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Morphological, Cultural and Pathogenicity Variation of *Exserohilum turcicum* (Pass) Leonard and Suggs Isolates in Maize (*Zea Mays* L.)

Daniel Abebe[1]* and Narong Singburaudom [2]

ABSTRACT

Surveying and collecting isolates of the fungus *Exserhilum turcicum* (Pass) Leonard and Suggs, the causal agent of Northern Corn Leaf Blight (NCLB), were conducted in two maize growing areas of Ethiopia in 2004 and used for studying cultural, morphological and pathogenicity varations. The results revealed that NCLB was widely distributed in almost all surveyed areas. Seventy isolates were studied for morphology and most of the conidia shapes were curved, spindle and elongated. The size of the conidia averaged 93.97 μm in length and 13.11 μm in width. The number of septa was found to range from 2 to 7. The study of cultural characteristics showed that variation existed among representatives of 28 isolates in colony growth, colony color and pigmentation. Conidial growth rate of 28 isolates was significantly different after growing the fungus on PDA. Twenty representative isolates were selected and evaluated for pathognicity on 11 seedlings maize varieties. A significant difference of disease reaction was found among tested isolates, varieties and isolates by varieties interaction. Lesion type varied in size from 0.69-2.91cm.The most virulent isolate, GOR, was found to cause disease on five varieties. Lesion size that was classified as resistance, was 0.69 to1.12cm^2. Susceptible lesion size was between 1.17-2.91cm^2. By applying the UPGMA in the SAHN program for cluster analysis, a pathogenicity dendogram of 20 isolates were grouped into five clusters of virulent patterns. The results of this study indicated wide variations existed among the fungi studied, therefore, the effective development of maize variety resistant to northern corn leaf blight should involve using virulent isolates, together with a mixed isolates, in order to test the disease interactions and select for resistant genotypes.

Key words: *Exserhilum turcicum*, northern corn leaf blight, cultural, morphological, pathogenicity variation

INTRODUCTION

In Ethiopia, maize (*Zea mays* L.) is the most important staple food crop ranking first in total production and yield among the cereals (CSA, 2001). However, productivity of maize in Ethiopia remains low due to a number of biotic and abiotic constraints. Among the biotic stresses to maize production in the country, are diseases such as northern corn leaf blight (NCLB). NCLB is a foliar disease of maize which is caused by the residue-borne fungus, *Exserohilum turcicum* (Pass) Leonard and Suggs. This disease has become a prevalent disease of maize worldwide and

[1] Gambella Agricultural Research center, P.O. Box 62, Gambella, Ethiopia.
[2] Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: dlabebe@yahoo.co.uk.

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particularly in areas where high humidity and moderate temperature prevail during the growing season (Adipola *et al.,* 1993,: Tilahun *et al.,* 2001). The presence of NCLB in Ethiopia was first recorded in 1952 in Kefa, Harerge, Shoa and Sidamo provinces but the economic importantance once was first reported by Mengistu (1990). The disease was found as low altitude at 500 m in the Gambella plain and high altitude at 2800 m in the Bale highlands (Assefa, 1995). In Ethiopia, out of many diseases identified on maize, NCLB is among the major foliar diseases of maize. Currently, NCLB can cause yield losses of 49.8%, as estimated on a susceptible variety under high disease pressures (Tilahun, 2001).

Robert (1952) studied the cultural and pathogenecity variability among isolates of corn leaf blight fungus, *Helminthosporium turcicum*, and found them to differ in virulence, but the differences were not uniform from year to year. The fungus is known to be highly variable in cultural characteristics and pathogenicity. Knox-Davies and Dickson (1960) reported sufficient evidence of heterokaryons and their perpetuation through the conidia and suggested that the high variability in the fungus population might be related to heterokaryosis. Previous studies by Merle *et al.* (1957) also indicated that seasonal variability could be major influenced factor in the screening of disease resistant variety. High seasonal variability might result variation in the pathogenecity of the causal organism. The variations among isolates could be due to variations in the resistance of the host plant and differences in the environment or from interaction among these variables. Adiopla *et al.* (1993) observed a similar response among NCLB resistant Uganda maize cultivars. Reactions to NCLB were clearly different in field trials in Uganda when NCLB was severe, but the reaction of most resistant cultivars could not be differentiated when conditions were less conducive for the development of NCLB. Jenkins *et al*. (1957) worked on leaf blight infection of 58 inbred lines for 4-6 years and could artificially induce an epidemic. It was determined that environmental condition was a major source of variation. The work of Assefa (1995) in Ethiopia indicated that there were significant differences among *E.turcicum* isolates in their virulence. Correlation analysis showed that the mean virulence rating was highly correlated with the mean lesion number and lesion type. Mean of virulence rating indicated that there were significant differences among *E.turcicum* isolates and were significantly correlated with spore length and rate of spore germination. Leavy (1991) showed that isolates from the difference areas were different in parasitic fitness as indicated by infection efficiency, sporulation and lesion size, while isolates of some locations showed less variation.

