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Short Communication: Resistance of eleven new hybrid maize genotypes to Turcicum leaf blight (*Exserohilum turcicum*)

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Abstract. *Setyawan B, Suliansyah I, Anwar A, Swasti E. 2016. Short Communication: Antidiabetic screening of some Indonesian marine cyanobacteria collection. Biodiversitas 17: 604-608.* Turcicum leaf blight (TLB) is a leaf disease caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs. In Indonesia, TLB was first discovered in North Sumatra in 1917 (Van Hall 1929), and now is found throughout Indonesia (Semangun 2008). Losses due to yield decrease will be greater when the plant is infected at the time of flowering and grain filling phase. Resistant varieties are the most effective way of controlling TLB. The purpose of this research was to test 11 new hybrid maize genotypes to determine the level of TLB resistance. The research was conducted in 2 season, using randomize complete block design, 3 replication and 2 control genotypes. Based on statistical examinations and CIMMYT (1999) scoring system, it could be concluded that 10 prospective genotypes (90.9%) which were SSU3X28871, SSU3X29131, SSU3X30735, SSU3X45172, SSU3X68276, SSUSX02791, SSUSX06145, SSUSX48274, SSUSX68849 and SSUSX76844 were significantly better than both control genotypes at LSD 5% (α=0.05).

Keywords: Disease, genotype, leaf blight, maize, turcicum
Abbreviations: CIMMYT: Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center), DAP: days after planting, DS: dry season, OPV: open pollinated variety, LR: less resistant, LSD: least significant difference, R: resistant, RS: rainy season, S: susceptible, TLB: turcicum leaf blight, VR: very resistant, VS: very susceptible.

INTRODUCTION

Turcicum leaf blight (TLB) is a leaf disease caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs. (Semangun 2008). TLB can result in decreased yield when infects during the flowering phase. The development of this disease is strongly influenced by the resistance of varieties, cultivation systems and the weather/climate (Carson 1995). The disease also infects the corn plant in India with a loss rate from 28% to 91% (Pant et al. 2001; Singh et al. 2012; Ishfaq et al. 2014; Nwanosike et al. 2015) and until 2005 had been found at least three races, i.e. Race 2, Race 3 and Race 4 (Dutta et al. 2005).

Right now the disease has spread throughout the world with several different races such as Race 0,1,2,3, N, 12,13,13N, 3N, 123.23, and 23N which were found in Kenya, Germany and Austria (Muiru et al. 2010). TLB was also found in Uganda (Castiano et al. 2012), Thailand (Wathaneeyawech et al. 2015), Argentina (Sartori et al. 2015) as well as other countries in Asia, Africa, Europe, Australia and America. In Indonesia, TLB was first discovered in North Sumatra in 1917 (Van Hall 1929). At this moment the disease has been widespreaded throughout Indonesia (Semangun 2008). Specific research had been conducted in South Sulawesi (Surtikanti 2009) and Batu, Malang (Latifahani et al. 2014).

TLB is potential in areas where the air temperature drops at night while the air humidity is high. The fungus releases many conidia at noon after a warm night with a relative humidity above 90%. The optimum temperature for the formation of conidia is 20-26^{o}C. Infection takes 6-18 hours at a temperature of 18-17°C. As known, this disease can infect plants from germination to harvest time. Losses due to yield decrease will be greater if the plants were infected during the flowering and grain filling phase (Semangun 2008). TLB damages or even kills the leaf tissue, and it will decrease the amount of chlorophyll where the carbohydrate, fat and protein are produced in plants. It was reported about 91% reduction in the rate of photosynthesis when severity of turcicum leaf blight incidence in maize exceeded 50% (Reddy et al. 2014). When the leaf area that die from this disease is quite large, yield will decrease. As a result of the breadth of green leaves die, the formation of starch will be retarded and the grains produced will be empty (chaffy). The leaves which withered as a result of this disease are not eligible to be used as animal feed (fodder) because it has lost all of the containing nutrients (Semangun 2008; Reddy et al. 2013). The degree of infection is determined by the disease resistance of the plants, because plant resistance can reduce the number of patches that cause chlorotic and necrotic (Semangun 2008). Growing resistant varieties is recommended because of it most effective way to control

this disease and safe for the environment (Pattaky 1992; Semangun 2008).

