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Disease Intensity and Distribution of *Exserohilum turcicum* Incitant of Northern Leaf Blight of Maize in Tanzania

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ABSTRACT

Maize (Zea mays L.) is widely cultivated across regions in Tanzania. To understand the relationship between maize and epidemics of northern leaf blight, surveys were conducted in 2012 and 2013. Disease incidence and percent severity were determined in 280 farmers' field. Each sampled point measuring about one acre at intervals of 500 meters was marked with global positioning system and used to construct the disease map. Data was subjected to ANOVA and means separated with Turkey's-kramer simultaneous test at P≤ 0.05. Northern leaf blight (NLB) disease was found in all the fields studied, however the level of virulence differed. The disease incidence (57.5 - 96 %) and percent severity (33.4 – 62.2 %) were significantly more severe in humid highlands of Mbeya and Arusha districts compared to incidence (11.8 - 61.3 %) and severity (3.9 – 34.5 %) in the lowland dry Coastal and Morogoro districts. Significant correlation coefficients (p ≤ 0.05) and coefficient of determination (R^2 = 0.36 and R^2 = 0.38) between disease incidence, percent severity and environmental factors confirmed that NLB spread in maize was proportional to high altitude, low temperature, high relative humidity and rainfall.The results from this study suggested that (i) Mbeya was a hotspot area for NLB and therefore can be used to screen germplasms for resistance to NLB, (ii) host resistance and susceptibility was influenced by location and environmental factors and (iii) frequent surveys in the studied areas and beyond will be imperative to identify source(s) of resistance and pathotypes/races of the pathogen in Tanzania.

Key words: *Maize, northern leaf blight, Exserohilum turcicum, distribution, environment, Tanzania*

INTRODUCTION

The northern leaf blight (NLB) of maize (Zea mays L.), also known as *Turcicum* leafblight is a fungal disease caused by *Exserohilum turcicum* (Pass.) K.J. Leonard and E.G. Suggs. The pathogen was formerly known as *Heliminthosporium turcicum* Pass[25,33]. Northern leaf blight disease is a major foliar disease of maize in most production areas worldwide[2,7]. It is more prevalent in humid areas with moderate temperatures[35,43]. The disease is widely distributed, however sporadic in nature and mostly depends on weather conditions, stage of plant growth and level of resistance in maize cultivars[46].

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The pathogen has wide host range and a high pathogenic variability[2,19,32,65]. However the epidemics originate from soil borne clamydospores, mycelia or conidia on an infected crop residue[26].

The disease attacks all parts of the plant but the most conspicuous lesions are found on the foliage. Lesions destroy the leaves, resulting to yield losses due to lack of carbohydrate to fill the grains. Heavily infected fields present a scorched or burnt appearance resulting in premature death of leaves[9,21]. Northern leaf blight causes extensive leaf damage and defoliation during the grain filling period[8,52]. Yield losses due to necrosis or chlorosis of leavespremature death of the leaves[7] and loss of nutritive value even as fodder[23,45] have been reported. The disease also reduces sugar content and viability of maize seeds[6] as well as predisposes the crop to stalk rots[17]. Yield losses of up to 28 to 91 % due to NLB have been reported[7,16,20], mostly when heavy infection occurred before tasselling[8].

Host plant resistance and tolerant hybrids have been reported to be the most efficient and sustainable approach for management of northern leaf blight[18,51].Such maize varieties delayed onset of the disease and reduced severity at crop maturity. However, the pathogen is known to produce new races that can overcome existing resistance[47]. Hence the alternative option to the rapid breakdown of resistance is to cultivate varieties that can withstand resistance for a long time in an environment favorable to NLB. Field sanitation and tillage ensures adequate handing of infected crop residue and deep ploughing[12]. Such measures reduced initial inoculum of the pathogen and incidence of early season disease development[28,43]. Adjusting date of planting have also been reported effective in the management of northern leaf blight disease of maize[15,17,37,39,48,55]. Crop rotation using soybean, beans and sunflower effectively reduced the NLB disease of maize[18,28,43]. Fungicides have been reported to inhibit growth of *E. turcicum*and development *in vitro* and *in vivo*[18,30,42,44,45,53,58,59].

Although the use of fungicide in the management of northern leaf blight has not been reported in Tanzania, evidence of indiscriminate use of fungicides which resulted to resistance to the fungicides and reduction of natural enemies of the pathogen hasbeen reported[68].