Gambella is one of the regional States in Ethiopia and located in western part of the country adjacent to the Republic of Sudan. It is characterized by ample precipitation, high humidity and warm temperature. These climatic conditions and the cultural practices in the region like zero tillage and continuous cropping system are favorable for the growth of the fungus. In the region, infection of the disease occurs before flowering for the late sown crop (personal observation). The surveys conducted in the past in order to determine the incidence of NCLB on maize crop in Gambella showed infection rates as high as 96% (Assefa, 1995).

Despite of the high NCLB incidence in Gambella regional State, no attempts have been made so far to characterize the variations among isolates in cultural and morphological characteristics, and the information on their reaction to different maize genotypes has been lacking. Therefore, the main objectives of the present work were to study the variation in cultural and morphological characteristics among isolates in the Gambella region and to examine their

pathogenecity among isolates of different origin, and finally to evaluate the variation on reaction of different maize genotypes to pathogen.

MATERIALS AND METHODS

Collection of isolates: Roadside surveys and collecting of maize leaf sample that showed the symptoms of NCLB was done in 2004 in the major maize growing areas of Western and South Western Ethiopia. A total of 70 leaf samples were collected and put in paper bags that were finally air dry and kept at room temperature for laboratory investigations at Holetta Agricultural Research Center (HARC).

Isolation of the fungus: Single conidial isolations were made by incubating infected leaves in a moist chamber for 72 hours under fluorescence light and single conidial selection was done with a sterile blood lancet using dissecting microscope. The single spores were first transferred to slant agar and maintained on PDA and then kept in a refrigerator to further study.

Morphological characteristics: Seventy pure culture isolates of *Exserhilum turcicum* that were identified for morphological variations such as shape, size of the conidia, and the number of septa. The size (length by width) of conidia was also measured (μm) with on ocular micrometer using a binocular compound microscope. Two hundred and ten conidia of 70 isolates were calculated for the average and range of size.

Cultural characteristics: The variations in cultural characteristics of *E.turcicum* were investigated by selecting 28 isolates of fungus. Each isolate was plated on potato dextrose agar (PDA). The qualitative features included, sporodochium production, colony color, and pigmentation of the culture after 9 days culturing. The colony color described in reference to standard color chart (Kornerup and Wanscher, 1967). The quantitative of cultural characteristic, conidial growth rate, of 28 isolates were investigated by plating conidia suspension on PDA in Petri dish and they were incubated for 9 and 13 days. Length of conidia was measured by micrometer and rate of length increased was calculated from three conidia of each isolate.

Pathogenicity variation: Eleven maize varieties were obtained from Bako National Maize Research Center and Gambella Research Center after previous field evaluations. Twenty isolates of *E.turcicum* were used for studying the pathogenic variation in the fungus population. From each variety, 4 seeds were placed in sterile soil in plastic pots. The seedlings were grown in the green house until the full emergence of the fourth leaf (4weeks old). The seedlings in each pot were thinned to two plants per pot in order to have healthy plants. Fertilizer and water were applied as needed to maintain optimum moisture for seed germination and growth of seedlings in the green house. Each treatment was arranged in a complete randomized block design. Disease reaction was evaluated 15 days after inoculation based on the reaction as suggested by Hooker (1961, 1963). Two selected lesions per test plant were determined after 15days as reported by Leath and Pedersen (1986). The reactions were classified as resistant or susceptible depending upon lesion length and width (cm).

