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Evaluation of soybean genotypes for Asian soybean rust (*Phakopsora pachyrhizi*) and common bacterial blight (*Pseudomonas syringae pv. glycinea*) at different phenological stages in Southwestern Ethiopia

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Abstract
Asian soybean rust caused by *Phakopsora pachyrhizi* and bacterial blight caused by Pseudomonas syringae pv. glycinea diseases are the two most limiting factors in soybean production. The use of resistant or tolerant varieties is one of the most suitable management options to control Asian soybean rust and bacterial blight. This study was carried out to evaluate the performance of soybean genotypes and to see the progress of the Asian soybean rust and bacterial blight disease over different Phenological stages of the crop. Thirty-six soybean genotypes were tested in triple lattice design. The result indicates that there was a significant ($P < 0.05$) difference in AUDPC value for Asian soybean rust among genotypes and genotypes by environment interaction. Mean soybean rust severity had progressively increased from 3.3% at 42 Days after sowing to 47.7% at 96 Days after sowing, while, bacterial blight severity increased progressively from seedling stage 3.4% at 29 Days after sowing to flowering stage 15.7%at 70 Days after sowing, and decreased after flowering 3.6 % at 96 Days after sowing. In this study, a total of 32 soybean genotypes showed TAN reaction while only four, genotypes showed Reddish Brown (RB) lesions. This study shows different performance of soybean genotypes against soybean rust and bacterial blight, but none of them shows immune in the future, it is recommended to evaluate large number of soybean genotypes and identifying the types of rust race is one of the key stages.

Keywords: AUDPC value, Phenological stages, Tolerance.

Introduction
Asian soybean rust caused by *Phakopsora pachyrhizi* and bacterial blight caused by *Pseudomonas syringae* pv. *glycinea* are the most destructive foliar diseases and worldwide problems in soybean production. The pathogen has been first reported in Japan in 1903 (Hennings, 1903) and distributed to other Asia countries (Hartman *et al*., 1999) and South America (Killgore and Heu, 1994). The first report of Asian soybean rust (ASR) in Africa has been in Uganda in 1996 and recently the disease is reported in several countries in Africa (Murithi *et al*., 2015). The first report of soybean rust in Ethiopia was reported in 2011 (Abush *et al*., 2016). Asian soybean rust has unique among other rust species, which have a diverse host range that infects more than 95 species from 45 genera (Bromfield,1984). The Asian soybean rust has been known to significantly reduce yields in areas where the pathogen occurs regularly; yield losses up to 80% have been commonly reported in several countries. In Ethiopia, yield loss due to soybean rust is 56% (JARC, 2017). Yield losses due to bacterial blight have been reported in a range from 4 to 40%, depending on the severity of the conditions in different countries (Hartman *et al*., 1999). However, there is no report on yield loss of soybean due to bacterial blight in Ethiopia. The southwestern part of Ethiopia has warm temperatures, a high relative humidity and rainfall, implying the pathogen is always present, due to ideal environmental conditions. Likewise, the southwestern part of Ethiopia is suitable for soybean production. However, the production of soybean in this area continues to suffer due to the diseases. Soybean yield losses to rust and bacterial blight have been reduced by application of chemicals; however, planting resistant and tolerant varieties is cost effective and environmentally sound for small scale farmers. The aim of the present study was to evaluate the performance of soybean genotypes for Asian soybean rust and bacterial blight in southwestern Ethiopia.

Materials and Methods

Description of study area: The field experiment was conducted at Jimma Agricultural Research Center and Mettu Sub Agricultural Research Center. Jimma and Mettu agricultural research centers are located in Oromia region, in South Western Ethiopia. Jimma agricultural research center is located at 12 km distance from Jimma town (1,754 m a.s.l, 7°40′N36°47′E), the place has a mean maximum and minimum temperature of 26.3 and 11.6°C , respectively, mean annual rainfall of 1,572 mm; characterized by reddish brown soil with a pH of 6.2- 6.8 and sub humid type of climate (JARC, 2013). Mettu sub agricultural research center is located at 8°3′Nand 30°E, at an altitude of 1,550 m a.s.l.) has a mean maximum and minimum temperatures of 28.9 and 12.7°C, respectively, and mean annual rainfall of 1,829 mm (JARC, 2013).