The search for effective options for managing the disease has recently focused on the cultural practices, host genetic resistance and the use of botanicals. These control measures are non- phytotoxic, biodegradable, do not have residual effect and are easily absorbed by the plants and cost effective[24,29,56,57].

It is not clear when northern leaf blight was first identified in Tanzania, but field surveys showed that the disease was widespread in maize fields and prevalent in the major maize zone of Northern and Southern Highlands and Lake Zones[5,38]. Since then little is known about the disease, but production growth of maize was estimated at 4.6 % with 2.4 % attributed to increased land area. Despite efforts by limited resources farmers' who are the major producers, average yield of maize remains low, fluctuates within 1.3 to 1.5 t/ha or less[31,54]. Nkonya*et al*.[38] referred *E. turcicum* as important disease and estimated 45% economic injury level (EIL) in Tanzania. Although empirical information on distribution of NLB of maize is not available in Tanzania, reports on severity and distribution are available from neighboring countries of Kenya, Uganda, Ethiopia, Zambia and South Africa[67]. This investigation therefore examined the current incidence, severity and distribution of NLB disease in maize in Tanzania.

MATERIALS AND METHODS

Study areas and sampling systems

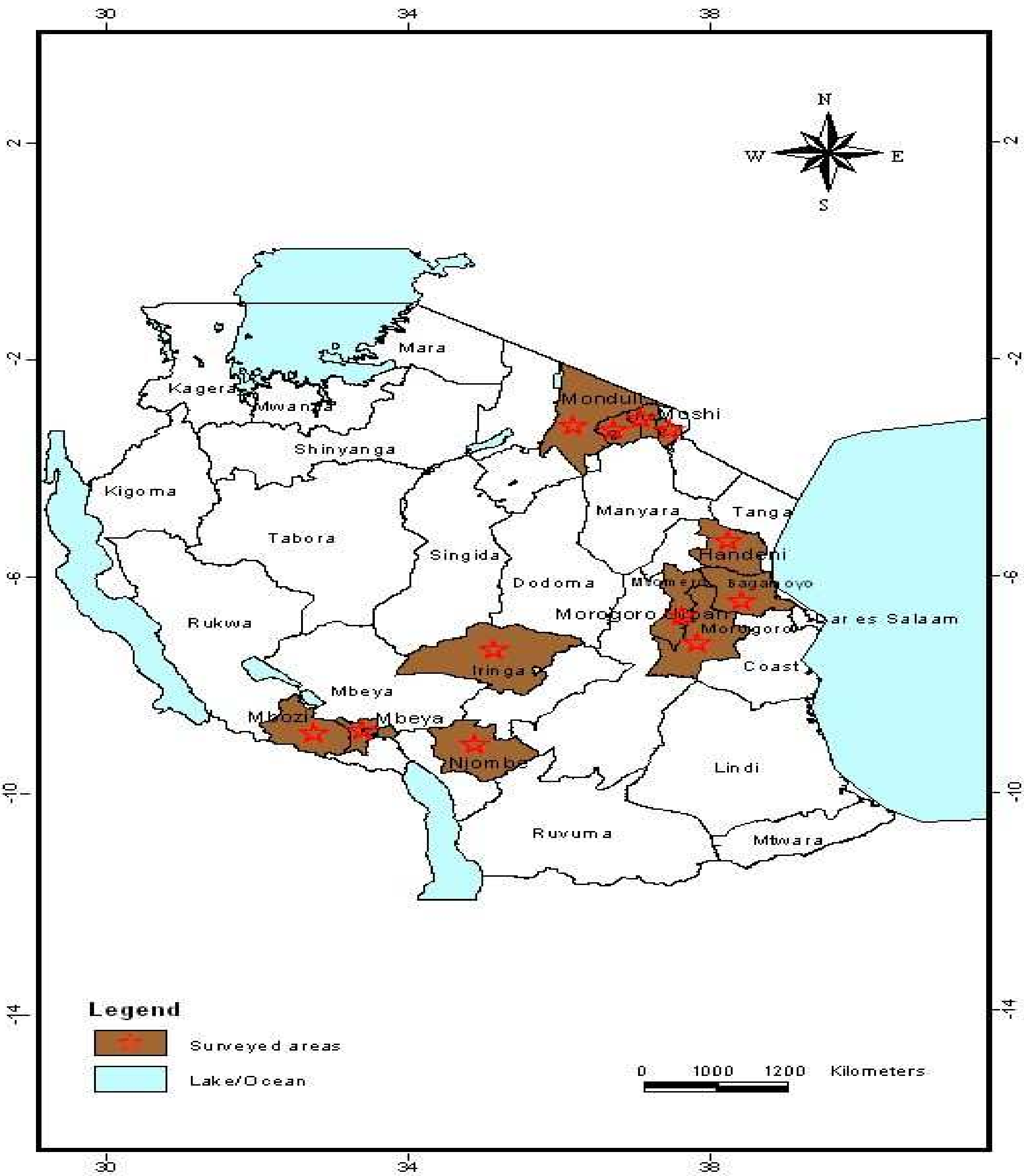
Field surveys were carried out in two growing seasons of 2012 and 2013 in Arusha, Kilimanjaro, Morogoro, Iringa, Mbeya and Coastal regions of Tanzania to determine the incidence and severity of northern leaf blight of maize.The regions and districts were based on differences in farming systems, soil factors, weather and climatic conditions, altitude and major vegetation cover[14,51,64]. However, some farming systems tend to overlap between districts.