Inoculation: Pure cultures of 20 isolates were grown for three weeks on potato dextrose agar in 90 mm Petri dishes. Concentrated inocula were prepared by washing conidia including the agar medium form 21 day old cultures that were grown in an incubator. Spore concentration was not standardized since a qualitative difference reaction among isolates was the principal criterion to be determined. Two to three drops of Tween 20 were added per liter of inoculum as suggested by Warren (1975) and spraying was made in the late afternoon when the plant reached leaf stages four to five or when the plant was four weeks old (Jeffers and Chapman, 1995).

Data analysis: Fifteen days after inoculation, lesion length was measured and subject to statistical analysis. The average lesion lengths were then categorized into a 1-5 scale in order to perform a cluster analysis for identifying the similarity of virulent patterns among isolates. For this analysis, a similarity matrix was derived with the Simqual Program (NTSYS pc. Version 1.7) using a simple matching of coefficients of similarity. A dendrogram was produced by the unweighted pair group method for arithmetic average (UPGMA) in the SAHN program.

Table 1 Occurrence and severity of NCLB in different maize growing Gambella and oromiya zones and regions in 2004.

Region	Zone	District	No. of leaf samples	NCLB severity**	NCLB severity**
Gambella	Zone I	Jikawo	8	High	High
		Itang	5	High	High
		Sub total	13 (18.6%)		
	Zone II	Abobo	5	High	High
		Gambella	5	High	High
		Pundgo	10	High	High
		Sub total	20 (28.6%)	High	High
Oromiya	Illuabbabor	Bure	2	High	High
		Sibu	2	High	High
		Uka	2	High	High
		Gore	2	High	High
		Metu	1	High	High
		Urumu	2	High	High
		Yayo	1	High	High
		Chora	2	High	High
		Dedesa	4	Moderate	Moderate
		Bedele	2	Moderate	Moderate
		Sub total	20 (28.6%)		
	East Wollega	Nekemte	5	High	High
		Sire	1	Moderate	Moderate
		Sayo	1	High	High
		Sub total	7 (10.0%)		
	West Showa	Bako	4	High	High
		Bako-Tibe	2	Moderate	Moderate
		Gudar	1	Low	Low
		Ambo	1	High	High
		Dandi	2	Moderate	Moderate
		Sub total	10 (14.3%)		

** Turcicum leaf blight severity rating by % infected leaf = $\frac{(\text{No of infected leaf } 100)}{\text{Total leaf}}$

>40% = incidence high, 20-30% = moderate and ≤ 10% = low severity

RESULTS AND DISCUSSION

Occurrence of NCLB in Ethiopia 2004. Survey of the occurrence and severity of NCLB in maize growing zones of Ethiopia conducted in 2004 and results are presented in Table 1. Seventy isolates were collected and isolated from infected leaf samples and it was revealed that NCLB was widely distributed in Gambella Regional State and western and southwestern zones of Oromiya Regional State. The survey was conducted in the areas at the altitudes ranging from 500 m at Gambella plain to 2500 m. at West Showa zones (Table1). The severity of disease seemed to follow the pattern of altitude and relative humidity that it was higher and more severe at lower altitudes and where higher relative humidity prevailed. About 70% of the leaf samples collected from these areas were infected by NCLB. The disease severity exhibited low incidence at locations with higher altitudes such as in East Wollega and West Showa zone, while the severity was consistently high in all districts of Gambella and low altitudes of Illuababor zone. Hence, the wide distribution and high severity of NCLB in Gambella could be attributed to its ample precipitation, high humidity and warm temperature. Moreover, the cultural practices in the region like zero tillage and lack of crop rotation were favorable for stimulating or predisposing the growth of the fungus.

Table 2 Proportion of conidial shapes, colonial gross and colonial color of *E.turcicum* isolates collected from different locations of western and southern Ethiopia in 2004.

