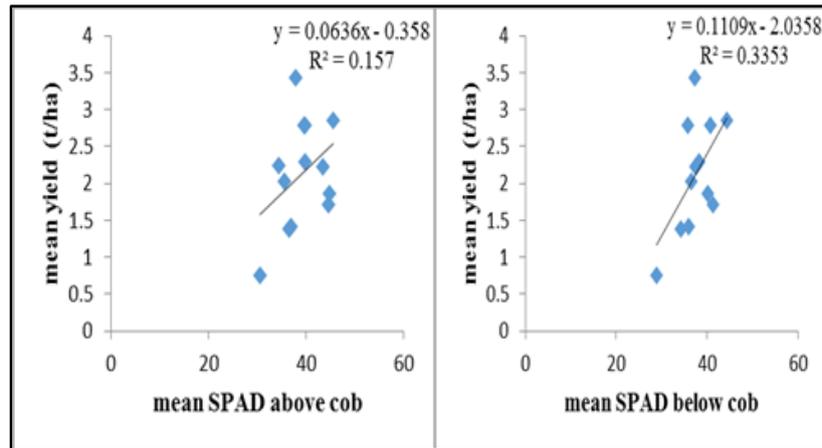


Figure 4. Estimated linear relationship between mean SPAD above/below cob and mean yield (metric ton ha⁻¹) of 13 maize genotypes in Paklihawa, Rupandehi, 2014

There was a significant ($P \leq 0.01$), negative, linear relationship between mean AUDPC and thousand kernel weight (g). According to linear regression equation, it can be said that if there is a unit increase in mean AUDPC, thousand kernel weight (g) would have been decreased by 2.10 times. According to the coefficient of determination, about 44.55% variation in thousand kernel weight (g) was due to mean AUDPC and remaining portion due to other factors as shown in Figure 5.

There was a significant ($P \leq 0.01$), negative, linear relationship between mean AUDPC and mean yield (metric ton ha⁻¹). According to linear regression equation, it can be said that if there is a unit increase in mean AUDPC, mean yield (metric ton ha⁻¹) would have been decreased by 0.03 times. According to coefficient of determination, about 52.98% variation in mean yield was due to mean AUDPC and remaining portion due to other factors as shown in Figure 6.

There was a significant ($P \leq 0.01$), positive, linear relationship between thousand kernel weight (g) and mean yield (metric ton ha⁻¹). According to the linear regression equation, it can be said that if there is unit increase in thousand kernel weight (g) on plant, mean yield (metric ton ha⁻¹) would have been increased by 0.0078 times. According to the coefficient of determination, about 30.42% variations in mean yield (metric ton ha⁻¹) was due to thousand kernel weight (g) and remaining portion due to other factors as shown in Figure 7.

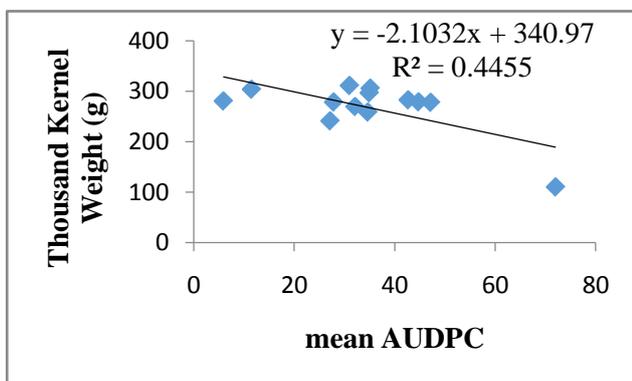


Figure 5. Estimated linear relationship between mean AUDPC and thousand kernel weight (g) of 13 maize genotypes in Paklihawa, Rupandehi, 2014

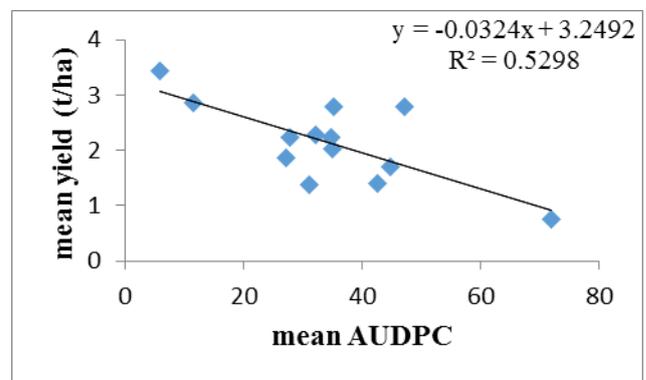


Figure 6. Estimated linear relationship between mean AUDPC and yield (metric ton ha⁻¹) of 13 maize genotypes in Paklihawa, Rupandehi, 2014

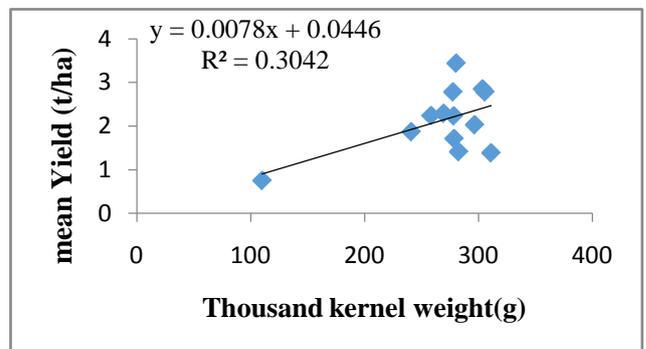


Figure 7. Estimated linear relationship between thousand kernel weight (g) and mean yield (metric ton ha⁻¹) of 13 maize genotypes in Paklihawa, Rupandehi, 2014

4. Summary

Southern leaf blight of maize, caused by *Bipolaris maydis* syn. *Helminthosporium maydis* (Telomorph: *Cochliobolus heterostrophus*) is considered as the most important one creating a threat to successful maize cultivation in Nepal during summer. Yield reduction up to 50% was recorded when “race O” inoculated into susceptible line ([12], [17]). Use of resistant varieties is a simple, effective, safe and economical means of controlling maize diseases. Resistant genotypes help to stabilize yield. Resistant levels should be updated each

year for each variety. In such context, identification of resistant genotypes/varieties would be good alternatives to manage SLB. Disease data in experimental trail and disease situation in farmer's field support the need for screening the genotypes against SLB [35]. The study was imperative to evaluate the level of resistance against this disease thereby predicting the effect of disease on yield of maize genotypes, if this disease occurs in epidemics.