A total of 280 fields were surveyed at silking stage of the crop. Two districts and a minimum of two villages were purposefully selected per region (Table 1).From each village, twenty fields, each averaging one acre in size were randomly selected at minimum distance of 500 meters interval. Each sampling point was marked with global positioning system (GPS) for altitude and co-ordinates.

Table 1: Surveyed areas for northern leaf blight disease and their altitudes in Tanzania

S/No	Region	District	Village	Altitude
1	Coastal area	Bagamoyo	Chalinze, Chahua, Pera	186.8
		Handeni	Kwabaya, kwamasaka	661.2
2	Morogoro	Urban/Rural	Pangawe,Mikese,Mkambarani	453.4
		Mvomero	Makuyu, Dibamba	421.1
3	Mbeya	Mbozi	Senjere	1442.7
		Uyole rural	Uyole	1843.8
4	Iringa	Iringa rural	Ismani, Kiponzilo	1409.5
		Njombe	Kisilo, Magoda	2134.1
5	Arusha	Urban	Ngaramtoni	1550.5
		Monduli	Ngarash, Sinon	1491.4
6	Kilimanjero	Hai	Nshara	1018.8
		Moshi	Himo, Uchira, Marangu	966.2

Fig. 1: Surveyed districts for northern leaf blight of maize in 2012 and 2013 growing seasons in Tanzania



In each field, 9 m² plots were marked out in three randomly selected points in a diagonal form using meter tap.Ten stands of maize in the middle of each plot were randomly selected and assessed for incidence and severity. Each plot was regarded as a replicate for disease assessment.

Assessment of northern leaf blight

Disease incidence was assessed as the proportion of plants showing symptoms in the field. The number of the ten randomly selected stands showing NLB symptoms were counted and expressed as a percentage of the total number of stands per plot. Based on the prevalence, incidence was categorized as described by Harlapur[18], where, 0-5 % = slight/trace infection, 5.1-15 % = light infection,15.1-30 % = moderate infection, 30.1-75 = severe infection, >75 = very severe infection. Disease severity on whole plant basis was rated using a visual scale of 0-5; where 0 is free from disease and 5 is completely blighted leaf.Scores for NLB were as follows, 0 = leaves free from infection, 1 = a few restricted lesions on the lower leaves (≤ 5 %), 2 = several small and large lesions on many leaves (5.1-10 %), 3 = numerous small and large lesions on many leaves (10.1-25 %), 4 = many enlarged and coalesced lesions on many leaves above the cob (25.1-50 %), 5 = several coalesced lesions, leaf showing wilting, tearing and blotching typical blight symptoms (> 50%)[10,13,33].

Severity scores were converted to percent disease index (PDI)[3,63]using the formula below;

$$PDI = \frac{\text{Sum of numerical grading}}{\text{Leaves examined} \times \text{maximum disease grade}} \times 100$$

Arbitrary graduation of 10 class scale, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5,4.0, 4.5 and 5.0 for accurate NLB severity[22]. Varieties with ratings; 0-1.4 = resistant (< 30%),1.5-2.4 = moderately resistant (≤ 50 %)and2.5-5 = susceptible (> 50 %)[35].Data on weather parameters (maximum, minimum temperatures, relative humidity and rainfall) were obtained from Tanzania Meteorological Agency and used to determine the relationship between disease incidence and severity in the regions.