Character types	Number of isolates	Percent of total isolate
Conidial shapes		
- Elongated	27	38.57
- Curved	27	38.57
- Spindle	16	22.86
Sub total	70	100
Colonial gross (dense)		
- Raised	6	21.42
- Apprised	10	35.74
- Sparse	6	21.42
- Tiny	6	21.42
Sub total	28	100
Colonial color		
Obverse side		
- Olivaceous gray	8	29.03
- Olivaceous brown	8	29.03
- Olivaceous	12	41.49
Sub total	28	100
Reverse side		
- Gray	15	53.20
- Green white	13	46.80
Sub total	28	100

Variation on morphological characteristics:

Shape and size of conidia: Morphological characteristics of *E.turcicum* as described by its shape and size of conidia varied among the 70 isolates collected from the different areas (Table 2). After conducting single conidia isolation, a pure culture of each isolate was cultured on potato dextrose agar and then the morphology of the conidia was observed under light microscope. The results indicated that the shape of conidia was distinctively elongated for some while others were curved and spindle shaped. In the present study, the curved (38.57%) and elongated (38.57%) shape of the conidia dominated in the populations of the fungus.

Variation of conidial size, in length and width and the number of septa of 210 conidia samples from 70 isolates of *E.turcicum* was studied. The results indicated an average size of conidia to be 92.68 μm in length and 15.46 μm in width and the number of septa averaged 5.3 septa (range 2-7 septa). The statistical analysis indicated the normal distribution of conidial width and length, and the number of septum and value of standard deviation (SD) and variance (S^2) indicated the variation of morphological characteristics among 70 isolates of *E.turcicum*.

Variation on cultural characteristics: Pure cultures of 28 isolates were investigated on cultural characteristics by categorizing the nature of the colony appearance on the medium. The results showed that the colonial growth raised, appraised, the colony density sparse and tiny were 21.42, 35.74, 21.42, and 21.42% of total isolates, respectively (Table 2). The color of the isolates generally belonged to the olivaceous color group including olivaceous gray and olivaceous brown, and on the reverse side of the fungus, pigments varied from gray to green-white was produced in the agar center (Table 2). Conidial growth rate was investigated by culturing conidia of 28 isolates on PDA and lengths of conidia were measured under microscope at 9 and 13 days after culturing. Statistical analysis indicated that there was a significant difference ($p = 0.01$) in conidial growth length among isolates of *E.turcicum*. The average conidial growth in length of the fungus isolates were 6.74 μm and 10.40 μm at 9 and 13 days of incubation, respectively (Table 3). The conidial growth rate was calculated based on rate of conidial length increased at one day intervals (Table 3). The average rate of increase ranged from 0.03-2.35 μm per day. Mean conidial growth rate of *E.turcicum* population was 0.92 μm per day. Isolates which collected from Gambella region (PUN1.1 and PUN1.2) produced significantly lower growth rate(r) recorded 0.03 and 0.22 μm respectively than isolates from Illubabor zones (BUR) which was recorded 2.35 μm of conidial growth rate.

Variations among isolates in morphological characteristics such as conidial shape and size, and cultural features such as colony growth rate and pigmentation were presented. Except in growth rate, variations occurred independently to the origin. Among tested isolates for growth conidia of per day, almost 65% of the isolates belonged to Gambella. Isolates which were collected from this area were slow growing and this was perhaps due to the low room temperature at Holleta (as compared with warm climate at Gambella) which did not favor conidial growth of those isolates. This investigation supported by different investigators such as Merle *et al*. (1957), Jenkins *et al*. (1957), Adiopla *et al*. (1993) concluded that the high variability of the environment might result in variation in the pathogenecity and this could lead to high variability in causal organism.

Variation on Pathogenicity: Twenty isolates that were tested for pathogenicity on eleven maize varieties in the greenhouse were pathogenic to all tested varieties and exhibited highly significant difference among isolates, maize variety and isolates by variety interactions (Table 4). Twenty representatives of *E.turcicum* isolates were found to be pathogenic on seedlings of 11

maize varieties but with certain variation in lesion size. There were two contrasting lesion types of disease reaction were observed. In green house inoculation with *Exserohilum turcicum* at 4-5 leaf stage, the initial symptoms appeared as minute white to light green fleck 7-10 days after inoculation, usually simultaneously on both resistant and susceptible leaves. On resistant

Table 3 Means of conidial length and conidial growth rate (R) μm of 28 isolates *E. turcicum* grown on PDA at two growth periods.