Experiment was conducted at Paklihawa Campus Rupandehi, Nepal during summer (March to July, 2014) under irrigated conditions. Seeds of thirteen maize genotypes POSILO-1, S03TLYQ-AB-02, S03TLYQ-AB-01, RAMPUR COMPOSITE, RAMPUR S03F04, ARUN-2, ACROSS 9942/ACROSS 9944, ZM-401, ZM-627, YELLOW POPCORN, RAMPUR HYBRID-2, RML-4/RML-17, RML-32/RML-17, originating from diverse sources, included in the study were obtained from National Maize Research Centre, Rampur as these were the promising maize lines for hybrid production. Yellow popcorn was taken as a susceptible check, being highly susceptible to SLB of maize in Nepal and were planted 2 rows around the whole field to provide a source of inoculums. Recommended cultivation practices including fertilization were adopted. Randomly 5 plants per plot were selected to identify the plant for disease assessment. Disease assessment i.e. disease scoring, was done by visual estimation of leaf area affected in each and every individual plant and were averaged for the plot. Yield and yield attributing parameters were also recorded. Disease scoring was done 3 times at an interval of 5 days starting from 63 days after sowing in field.

Symptoms of SLB disease started to appear first in Yellow popcorn (63.67) on 1st June, 2014. Symptoms appeared in all maize genotypes within 63.67 to 79 DAS. Yellow popcorn showing the symptoms earlier than other genotypes also showed highest disease severity, and least severity was observed in RML-32/RML-17.

The AUDPC values of 13 maize genotypes were calculated and found to be variable and differed significantly in all dates of observation. Yellow popcorn showed the highest level of disease increment at all dates while least was observed in RML-32/RML-17. On the basis of mean AUDPC value, the 13 genotypes were categorised into four categories of resistance i.e. resistant, moderately resistant, susceptible and highly susceptible. S03TLYQ-AB-01, S03TLYQ-AB-02, Across 9942/Across9944, ZM-627, Arun-2, ZM-401, Posilo-1 were moderately resistant and Rampur Composite, Rampur Hybrid-2, Rampur S03F04 were susceptible. Out of 13 genotypes RML-32/RML-17 was found to be most resistant followed by RML-4/RML-17 whereas Yellow popcorn was most susceptible.

Mean AUDPC and appearance of symptoms DAS varied significantly among the genotypes. Maximum mean AUDPC (71.99) was recorded in Yellow popcorn, while minimum mean AUDPC (5.90) was recorded in RML-32/RML-17. However, mean AUDPC of RML-4/RML-17 (11.50) and RML-32/RML-17 (5.90) did not differ significantly. Appearance of symptoms DAS differed significantly among the thirteen maize genotypes in the field. The appearance of symptoms DAS ranged from 63.67 to 79. Highest DAS (79) was found in RML-32/RML-17 and lowest in Yellow popcorn (63.67).

Maximum SPAD value above cob was recorded in RML-4/RML-17 (45.62) followed by S03TLYQ-AB-01 (44.88) while minimum in Yellow popcorn (30.60). So, Yellow popcorn has the highest total AUDPC above cob (3.16) and RML-32/RML-17 (0.08) lowest total AUDPC above cob. Similarly maximum SPAD value below cob was recorded in RML-4/RML-17 (44.37), while minimum in Yellow popcorn (28.82). So, Yellow popcorn has the highest total AUDPC below cob (8.750) and RML-32/RML-17 (0.41) has lowest total AUDPC below cob.

Grain yield (metric ton ha⁻¹) varies significantly among the genotypes. Maximum yield was recorded in RML-32/RML-17 (3.43 metric ton ha⁻¹) while minimum yield was recorded in Yellow popcorn (0.75 metric ton ha⁻¹) and is directly proportional to the SPAD value. However, the yield of RML-32/RML-17 (3.43 metric ton ha⁻¹) and RML-4/RML-17 (2.85 metric ton ha⁻¹) does not differ significantly. The TKW ranged from 110.1g to 311 g. Highest TKW was found in Across 9942/Across 9944 (311g) and lowest in Yellow popcorn (110.1 g).

5. Conclusions and Recommendation

Since the performance of maize genotype varied highly with SLB disease, screening of genotypes seemed to be one of the important techniques for finding the sources of resistant and susceptible genotypes. From our research following conclusions could be drawn;

- Among the 13 genotypes, RML-4/RML-17 and RML-32/RML-17 appeared resistant to SLB with maximum yield (metric ton ha⁻¹). These genotypes could be used as the sources of resistance in breeding program and could be developed to resistant varieties under tropical and subtropical climatic conditions during summer season in Nepal.
- The genotype Yellow popcorn appeared highly susceptible to SLB with a maximum mean AUDPC and minimum yield (metric ton ha⁻¹). This genotype can be used as susceptible check for breeding purpose and different varietal screening.

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