Data analysis

Combined analysis of variance(ANOVA), simple correlation analyses (r) and coefficient of determination (R^2) of the two year data on the incidence and severity were analyzed usingGenStat 14th Edition. XLSTAT 2015 version software was used to display the correlation between incidence and percent disease severity of northern leaf blight and weather parameters. Means with significant differences were compared using Turkey's-kramer simultaneous test for data at $P \leq 0.05$[60]. Disease map and distribution were analyzed using Arcview GIS version 3.2.

RESULTS

Incidence and severity of northern leaf blight on maize

Combined/pooled analysis indicated significant differences ($p \leq 0.05$) in disease incidence and percent disease severity within regions and districts as well as the interaction and year (Table 2). Northern leaf blight was found in all the maize fields visited, however degree of the disease varied from one location to another (Table 3).

The incidence of NLB significantly ranged from 11.8 % (Bagamoyo district) to 93.8 % in Mbeya Rural district with average incidence of 59.5 %. The percent disease severity varied from 3.9 % to 62.2 % in Bagamoyo and Arusha Municipal districts, respectively, with mean of 31.4 %. Results also showed that disease incidence in highlands of Arusha Municipal, Mbozi, Mbeya Rural districts and PDI for Arusha Municipal and Mbeya Rural districts (Table 3) were significantly higher. Although Iringa region is within the Southern highlands (Table 3), low percent disease index (14.3 – 26.5 %) observed in the two districts may be attributed to relatively high minimum (16°C), maximum temperature (27°C) and moderate relative humidity (Table 4).

Table 2: Combined analysis of variance (ANOVA) for incidence and percent disease severity of northern leaf blight of maize in six regions of Tanzania during the 2012/2013 growing seasons

Source of variation	d.f.	s.s.	m.s.	F[a]	F pr.
Disease incidence					
Region	5	560920.3	112184.1	256.95	<.001
District	1	43450.1	43450.1	99.52	<.001
Region.District	5	221758.7	44351.7	101.58	<.001
Year	1	20626.7	20626.7	47.24	<.001
Residual	1425	622161.9	436.6		
Total	**1439**	**1469213.3**			
Percent disease index					
Region	5	346376.9	69275.4	270.15	<.001
District	1	11055.6	11055.6	43.11	<.001
Region.District	5	117652.1	23530.4	91.76	<.001
Year	1	15405.6	15405.6	60.08	<.001
Residual	1425	365412.3	256.4		
Total	**1439**	**855958.2**			

[a]*Statistical significant differences = p ≤ 0.05, DF degree of freedom; SS sum of squares; MS mean squares*

Table 3: Incidence and percent disease index of northern leaf blight of maize in major maize growing districts in Tanzania

			Incidence (%)			Percent disease index (%)		
S/No	**District**	**Altitude**	**2012**	**2013**	**Pooled**	**2012**	**2013**	**Pooled**
1	Bagamoyo	186i	11.5d	12.2f	11.8f	3.2f	4.5f	3.9f
2	Handeni	661g	56.5b	66.2bc	61.3bc	33.4c	35.6bc	34.5c
3	Morogoro rural/urban	453h	36.8c	45.5d	41.2de	15.8de	21.6e	18.7e
4	Mvomero	421h	43.2c	50.5d	46.8d	15.7de	19.1e	17.4e
5	Mbozi	1443d	85.5a	91.0a	88.3a	52.0b	55.7a	53.8b
6	Mbeya rural	1840b	91.5a	96.0a	93.8a	61.7a	61.0a	61.4a
7	Iringa rural	1494cd	38.2c	46.0d	42.1d	11.2ef	17.3e	14.3e
8	Njombe	2134a	60.5b	75.7b	68.1b	21.7d	31.2cd	26.5d
9	Arusha Municipal	1550c	86.5a	92.2a	89.3a	60.4ab	63.9a	62.2a
10	Monduli	1523c	56.2b	57.5cd	56.4c	34.8c	27.5cde	33.4cd
11	Hai	1064e	34.7c	30.2e	32.4e	14.1de	23.3de	18.7e
12	Moshi	966f	39.5c	49.7d	44.6d	19.4de	44.1b	31.7cd
	Mean	1162 ±9.9	53.5±2.2	59.5±2.7	56.3± 1.9	28.9±1.6	34.1±2.1	31.4±1.5
	CV	8.3	39.5	34.9	37.7	53.8	47.2	52.1

Means followed by the same letter in the same column are not significantly different according to Turkey's 95 % level of confidence.