To determine the resistance of a certain cultivar to this disease can be carried by research. The research can be established in the field, in the greenhouse or utilizing molecular marker (Inghelandt et al. 2012). Research regarding TLB had been done in Indonesia. These researchs were purposed to determine the resistance of existing cultivars on the market of South Sulawesi Province and East Java Province. Both researchs were conducted in the field and the laboratory. Isolates used were common TLB isolates which were taken from the farmer fields (uncharacterized/ unknown races). The results showed higher virulence and decreased yield (Surtikanti 2009 and Latifahani et al. 2014).Therefore, the resistance research of maize varieties against TLB is a mandatory.

The purpose of this research was to determine the resistance level of 11 (eleven) prospective genotypes (tested genotypes) to TLB infection. Prospective genotypes which had resistance level equal to or more superior than BISI 18 would be included in multi-location trials in order to release national new superior varieties.

MATERIALS AND METHODS

Research materials

This research used 13 materials. The materials of this research consisted of 11 new prospective hybrid corn varieties (genotypes) with two control varieties that had already existed in the market, namely BISI 18 and Sukmaraga. BISI 18 was representing less resistant (LR) and hybrid cultivars, while Sukmaraga was representing the resistant (R) ones (Ministry of Agriculture 2013). Sukmaraga also represented OPV cultivars due to its progenitor random cross pollination during the production of the seed.

The above mentioned 11 prospective genotypes consisted of six threeway cross hybrids and five single cross ones. These prospective genotypes were the outcome of the author breeding program which was began in 1997. The prospective genotype progenitors were inbred lines which were extracted from landrace populations introduced from 7 countries (USA, Mexico, Colombia, India, Thailand, Malaysia, Philippines) and some indigenous landraces of some areas in Indonesia. Based on their progenitors resilience, these 11 prospective genotypes were expected to be classified as resistant (R) or very resitant (VR) cultivars according to CIMMYT (1999) scoring system which is being adopted by The Variety Assessment and Release Team of The Republic of Indonesia. The complete data on 11 new prospective genotypes and the control ones, is presented in Table 1.

Methods

The research used randomized complete block design with three replications. Each plot size of 5 m x 2.8 m was tillaged with a complete tillage system (first plowing, second plowing after 14 days interval and harrowing 14 days after second plowing). Each plot consisted of 4 (four) rows with a spacing of 70 cm x 20 cm. Research material were planted in the plot with 2 (two) seeds per hole, therefore 200 plants per plot were expected at planting time (50 seeds per row x 4 rows). The first thinning was done before the first fertilization by cutting unwanted plants especially at holes which consisted 2 plants. At this time 120 plants remained per plot (30 plants per row x 4 rows) regardless plant-count per hole. Second thinning was done before the second (last) fertilization by same method with the first thinning. At this time until the time of observation 100 plants had to be remained in one plot (25 plants per row x 4 rows) regardless plant-count per hole.

Fertilization were done two times during planting period. The first fertilization was done 14 days after planting (DAP), using Urea, SP-36 and KCl at a dose per hectare 250 kg, 100 kg and 50 kg respectively. Second fertilization is done when the plants were 30 DAP, using urea at a dose of 100 kg per hectare. The dose of fertilization was adapted from local farmers who experienced growing hybrids corn. Weeding was done right after fertilization, while irrigation was utilizing rainfall.