Experimental design and treatments: A total of 36 soybean genotypes introduced and released varieties obtained from Jimma Agricultural Research Center were used for this study. The experiment was laid out in a 6 x 6 triple lattice design. Each plot had four rows each measuring 4 m long, with a spacing of 0. 6 m between rows and 0.05 m between plants. The distance between plots and between the blocks was 0.6 and 1.5 m, respectively. DAP fertilizer, which consists of 46% P_20_5 and 18% N with a rate of 100 kg per ha was applied for each location at sowing time.

Data collection

Disease severity: Soybean rust and bacterial blight disease severity score was taken using a scale of 0 to 90% (Vincellin and Hershman, 2011) from five randomly marked plant in 10- day intervals except the first and the second observations which was taken in 15- day intervals started from 4 weeks from planting until R7 (maturity). Severity data were converted into PSI and an area under the disease progress curve (AUDPC) was calculated from PSI using the formula proposed by Campbell and Madden using standard interactive procedures (Shaner and Finney, 1977).

The soybean genotypes were also assessed for rust reaction types based on lesion color on the leaves. Weather data (temperature, rainfall and relative humidity) were collected under the tested sites from planting to the final harvesting. And the total seed yield from central two rows was measured and converted into ton per hectare.

Statistical data analyses: Before conducting individual location statistical analysis, data were checked for the normality and all the data meet the assumption. Thereafter, homogeneity of error variance was tested using Levene's test, and all traits met the assumption of combined analysis. The data collected was subjected to analysis of variance (ANOVA) as per the method of Gomez and Gomez (1984) using the SAS Software to assess the significance of the difference between the genotypes. Least significant difference (LSD) test at $P< 0.05$ were employed to identify genotypes that are significantly different from each other.

Results and Discussion

Reaction of tested soybean genotypes to soybean rust disease: The analysis of variance indicates that there was a significant ($P < 0.05$) difference in AUDPC value of Asian soybean rust among the tested soybean genotypes (Table 2). This result indicates the presence of sufficient variation among the soybean genotypes in response to Asian soybean rust. The AUDPC value ranged from 398.1 (PI605773) to 691.4% days(LD13-08470) with a mean value of 535.5 % days. The AUDPC value of 12 genotypes was less than the check variety (Nyala), and only five genotypes showed a higher value of AUDPC than the check (Afgat). Among the checks, the lowest AUDPC value was found in Nyala, and the highest AUDPC value was recorded in Afgat.

There were significant genotypes by environment interaction for AUDPC value ($P < 0.05$). Cherif *et al*. (2010) also reported similar result that the AUDPC value is influenced by genotype by environment interaction. This indicates that the AUDPC value is not consistent across locations, which might be mainly due to the variation of environmental factors that affects the occurrence of the disease at each location. Jimma and Mettu have warm temperatures, high relative humidity and high rainfall, which favors rust and bacterial blight pathogen (Table 1).

Table 1. Weather data of Jimma and Metu in soybean growing months in the 2017 cropping season

	Jimma					Metu		
Month	RF (mm) total	T ºC Max	T ºC Min	RH mean	Dew (h) mean	RF (mm) total	T ºC Max	T ºC Min
Jun	148.2	26.0	10.2	74.5	14.9	187.5	28.1	11.08
July	181.8	25.5	10.7	78.6	14.9	105.4	24.8	11.1
August	169.1	26.8	11.0	80.3	13.8	171.6	26.3	11.2
September	359.0	26.4	11.2	73.1	15.9	299.2	27.11	11.0
October	319.1	26.4	10.9	76.18	14.7	127.3	28.0	11.0
November	27.6	28.4	10.2	75.0	15.8	36.4	27.5	10.0

RF = rainfall, RH = relative humidity; T = temperate, Dew = dew period.
Source: Jimma Agricultural Research Center.

Resistant genotypes to ASR are expected to have a lower number of AUDPC values, and reddish brown (RB) lesion type. However, in the present study, genotypes with high AUDPC value have shown high grain yield which is beyond expectation. This shows that such genotypes are tolerant to the disease, though they are not resistant.