	Conidial length (μ) at		
Isolates	9 Days	13 Days	R
BUR-2	7.48 a[1/]	9.69 b	0.55
PUN2.2	8.73 a	11.38 ab	0.66
BUR-3	8.01 a	17.40 a	2.35
NEW-1	7.81 a	9.69 b	0.47
PRE-1	5.60 ac	10.34 b	1.19
AMB-95	8.73 a	10.25 b	0.38
PUN1.4	5.54 ac	9.30 b	0.94
JEJ-1	8.60 a	10.06 b	0.37
GIN-98	5.15 ac	8.93 b	0.95
CHO-73	8.60 ac	10.51 b	0.48
SIB-1	6.43 ab	7.17 b	0.19
ELI-3	4.41 a	10.06 b	1.41
DED-2	6.95 a	10.50 b	0.89
PUN1.1	7.16 a	7.27 b	0.03
GOR	8.66 a	10.03 b	0.34
PUN1.2	8.53 a	9.40 b	0.22
MEN14.1	7.16 a	12.04 ab	1.22
BAR-002	7.79 a	11.48 ab	0.92
GIN-99	8.00 a	12.18 ab	1.05
CHO-71	7.74 a	11.94 ab	1.05
PUN1.3	5.08 ac	11.66 ab	1.65
BAT-1	5.79 ac	10.81 ab	1.26
ITA	8.00 a	10.34 b	0.59
ELI-3	8.13 a	10.52 b	0.6
PUN3.1	2.62 bc	7.63 b	1.25
GAM	4.64 ac	10.81 ab	1.54
NEK	1.99 c	8.34 b	1.59
GAM-3	5.26 ac	11.56 ab	1.58
Mean	6.74	10.40	0.92
Median	7.32	10.34	0.93
Mode	7.16	9.69	1.05
SD	1.858	1.930	0.547
S^2	3.453	3.725	0.299

[1/] Figures with the common letters are not significantly different at P = 0.01 probability level of significance

Table 4 Average lesion sizes (cm^2) produced by 20 *E.turcicum* isolates on eleven maize varieties after 14 days of inoculation.