Table 1. Research materials

Code of Genotypes	Cross	Pedigree		TLB resistance		Expected F_1 TLB resistance	Remark
		Female parent	Male parent	Female parent	Male parent		
SSU3X17782	Threeway	SSU3X17782FF	SSUSX02791M	S	R	R	Tested
SSU3X28871	Threeway	SSU3X28871FF	SSUSX76844M	R	VR	VR	Tested
SSU3X29131	Threeway	SSU3X29131FF	SSUSX68849M	R	VR	VR	Tested
SSU3X30735	Threeway	SSU3X30735FF	SSUSX48274M	R	R	R	Tested
SSU3X45172	Threeway	SSU3X45172FF	SSUSX06145M	LR	R	R	Tested
SSU3X68276	Threeway	SSU3X68276FF	SSU3X68276M	LR	R	R	Tested
SSUSX02791	Single	SSUSX02791F	SSUSX02791M	R	R	R	Tested
SSUSX06145	Single	SSUSX06145F	SSUSX06145M	R	R	R	Tested
SSUSX48274	Single	SSUSX48274F	SSUSX48274M	LR	R	R	Tested
SSUSX68849	Single	SSUSX68849F	SSUSX68849M	VR	VR	VR	Tested
SSUSX76844	Single	SSUSX76844F	SSUSX76844M	R	VR	VR	Tested
BISI 18	Single	-	-	-	-	LR	Control
Sukmaraga	OPV	-	-	-	-	R	Control

Note: The expected TLB resistance level of BISI 18 and Sukmaraga were based on Ministry of Agriculture (2013)

Innoculation of the disease was utilizing spreader rows (sweet corn) which very susceptible to TLB. These spreader rows were planted 4 weeks prior the planting of research materials. Spreading of disease relied on nature. It was done because the research location had been classified as endemic to TLB and the research had to be conducted in the field/not at laboratory (National Seed Board 2008). The outcome of this research would be included as part of multilocation trial data in order to release new superior hybrid corn cultivars. Innoculation utilized specific race isolates was not possible to be done because characterization on TLB had been never done before this research.

Observation or data collections in this research was done through visual observation at the end of flowering stage by using scoring system (CIMMYT 1999 and National Seed Board 2008). Observations were made on the entire plot and all plants in the plot (100 plants) individually. It meant that every single plant in the plot was examinated for TLB infection and the score was given. The plot score was the average of all plants score in the respective plot. Score 1 was the best (VR) while score 5 was the worst one (VS). Scoring was based on TLB degree of infection with the guidelines presented in Figure 1 (CIMMYT 1999).

Figure 1 can be explained as follows: (i) Score 1 (very resistant/VR): there are no infections on any leaves, (ii) Score 2 (resistant/R): 2-3 leaves under ear are infected, (iii) Score 3 (less resistant/LR): infectious disease reaching 2-3 leaves upper the ear, (iv) Score 4 (susceptible/S): infection reached almost all the leaves except the 2-3 upper leaves of the plant, (v) Score 5 (very susceptible/VS): all the leaves of the plants are infected.

Location and time of research

The research was conducted at experimental field located in The Village of Kuta Kendit, District of Mardingding, Karo Regency, North Sumatra Province, Indonesia. It was conducted in two seasons, the dry season (DS) 2015 and the rainy season (RS) 2015/2016.

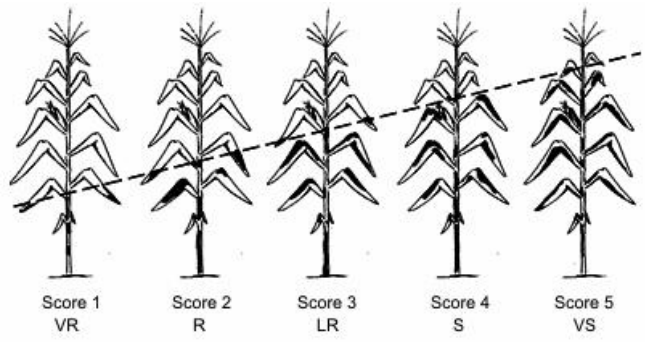

Figure 1. TLB scoring system (CIMMYT 1999)

RESULTS AND DISCUSSION

Transmission of TLB infection on this research had been going well at both season. It could be proven by the actual TLB degree of infection on BISI 18 and Sukmaraga (Table 2) which were lower than their expected level of resistance stated in Table 1 which based on Ministry of Agriculture (2013). This deteroriation was probably due to the TLB virulence level of infection which continued to be more severe over the time. This was in line with the research of Dutta et al. (2005) and Muiru et al. (2010) which stated that new races of TLB is always found all the time. Based on the above facts, it could be ascertained that the occurence of stress escape phenomenon could be avoided in this research.