Table 2. Soybean rust and bacterial blight area under disease progress curve (AUDPC) and reaction type of soybean genotypes in field experiment over two locations (Jimma and Mettu, Ethiopia) in the 2017 cropping season

Soybean genotypes	AUDPC		Yield (t/ha)	Soybean rust Reaction type
	Rust	**BB**		
LD13-00833	522.9a-d	307.2	1.89	T
LD11-10649	554.7a-d	631.8	1.61	T
SA13-3135	589.2a-d	498.0	2.04	T
LD13-08470	691.4a	390.0	1.54	T
LD13-03483	546.4a-d	154.3	1.50	T
LD13-08466	683.0a	534.7	1.92	T
LD13-06216	576.3a-d	599.0	1.81	T
LD10-10198	579.3a-d	569.5	1.83	T
LD13-07022	588.0a-d	584.7	2.19	T
F6LG06-5920 x LG04-6000	473.9a-d	341.1	2.10	T
LG04-4468 x U02-242055	585.7a-d	380.9	1.98	T
LG04-5993 x LG04-5196	661.8ab	390.8	1.81	T
F6LG04-4717 x LG05-4292	602.2a-d	610.1	2.01	T
PI200466	616.3a-d	405.0	2.36	T
PI587905	428.3b-d	397.2	2.54	T
PI416778	506.8a-d	274.1	1.43	RB
PI459025B	454.4a-d	339.3	2.61	T
PI594149	519.0a-d	574.0	2.43	T
PI417126	472.8a-d	456.7	2.15	RB
PI507005	493.8a-d	364.7	1.83	T
PI615437	492.8a-d	509.1	1.63	T
PI628932	470.3a-d	561.3	2.07	T
PI462312	534.1a-d	624.7	1.86	T
PI605773	398.1d	455.7	1.63	T
PI605854B	462.5a-d	509.8	1.92	RB
PI594767A	643.1a-c	362.7	1.80	T
PI416873B	591.2a-d	561.0	2.22	T
PI567180	453.8a-d	461.4	1.61	T
PI423960B	409.8cd	773.3	2.40	T
PI635999	408.3cd	424.1	2.29	RB
PI605865B	565.7a-d	660.8	2.11	T
PI423960A	515.7a-d	607.3	2.14	T

AFGAT	606.8a-d	474.6	2.03	T
Nyala	499.7a-d	233.2	2.26	T
Nova	552.5a-d	362.9	2.28	T
Clark 63 K	526.3a-d	452.0	1.65	T
Mean	535.5	467.7	1.99	
Min	398.1	154.3	1.43	
Max	691.4	773.3	2.61	
MSL	4748186.05**	11228772**		
MSG	30921.37**	74983.9ns		
MSGXL	30358.87**	80397.16ns		
MSE	11103.17	92522.48		
CV(%)	19.67	58.4		
LSD (5%)				

Where; RB = reddish brown, T = tan, NS = not significantly different * = significant at (P < 0.05).