	Varieties											
ISOLATE	Ab0bako	BH-140	BH-540	BH-660	BH-670	GIBE	GUSSAU	GUTTO	LOCAL-M	KULENI	QPM	MEAN
BURE-1	1/ 0.73a-j[1/]	0.73a-j	0.33a-f	2.20p-w	0.13ab	0.10a	2.20p-w	1.11a-k	1.66c-n	0.89a-k	0.68a-j	0.97 [2/]
PUN2.1	2.00j-w	0.70a-j	0.75a-j	1.50a-m	1.30a-l	1.63b-w	1.97a-m	1.33b-n	2.00a-k	0.58a-j	0.86a-k	1.32
BUR	0.78a-k	0.80a-j	0.93a-k	1.13a-k	1.00a-k	1.13a-k	1.86a-n	1.23a-n	1.86a-n	0.66a-k	0.90a-k	1.11
NEW	3.00 wx	2.66v-x	0.86a-m	0.53a-j	0.20a-i	0.23a-d	1.66a-m	0.20a-i	1.66a-k	0.77a-k	1.66a-m	1.39
PRE	0.86a-m	2.66v-x	0.86a-n	0.66a-k	0.66a-k	1.36a-k	1.77a-k	2.00j-w	1.83a-n	1.06a-m	0.66a-j	1.24
GIN-98	2.00j-w	2.00j-w	0.86a-k	0.66a-k	2.40a-m	3.00w-x	1.73d-w	2.06d-n	1.80e-w	0.72a-m	1.68a-m	1.71
BED-70	2.00j-w	1.26a-m	1.60a-m	1.68a-k	1.68a-k	2.36r-w	1.54a-m	1.33a-m	1.33a-m	0.58a-k	1.66a-m	1.28
PUN1.1	1.16a-l	1.13a-k	1.33a-m	1.33a-m	1.06a-k	2.36r-w	1.49a-m	0.66a-k	1.33a-k	0.54a-k	1.86a-m	1.29
PUN1.5	1.40a-m	0.88a-k	1.69d-n	0.45a-i	1.11a-w	1.46a-m	1.49a-m	1.50a-m	1.50a-m	1.62b-w	2.00l-w	1.37
BAD3.2	0.93a-k	0.94a-m	1.45a-i	0.46a-k	0.46a-m	0.93a-s	1.50a-m	1.46b-n	1.46b-n	0.60a-k	0.96a-k	1.01
GAM-2	1.78e-n	0.86a-k	1.62b-n	1.18a-m	1.33a-m	1.00a-k	1.52a-m	1.50a-m	1.80e-w	0.73a-j	0.73a-k	1.44
NEK-81	1.40a-m	0.70a-j	0.86a-k	1.56a-n	1.93h-w	2.16u-y	1.50a-m	0.16a-c	1.50a-m	0.54a-j	0.97a-k	1.20
GAM-1	3.73y	0.66a-j	0.90a-k	1.00a-k	2.60t-x	1.60a-n	1.58a-l	1.16a-l	2.13n-w	0.66a-i	1.24a-m	1.56
ELI-4	0.40a-g	1.04a-k	0.36a-f	0.63a-g	0.26a-d	1.60a-k	0.26a-d	0.56a-j	0.50a-j	0.33a-f	0.49a-j	0.58
ITA1.2	2.00j-w	1.10u-k	0.52a-f	0.33a-f	0.93a-s	0.66a-j	2.16u-y	1.10 a-u	2.16 0-w	0.64a-i	0.74a-k	1.12
BAT	0.30a-e	0.53a-j	1.53a-m	0.73a-k	0.93a-s	2.66v-x	2.16u-y	1.37am	2.160-w	0.84a-k	0.80a-k	1.27
PUN3.1	1.33a-m	2.00l-w	1.06a-k	0.36a-g	1.10a-u	0.93a-s	1.84f-w	1.40a-v	2.10e-w	0.85a-k	0.80a-k	1.25
PUN1.3	3.83xy	3.83xy	0.36a-k	0.60a-g	1.10a-u	1.16a-u	1.71f-n	1.33a-m	1.80e-w	0.30a-e	1,63b-w	1.60
GIN-1000	1.80a-k	1.06a-k	0.36a-g	0.73a-j	1.00a-k	4.50y	2.16u-y	1.10a-u	2.160-w	0.44a-i	2.00j-w	1.57
GOR	0.43a-j	0.70a-j	2.00j-w	0.41a-h	2.44s-w	2.64u	2.16u-y	0.80a-k	2.160-w	0.38a-g	0.93a-k	1.36
MEAN	2.89	1.29	0.93	0.91	1.19	2.89	2.91	1.17	2.62	0.69	1.12	

[1/] in a column, means follow by common letters are not significantly different at 5% level of probability

[2/] the lesion size data measured in the greenhouse represent the mean of three replications.

leaves, lesions were smaller and slightly elongated chlorotic resistance type of lesions. Susceptible plants expressed lesion that were elongated grayish-green-white spot to tan necrotic lesions showing no evidence of chlorosis. Susceptible lesion varied in size averaging 2.91, 2.89, 2.89 to 2.62 cm^2. Some varieties like Local-M, Gussau, Gibe and Gutto showed significantly ($p<0.05$) higher lesion sizes of 1.75, 1.68, 1.57 and 1.46cm^2 respectively (Table 4). Similarly, significantly ($p<0.05$) smaller lesion size 0.69 cm^2 was recorded for seedling of variety Kuleni.

E.turcicum isolates, PUN1.3 and GIN-98 caused higher mean lesions of 1.60 and 1.57 cm^2, respectively, than isolate ELI-4 with 0.59 cm^2. The isolate GIN-98 was the most aggressive one on three maize varieties whereas, isolate ELI-4 was non aggressive or week to all varieties (Table 4). Isolate BAD3.2 was less aggressive on varieties BH-140 and Abobako than isolate PUN 1.3 on the same variety. However, BAD3.2 and PUN 1.3 had less aggressive or week virulence on varieties BH-660 and Kuleni. Isolate BAD3 and PUN-1 was moderately aggressive on most varieties.

Statistical analysis indicated that there was variation in pathogenicity among isolates tested on 11 maize varieties. Higher value of variance (S^2) and standard deviation indicated higher variation occurring among lesions size produced by 20 isolates of *E.turcicum*. From table 5, it indicated that Kuleni variety was the most resistant to 20 isolates of *E.turcicum* due to all statistic indicators which were lowest value such as SD and S^2 means of 0.69, 0.292 and 0.085, respectively. As compared to variety Abobako, it was the most variability on pathogenic to all isolates of *E.turcicum* in this test. Although mean of lesion size was not the highest but other statistic indicators were highest such as SD and S^2 1.057 and 1.118 respectively.