Unfortunately, unlike downy mildew, the characterization of TLB had been never conducted in Indonesia, therefore specific race isolates were not available yet. Four major genes (Ht1, Ht2, Ht3 and HtN) are responsible for the resistant of TLB in maize plant. Actualy, 1 gene (HtNB) of which responsible for resistant of TLB Race 1 were discovered from Indonesian landrace named "Bramadi" (Wang et al. 2012), but the isolate of TLB Race 1 was not taken from Indonesia. It provided by the Plant Pathology Laboratory of Huazhong Agriculture University.

At the beginning of this research, authors tried to find "Bramadi" for resistant cultivar control, but beside it was not registered in Ministry of Agriculture (2013), it also could not be found throughout Indonesia. It might be confused with "Permadi", an OPV cultivar which was registered in Ministry of Agriculture (2013). But "Permadi" which was released in 1966 (Ministry of Agriculture 2013), was not available anymore. Base on the above mentioned facts, data of this research was base on general (unspecific) TLB resistant.

Analysis of variance stated that both control genotypes showed uniform genetic stability in both seasons. In the dry season the rainy season, control genotype Sukmaraga had P-value = 1.83594 and 0.46738 repectively. Control genotype BISI 18 possesed P-value = 1.18633 in the dry season, while in the rainy season P-value = 1.49958. Both genotype controls also showed uniform genetic stability of inter-block in every season. Meanwhile, most of all prospective genotypes in the rainy season showed ununiform genetic stability except prospective genotype SSUSX68849 (P-value = 0.49228). In the dry season, the genetic stability relatively uniform except on the prospective genotypes SSUSX76844 (P-value = 1.59782), SSUSX02791 (P-value = 0.00005) and SSU3X17782 (P-value = 0.01220).

Base on the data presented in Table 2 and Figure 2, it also could be stated that there were no variation among replications in the dry season (P-value = 0.142883). In the rainy season variation among replications were significant (P-value = 0.040443). It probably happened due to ununiformity of soil fertility as a result of the movement of nutrients from the higher plots to the lower ones. This movement was mainly caused by rainfall which often

exceed 250 mL per day during the rainy season. In the dry season, rainfall was rarely exceeds 100 mL per day so it was not strong enough to move nutrients from the upper plots. The research location was a hilly area on the plateau (990 meters above sea level) with average slope more than 6%. This phenomenon was in line with Carson (1995) and Treikale et al. (2014) that the development of TLB was strongly influenced by the resistance of varieties, cultivation systems and the weather/climate.