Distribution of northern leaf blight of maize in Tanzania

Disease incidence, percent disease severity and GPS records were used to construct disease map in districts. Results showed spatial pattern of epidemics with the highest incidence (Fig. 2) and Percent disease severity (Fig. 3) recorded in the Highlands of Mbeya and Arusha districts (red) while the lowest disease incidence and disease severity index were recorded in the lowland Bagamoyo district (green), Coastal area of Tanzania. The high disease incidence and severity index in the highlands of Mbeya, Mbozi and Arusha may be due to conducive climatic conditions (Table 4) and adoption of susceptible varieties (Nwanosike unpublished). Although NLB disease incidence indicated severe infection (Fig. 2) in Handeni, Moshi and Monduli districts, the severity index were moderately low (Fig.3). Generally, incidence and severity was severe in the highlands as compared to relatively dry lowland regions.

Fig. 2: Incidence of northern leaf of maize in Tanzania

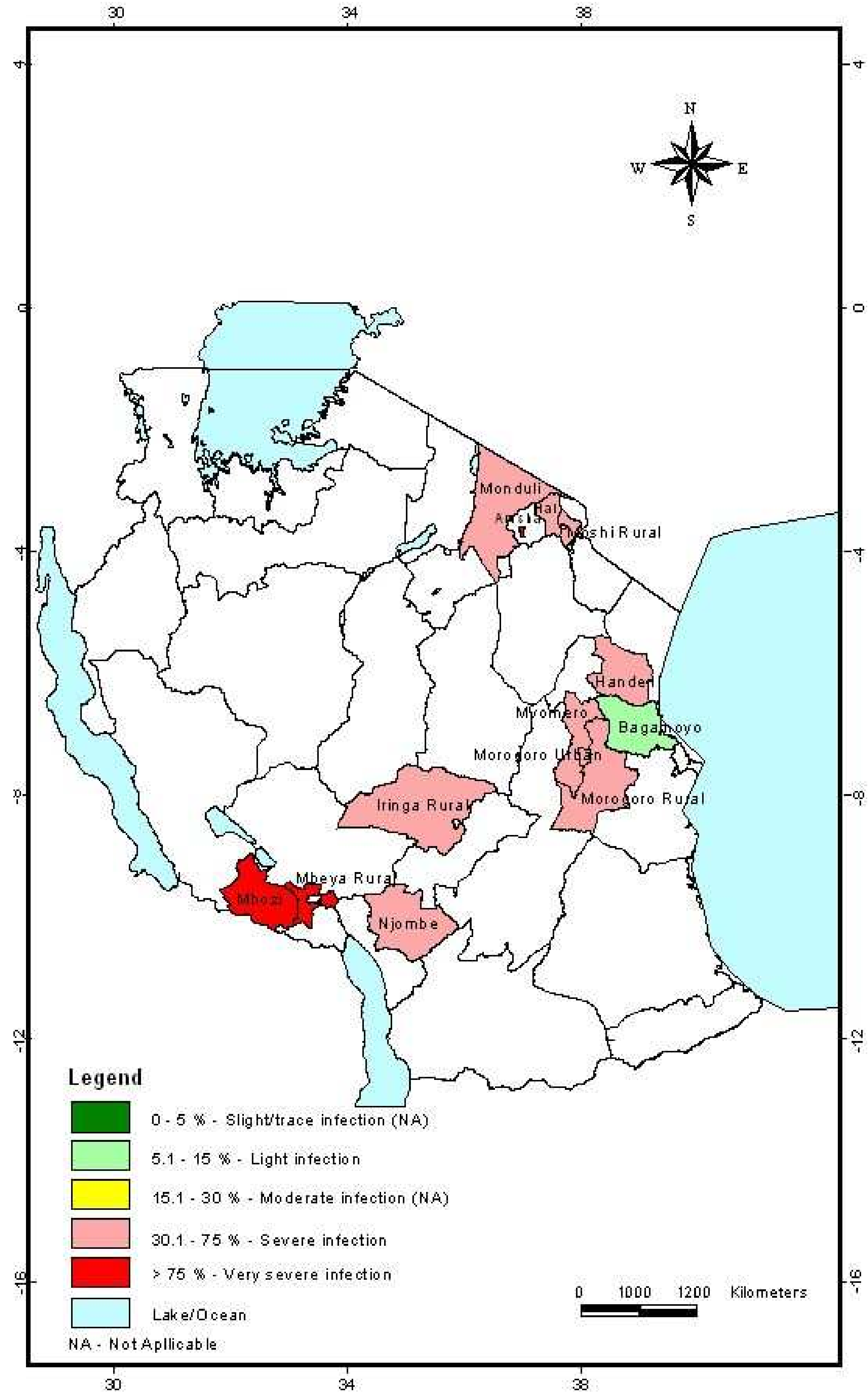
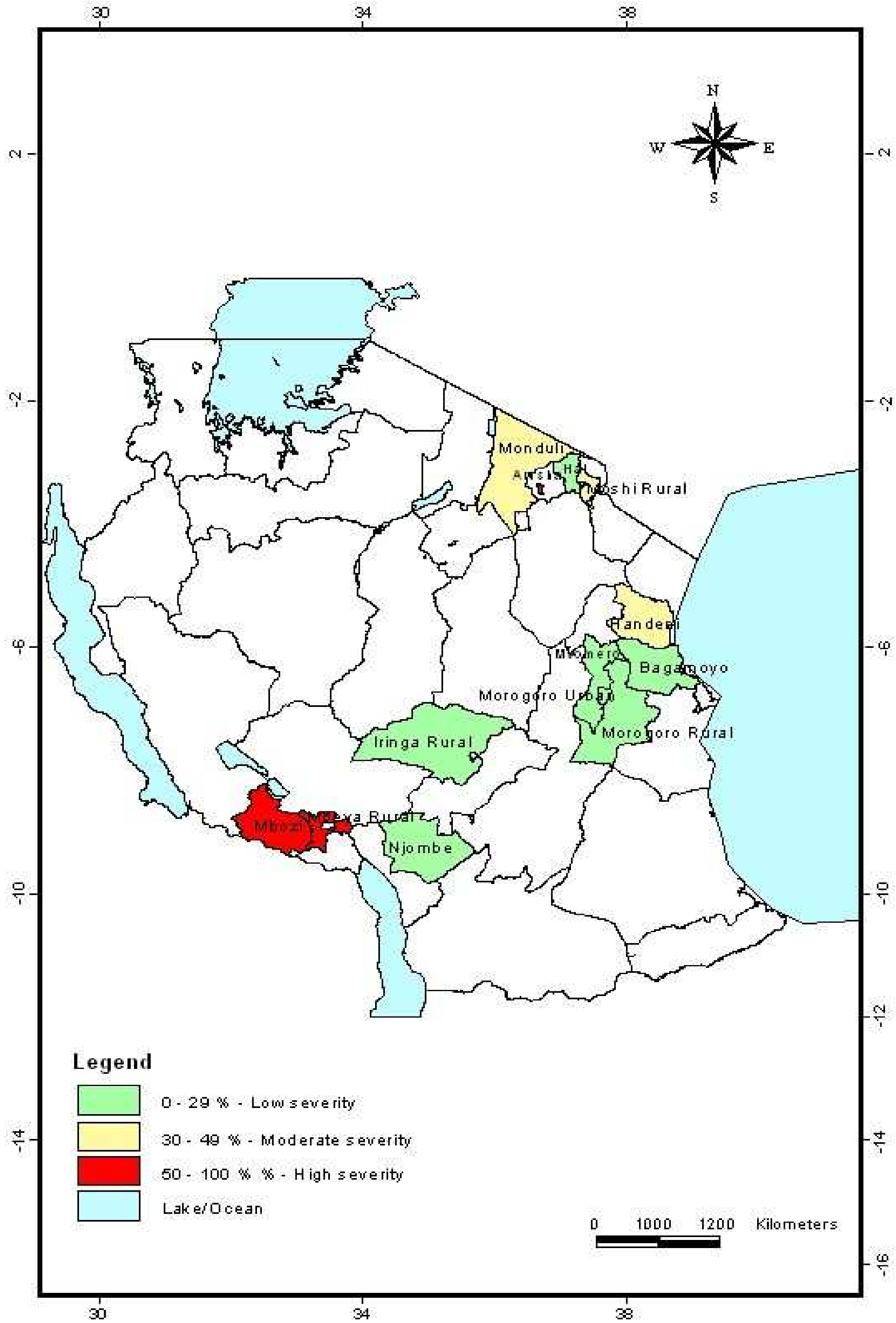