The virulent phenotypes of the 20 isolates *E.turcicum* were divided into 5 groups. The results indicated that there were five virulence patterns exhibited on 11 maize varieties after inoculating with 20 isolates of *E.turcicum* (Table 5). The most virulent isolates GAM 1 and PUN 1.3, GIN-100 that were highly pathogenic on Abobako, BH-140 and GIBE. The other groups of isolates exhibited a lower virulence and a virulent pattern on 20 maize varieties.

Based on lesion size data of *E.turcicum* isolates on 11 maize varieties, cluster analysis of virulence data with UPGMA revealed 5 clusters with similarity coefficient of 0.57 (Figure 1). Cluster I contained one isolate, NEW, causing an average lesion size of 1.39 cm. Cluster II containing isolate GOR, caused average lesion size 1.36cm, otherwise, isolate GOR in cluster II was most aggressive as compared to isolate NEW in cluster I due to a larger number of susceptible varieties were obtained. Cluster III contained 4 isolates, showing the same virulence pattern on the same varieties, but different reactions to different varieties. Cluster III was identified as moderate in virulent pattern. Cluster IV contained 12 isolates collected from different locations in Ethiopia. There was wide variation in lesion size which rangned from 1.01cm to 1.71cm in the cluster. Isolates in cluster IV fell into subgroups. Isolates BED -70 and PUN1.1 separated from others at the similarity coefficient of 0.91 because of its virulent reaction on all maize varieties except Kuleni. Cluster V contained two isolates BURE-1 and ELI-4 that exhibited weak or non-virulent patterns on most varieties tested.

CONCLUSION

For cultural characteristics, there was variation in conidial growth rate among isolates of *E.turcicum*. The fungus islolates could be divided into 2 groups, slow and fast growth rate. Most of isolate grouped into slow growth rate belonged to isolates collected from warm areas. Hence, variation on conidial growth rate like morphology characters were also depended on environment condition.

In this study, virulence patterns were

Table 5 Virulent phenotype pattern of 20 *E.turcicum* isolates on eleven maize varieties after 14 days inoculation.

	Disease reaction [1/]											
ISOLATE	Ab0bako	BH-140	BH-540	BH-660	BH-670	GIBE	GUSSAU	GUTTO	LOCAL-M	KULENI	QPM	Mean
BURE-1	2	2	1	4	1	1	4	3	3	2	2	2.24
PUN2.1	3	2	2	3	3	3	3	3	3	2	2	2.64
BUR	2	2	2	3	2	3	3	3	3	2	2	2.45
NEW	4	4	2	1	1	1	3	1	3	2	3	2.27
PRE	2	4	2	2	2	3	3	3	3	3	2	2.64
GIN-98	3	3	2	2	4	4	3	4	3	2	3	3.00
BED-70	3	3	3	3	3	4	3	3	3	2	3	3.00
PUN1.1	3	3	3	3	3	4	3	2	3	1	3	2.82
PUN1.5	3	2	3	1	3	3	3	3	3	3	3	2.73
BAD3.2	2	2	3	1	1	2	3	3	3	2	2	2.18
GAM-2	3	2	3	3	3	2	3	3	3	2	2	2.64
NEK-81	3	2	2	3	3	4	3	1	3	1	2	2.45
GAM-1	5	2	2	2	4	3	3	3	4	2	3	3.00
ELI-4	1	2	1	2	1	3	1	2	1	1	1	1.45
ITA1.2	3	3	1	1	2	2	4	3	4	2	2	2.45
BAT	1	1	3	2	2	4	4	3	4	2	2	2.55
PUN3.1	3	3	3	1	3	2	3	3	4	2	2	2.64
PUN1.3	5	5	1	2	3	3	3	3	3	1	3	2.91
GIN-100	3	3	1	2	2	5	4	3	4	1	3	2.82
GOR	1	2	3	1	4	4	4	2	4	1	2	2.55
MEAN	2.89	1.29	0.93	0.91	1.19	2.89	2.91	1.17	2.62	0.69	1.12	2.57