Table 2. TLB degree of infection

Code of genotypes	Dry season				Rainy season				Aggregate	Resistance level*
	Rep 1	Rep 2	Rep 3	Average	Rep 1	Rep 2	Rep 3	Average		
SSU3X17782	3.69	3.79	3.52	3.67^{b}	4.01	4.09	4.14	4.08	3.87	S
SSU3X28871	1.41	1.48	1.53	1.47^{ab}	1.34	1.42	1.40	1.39^{ab}	1.43^{ab}	R
SSU3X29131	1.55	1.49	1.58	1.54^{ab}	1.38	1.40	1.29	1.36^{ab}	1.45^{ab}	R
SSU3X30735	1.77	1.83	1.68	1.76^{ab}	1.31	1.40	1.46	1.39^{ab}	1.58^{ab}	R
SSU3X45172	1.86	1.96	1.83	1.88^{ab}	1.45	1.39	1.38	1.14^{ab}	1.65^{ab}	R
SSU3X68276	2.74	2.91	3.07	2.90^{b}	2.93	3.05	2.84	2.94^{b}	2.92^{b}	LR
SSUSX02791	1.85	1.92	1.70	1.82^{ab}	1.38	1.52	1.37	1.42^{ab}	1.62^{ab}	R
SSUSX06145	1.68	1.64	1.79	1.70^{ab}	1.62	1.74	1.73	1.70^{ab}	1.70^{ab}	R
SSUSX48274	1.67	1.86	1.52	1.68^{ab}	1.42	1.44	1.34	1.40^{ab}	1.54^{ab}	R
SSUSX68849	1.00	1.00	1.00	1.00^{ab}	1.01	1.00	1.00	1.00^{ab}	1.00^{ab}	VR
SSUSX76844	1.10	1.30	1.29	1.23^{ab}	1.34	1.33	1.36	1.34^{ab}	1.29^{ab}	R
BISI 18	3.96	3.87	4.07	0.97	4.04	3.82	3.97	3.94	3.96	S
Sukmaraga	2.75	2.92	2.99	2.89^{b}	2.91	3.03	2.94	2.96^{b}	2.92^{b}	LR

Note: a = significantly better than Sukmarga at LSD 5%, b = significantly better than BISI 18 at LSD 5%, * = according to CIMMYT (1999).