The soybean genotypes reaction to soybean rust data at the different growth stage of the crop combined over the two locations is presented in Figure 1. Asian soybean rust affects soybean at any developmental stage, when conducive environment, virulent urediniospores and susceptible hosts coincide with each other. For the occurrence of soybean rust requires a continuous dew period of between 6-7 hours, and for the optimum germination of the urediniospores requires temperatures of between 17-25 °C and relative humidity of above 70%, and rainfall is the most important factor causing a rapid increase in severity and disease spread (Melching *et al.,* 1989). Mean rust severity among the tested soybean genotypes at 42, 56, 86 and 96 days after sowing (DAS) were not significant (P < 0.05), but the differences in mean rust severity among the genotypes at 66 and76 DAS, were highly significant (P < 0.001) over the combined location (Appendix1). The mean rust severity for the tested soybean genotypes at 42, 56, 66, 76, 86 and 96 DAS were 3.33, 3.36, 12, 25.6, 31.8 and 47.7% (Figure 1). This result indicates soybean rust severity had progressively increased on the soybean genotypes, as they tend to mature. A similar result was reported by Mcebisi (2013); hence, evaluation and selection of soybean genotypes for rust at the seedling stage does not assure adult plant resistance. Because, under field conditions the multiple cycles of re-infection, promote greater pathogen virulence, which is able to challenge the true resistance potential of a genotype (Ribeiro *et al*., 2007). Under field condition Asian soybean rust symptoms and severity increases at reproductive stage, thus, to evaluating soybean genotypes is ideal during the reproductive stage as variation in severity is greatest during this period (Miles *et al*., 2006; Walker *et al*., 2011). In this study, a total of 32 soybean genotypes showed TAN reaction while only four, PI genotypes (PI635999, PI605854B, PI416778 and PI417126) showed Reddish Brown (RB) lesions, and have less sporulation and sparse uredinia, indicating partial resistance of the genotypes to ASR. However, none of the tested soybean genotypes were immune to ASR. Soybean genotypes without any visible rust symptoms were considered to be immune indicating the presence of complete resistance, those with red-brown lesions were partially resistant while lines with tan-colored lesions (TAN) were susceptible (Bromfield, 1984; Miles *et al*., 2005). Reddish Brown (RB) lesions are formed, because of the hypersensitive response of the soybean crops to *P. pachyrhizi*. This reaction will inhibit the fungus development. Lesion color is known to be controlled by resistance genes of Rpp1- Rpp6, and thus should be considered when selecting resistant genotypes (Yamanaka *et al*., 2010; 2013).

Reaction of tested soybean genotypes to soybean bacterial blight disease: The study result indicates that soybean genotypes were not significantly different from AUDPC value among the 36 soybean genotypes and genotype by environmental interaction at (p = 0.05) level of significance for bacterial blight disease (Table 2). The AUDPC value of soybean genotypes for bacterial blight ranges from 154.3 (LD13-03483) to 773.3 (PI423960B) with a mean value of 467.7 percent days. The differences in mean rust severity among the genotypes at 44, 59, 70, 81 and 92 days from sowing were not significant, but the differences in mean bacterial blight among genotypes at 29 days from sowing were highly significant (P < 0.001)(Appendix1).The mean severity for the tested soybean genotypes at 29 days from sowing was (3.4%), 44 days from sowing (13.4%), 59 days from sowing (15.3%), 70 days from sowing (15.7%), 81 days from sowing (13.7%) and 92 days from sowing (3.6%) (Figure 2). This indicates that the soybean bacterial blight severity increased progressively from seedling stage to flowering stage, and decreased after flowering for all the tested genotypes

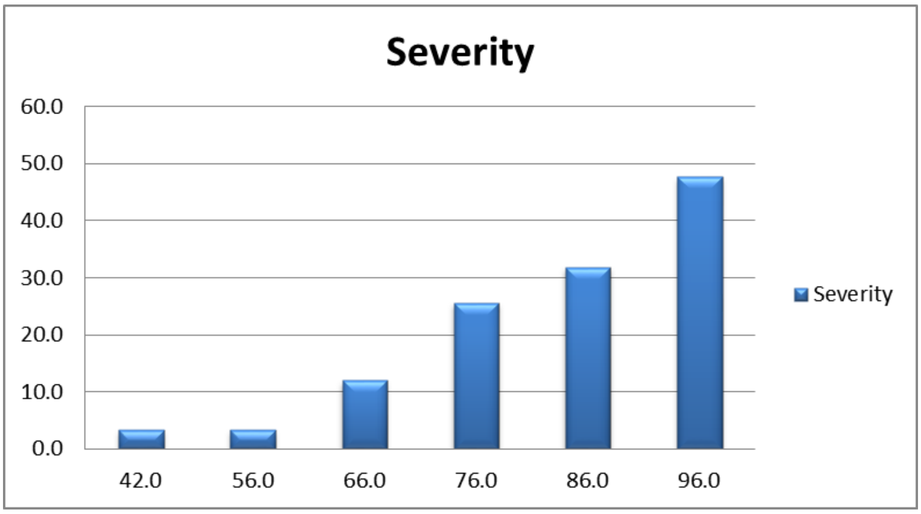

Figure 1. Severity mean value of soybean genotypes to soybean rust at different growth stage at Jimma and Mettu in the 2017 cropping season.