Fig. 3: Percent disease index of northern leaf of maize in Tanzania



Effect of weather parameters on development of northern leaf blight

Results showed that increased maximum, minimum temperatures and decrease in relative humidity reduced infection of maize by *E. turcicum* in Morogoro, Iringa, Moshi and coastal areas. Data also revealed that low maximum, minimum temperatures and increased relative humidity favoured infection of northern leaf blight in Mbeya, Mbozi and Arusha districts. Infection was also observed to increase with increased rainfall. Although cumulative rainfall (447.2 mm) was high in the Coastal areas, the high temperature (21-31.7°C) may have been responsible for low disease incidence and percent severity in the Coastal areas (Table 4). Generally, NLB disease in maize was favoured by maximum temperatures of 24.1-24.9°C, minimum temperatures of 13.7-15.9°C, relative humidity of 67-85.6 % and rainfall range of 325.3-679.2 mm during the growing seasons.

Table 4: Mean weather reports, incidence and percent severity of northern leaf blight of maize during 2012 and 2013 growing seasons in Tanzania

Region	Max. temp	Min.temp.	RH	RF	Incidence	% severity
ARUSHA	24.9	15.9	85.6	325.3	72.9	47.8
MOROGORO	30.1	19.7	60.0	251.1	44.0	18.0
MBEYA	24.1	13.7	67.0	679.2	93.5	57.6
COASTAL AREA	31.7	21.0	77.2	447.2	36.6	19.2
IRINGA	27.3	16.0	65.5	347.9	55.1	20.4
KILIMANJARO	28.3	19.5	62.9	400.7	38.5	25.2
Mean	27.7	17.6	69.7	408.6	56.8	31.4
Std dev.	2.9	2.8	9.7	148.4	22.4	17.0

Results showed significant correlation coefficient ($p \leq 0.05$) between NLB incidence, severity and weather parameters (Table 5).It was found that altitude, relative humidity and rainfall increased with PDI and disease incidence. Results also revealed that disease incidence and PDI increased with reduced maximum and minimum temperatures. This is evident in the negative significant relationship (Table 5). Regression analysis also indicated strong relationship between environment factors and NLB development. The coefficient of determination ($R^2 = 0.360$, $R^2 = 0.382$) between weather parameters, NLB incidence and percent disease index explained that 36 % disease incidence and 38 % percent disease index were associated with altitude and climatic factors among other factors (Table 6).

Table 5: Correlation coefficient between northern leaf blight of maize and weather parameters in 2012 and 2013 growing season

Variables	Incidence	Severity	Altitude	Max.temp.	Min. temp.	RH	Rainfall
Incidence	1	0.857**	0.530**	-0.565**	-0.456**	0.157**	0.362**
Severity		1	0.445**	-0.563**	-0.420**	0.243**	0.407**
Altitude			1	-0.814**	-0.545**	0.135**	0.302**
Max.temp.				1	0.551**	-0.213**	-0.434**
Min.temp.					1	-0.488**	-0.705**
RH						1	0.010

***significant at $p \leq 0.01$Maximum and minimum temperature (°C), Rainfall (mm), Relative humidity, Incidence and severity (%)*

Table 6: Multiple regression coefficient of determination (R^2) for weather parameters in relation to northern leaf blight incidence and percent disease index in Tanzania

Weather parameter	Disease incidence (%)			Percent disease index (PDI) (%)		
	Slope	SE	t value	Slope	SE	t value
Altitude	0.010	0.002	4.458***	0.005	0.002	2.964**
Max.temp.	-3.634	0.482	-7.536***	-3.465	0.362	-9.581***
Min.temp.	-1.791	0.739	-2.425*	2.955	0.554	5.335***
RH	0.024	0.114	0.215	0.735	0.085	8.637***
Rainfall	0.021	0.009	2.199*	0.069	0.007	9.830***
	$R^2 = 0.360$			$R^2 = 0.382$		

****significant at $p \leq 0.000$,**significant at $p \leq 0.01$, * significant at $p \leq 0.05$Maximum and minimum temperature (°C), Rainfall (mm), Relative humidity (%), Figure in bracket represents the constant.*

DISCUSSION

Northern leaf blight was widely distributed in all the maize fields studied, however the levels of virulence varied. Earlier reports have shown that northern leaf blight disease had a nationwide distribution with disease severity over 45%[4].Extreme impact of NLB on maize in the highland agro ecologies have also been reported in Uganda, Kenya, Ethiopia and Zambia and South Africa[49].