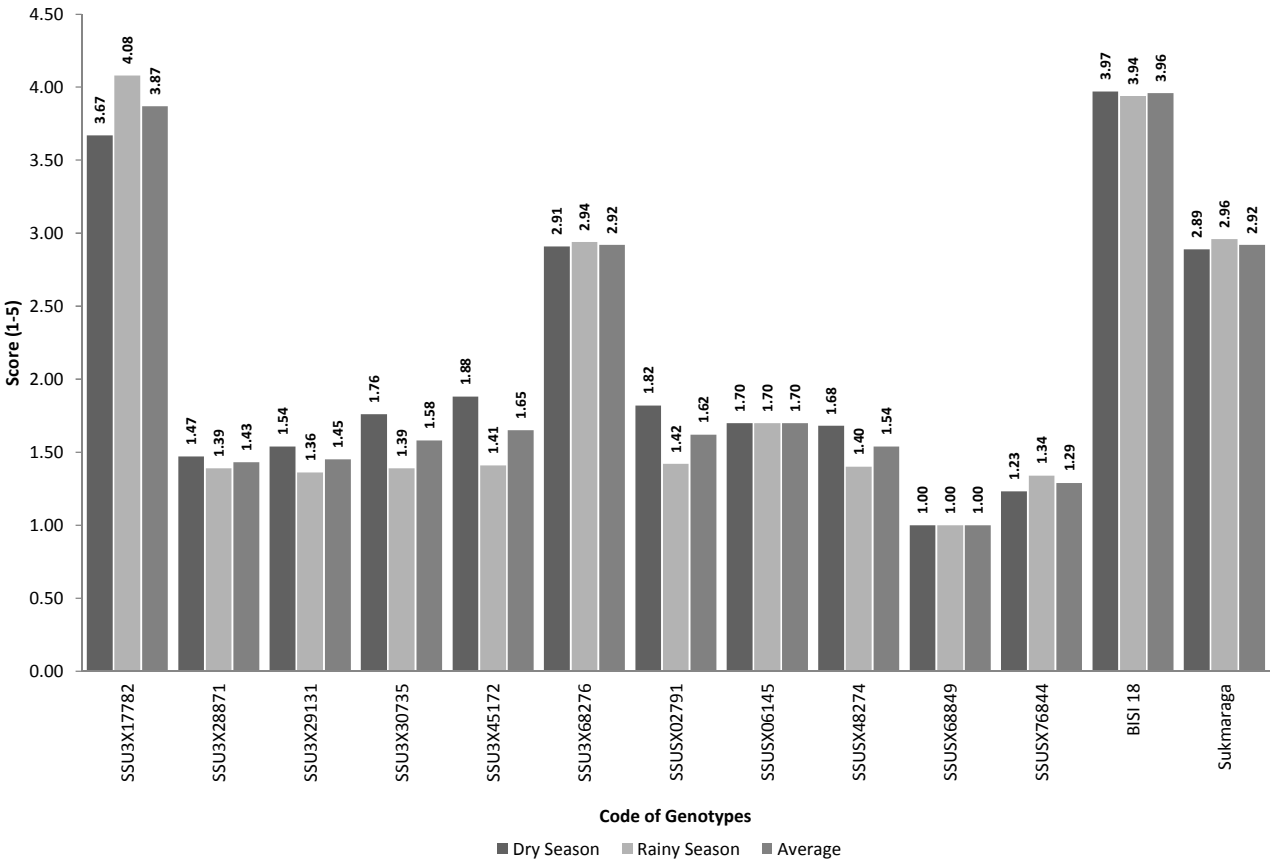

Figure 2. TLB degree of infection

In general, most of the prospective genotypes (90.9%) have genetic stability againt seasons alteration, except SSU3X17782. This prospective genotype was more susceptible to the TLB during the rainy season. In the dry season, resistance level of SSU3X17782 was equal to Sukmaraga and significantly better compared to BISI 18 at LSD 5% (α=0.05). Otherwise, in the rainy season resistance level of SSU3X17782 was significantly lower than BISI 18 and Sukmaraga. This trait was probably inherited from its male parent SSUSX02791M. Prospective genotype SSUSX02791 which shared same male parent with this prospective genotype, showed similar genetic instabilitity during both season.

Besides SSU3X17782, in the dry season, resistance level of prospective genotype SSU3X68276 was equal to resistance level of Sukmaraga and significantly better than BISI 18 at LSD 5%. However, in contrast to SSU3X17782 this prospective genotype was genetically remained stable and still had the same resistance level during the rainy season. The remaining 9 prospective genotypes (81.8%) were significantly better than Sukmaraga and BISI 18 at LSD 5% in the both season.

Based on CIMMYT (1999) scoring system, 7 prospective genotypes (63.6%), which were SSU3X30735, SSU3X45172, SSU3X68276, SSUSX02791, SSUSX06145, SSUSX48274 and SSUSX68849 had actual resistance level (Table 2) equal to their expected resistance level (Table 1). Prospective genotypes SSUSX68849 was classified in the range of very resistant (VR), while the other 6 prospective genotypes (54.5%) were classified in the range of resistant (R). Five prospective genotypes (45.5%) which were SSU3X17782, SSU3X28871, SSU3X29131 and SSUSX76844 had actual resistance level (Table 2) lower than their expected resistance level (Table 1). This deteriorization was probably due to epistasis phenomenon. Resilience to TLB is controlled by many genes (polygenic), so that many genes interact each other during the crossing between the progenitors (Muiru et al. 2010; Castiano et al. 2012; Wathaneeyawech et al. 2015 and Sartori et al. 2015).

Prospective genotype SSUSX68849 was very resistant (VR) to TLB. It was proven by 299 plants out of 300 plants on all plots and all replications, were not infected at all by the fungus E. turcicum. Score 2 (2-3 leaf below the ear infection) occured only 1 time in this prospective genotype. Score 2 was found in replication-1, line 4, plant number 21. This phenomenon was probably caused by outcrossing from susceptible line during the crossing of prosopecrive genotype SSUSX68849 or natural mutation in one or both its parents. The both parents of this prospective genotype possesed excellent resistance to the TLB. It was similar with Hurni et al. (2015) that showed mutant cultivars were more susceptible than their progenitors.

Based on statistical tests and CIMMYT (1999) scoring system, it could be concluded that 10 prospective genotypes (90.9%) which were SSU3X28871, SSU3X29131, SSU3X30735, SSU3X45172, SSU3X68276, SSUSX02791, SSUSX06145, SSUSX48274, SSUSX68849 and SSUSX76844 had passed the preliminary examination of the TLB infection. Therefore, these 10 prospective genotypes could be included in multi-location researchs in order in order to release national new superior varieties.

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