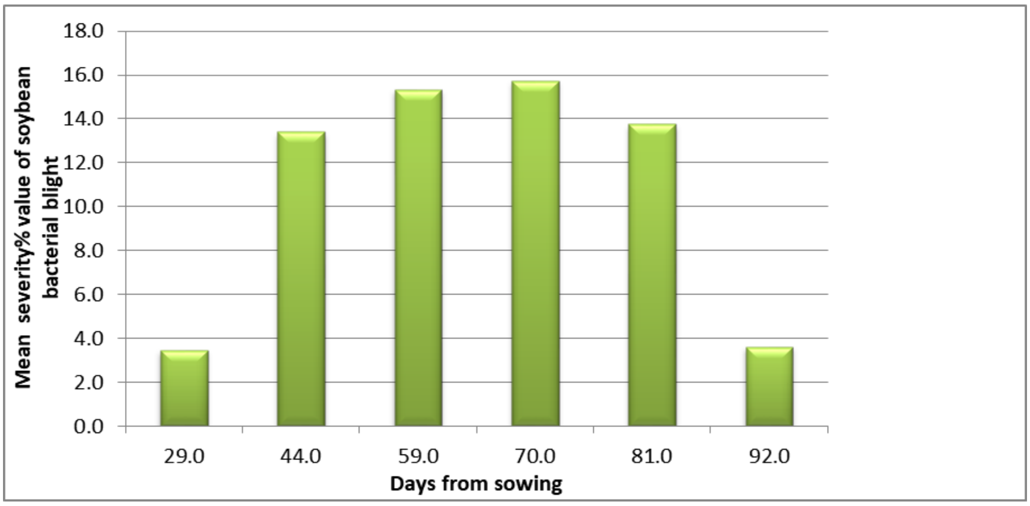

Figure 2. Mean severity value of soybean genotypes to soybean Bacterial blight at different growth stage at Jimma and Mettu in the 2017 cropping season.

Conclusions

Asian soybean rust and bacterial blight disease regularly occurred under favorable environmental conditions and are the most important constraints in soybean production. Disease establishment, development and severity are highly dependent on the environmental condition. There was a significant difference for AUDPC value for Asian soybean rust among the tested soybean genotypes and significant difference for genotypes by environment interaction was observed in both Asian soybean rust and bacterial blight. Asian soybean rust and bacterial blight can occur at any development stage, in this study bacterial blight occurred at the seedling stage and increased up to flowering then declined; whereas, soybean rust started infection at flowering and showed progressive incremental severity throughout the soybean's growth.

Recommendations

The genotypes which have been identified as partial resistance to soybean rust can be advanced to further testing and used as parental material for hybridization. In the future, it is recommended that including a large number of soybean genotypes and study based on their maturity group for the performance of for Asian soybean rust and bacterial blight. Genotypes in this study, which show RB reaction to soya bean rust may or may not show a similar reaction in other locations; therefore, multi-location variety trial in known rust prone areas will be done in the future to release rust tolerant lines. Identification of rust races need future attention, for proper recommendation of rust resistant/tolerant soybean genotypes.

Conflict of Interest

The author declares no conflicts of interests.

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Appendix1. Analysis of variance summary of rust and bacterial blight severity at different growth stages of soybean over the tow combined location

	Asian soybean rust days from sowing					
Source of Variation	42	56	66	76	86	96
MSL	0.11	0.01**	15623.5**	1680.4**	15082.8**	0.01**
MSG	0.025	0.07	12.9**	56.9*	48.5	149.6
MSGXL	0.02	0.08	10.04*	67.9 **	66.8	0.001
MSE	0.02	0.06	4.3	32.1	61.3	41.8
	Bacterial blight days from sowing					
Source of Variation	29.0	44.0	59.0	70.0	81.0	92.0
MSL	0.44	20734**	31075.2**	32651**	23558**	0.01
MSG	0.3**	17.4	43.8	31.0	9.57	1.08
MSGXL	0.33**	15.2	39.6	29.5	10.64	0.001
MSE	0.17	12.6	37.3	33.5	15.37	0.44