The NLB disease incidence of 36.6-91 % and severity index of 18.0-57.6 % were relatively high in the highlands of Mbeya and Arusha districts compared to Morogoro and Coastal regions, indicating higher resistance to NLB in the maize genotypes cultivated in the lowland dry areas. The high incidence and severity index in Mbeya and Arusha may be attributed by favourable climatic conditions (Table 4), high altitude (Table 2), susceptible maize varieties grown and possibly disease pressure from the available pathotypes of the pathogen. It is therefore clear that Mbeya is a hotspot for northern leaf blight of maize, therefore could be used for screening germplasms for resistance against the disease.

The study agreed with Adipala*et al*.[1] and Ramathani *et al*.[51] who reported prevalenceof NLB in highlands and wetter areas of the Kenya and Uganda. Previous reports have also shown that *E. Turcicum* is a serious disease in highlands associated with cool, high relative humidity, mid-altitudes and cloudy weather conditions[27,35,40,41]. Similar spatial pattern of *E. turcicum* epidemics was reported by Ramathani[50] where NLB severity varied from 8 % in warmer zones to 60 % in humid areas in Uganda. Mwangi[36] reported that high annual rainfall of about 1100 mm with high humidity and cool temperatures of between 11 to 27°C create ideal conditions for infection and dispersal of *E. turcicum* in Kenya.

Muiru*et al*.[34] reported that consistent cultivation of susceptible maize varieties resulted to 90-100 % NLB prevalence and incidence 10-90 % in different agro ecological zones in Kenya. Zadok and Schein[66] also reported that host-pathogen interaction is a reflection of human activities. This may also be advanced for high infection of NLB the highland districts. Personal observation showed that sole cropping, continuous cropping and poor stover management were commonly practiced in Mbeya and Arusha. Such cropping and management system increases inoculum reserviour and transfer to subsequent seasons[62].

Planting of maize and sorghum genotypes in the same farming system together with related and wild plant species growing within or adjacent to agricultural fields have been reported as source of new fungal inoculum and reservoirs for constant presence of *E. turcicum*[11,51,61]. Similar field observations were made in Kwabaya village, Handeni district, where farmers intercropped and cultivated maize adjacent to sorghum fields. Such cropping pattern in a mid altitude (661 masl) Coastal area may be responsible for the severe incidence (61.3 %) of NLB.

The positive significant correlation coefficients ($P \leq 0.05$) between altitude, rainfall and relative humidity and negative significant correlations between minimum, maximum temperature and NLB disease incidence and severity index indicated that environmental factors were directly related to the development of the disease in maize. The significant regression coefficients also confirmed strong relationship between environment factors and NLB development. The coefficient of determination (R^2) explained that 36 % disease incidence and 38 % disease index were associated with altitude and climatic factors. Findings agreed with Harlapur*et al*.[18] who reported positive significant relationship between cumulative rainfall, relative humidity and disease intensity and significantly negative correlations between minimum and maximum temperature and disease intensity. This study also confirmed the reports of Pandurangegowda *et al*.[42], Harlapur*et al*.[20] and Rai and Kumar[48] who reported that frequent rainfall, high relative humidity and low temperature favoured vertical and horizontal spread of NLB on maize.

The study established that NLB of maize is widely distributed, and the level of intensity varied based on location in Tanzania. Among other factors, environmental factors (altitude, temperature, relative humidity and rainfall) were significant in the spread of the disease in maize. Being the first extensive surveys on northern leaf blight of maize in Tanzania, there is a need for successive survey in all the maize producing areas to understand the disease change over time, identify sources resistant genotypes and the pathtypes/ available in Tanzania. The later will facilitate design for sustainable management strategy for the disease.

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