[1/] = Disease reactions were categorized according to lesion size:
1 = highly resistance (HR) with lesion size 0.0-0.5 cm^2
2 = Resistance (R) with lesion size 0.6 – 1.0 cm^2
3 = moderately resistance (MR) with lesion size 1.1 – 2.0 cm^2
4 = Susceptible (S) with lesion size 2.1 – 3.0 cm^2
5 = highly susceptible (HS) with lesion size more than 3.0 cm^2

identified patterns by using cluster analysis to compliment the phenetic or DNA finger print. Cluster analysis of virulence data with UPGM revealed 5 clusters, with similarity coefficient of 0.57.

Wide variations among the fungus populations of *E.turcicum* were found. NCLB was a common disease of maize in Ethiopia and most maize genotypes lacked an adequate level of resistance to the pathogen. Hence, the maize breeding program at Ethiopia should aim at developing resistance genotypes using virulent isolates, together with a mixture of isolates, in order to test the disease interactions and select for maize genotypes.

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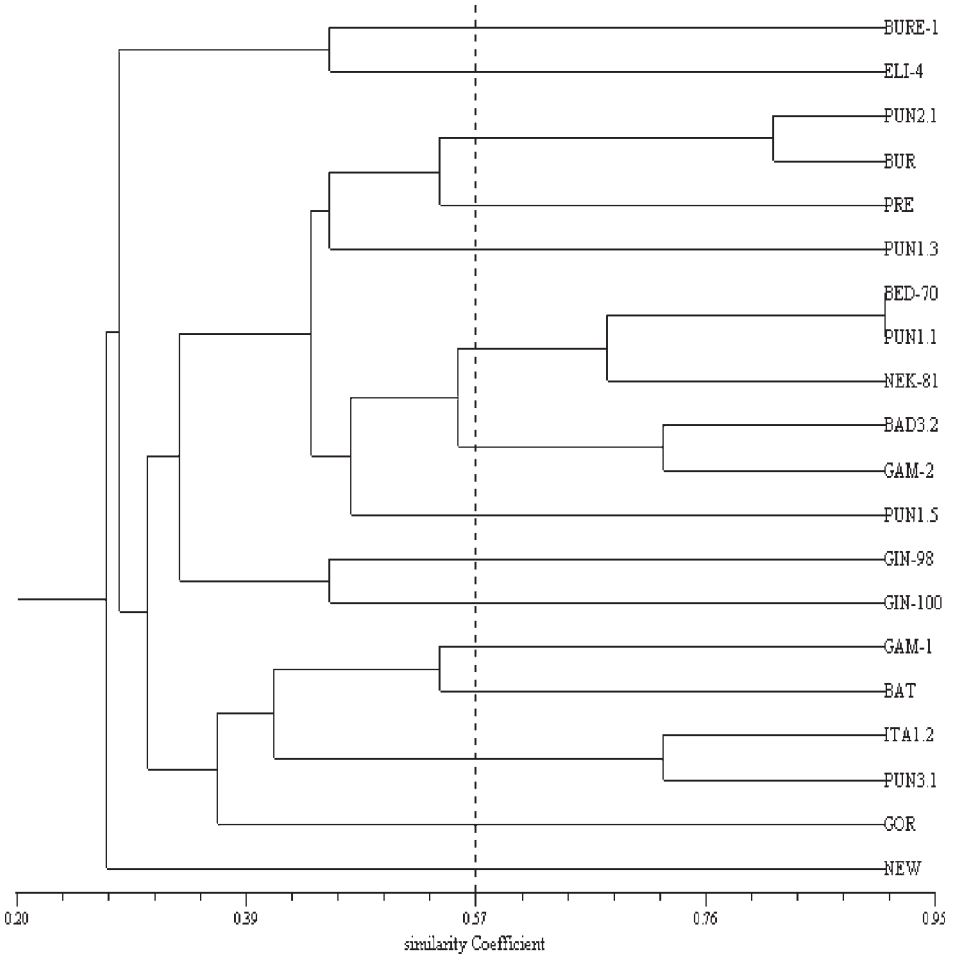

Figure 1 Dendrogram showing virulence similarity and successive clustering of 20 isolates of *E.turcicum* on11 maize varieties.

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