



## Original article

# Inheritance of resistance against northern leaf blight of maize using conventional breeding methods



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## ABSTRACT

Maize (*Zea mays* L.) is one of the important cereal crops along with wheat and rice worldwide. The purpose of this study was to use classical genetic approaches to assess the resistance of various maize parents and hybrids to the northern corn leaf blight (NCLB) disease in two different locations in Egypt. Eight parents, 28 F1, and 2 check hybrids were evaluated. The analysis of variance showed high significant variations between maize parents and their hybrids for the studied parameters and NCLB disease, besides there are significant variations between both locations. Results of maize parents showed that Sids 63, Giza 602, and Giza 628 cultivars exhibited the highest values and were resistant to NCLB in both locations comparing with Nubaria 39 and Gemmiza 18 that were susceptible to NCLB disease. Concerning the maize hybrids, analysis of variance and mean squares of growth characters in both locations indicated high significant variations between the maize hybrids including the check hybrids. When combined between the two locations for current parameters against NCLB, the data pointed that the Sakha location values for maize hybrids were much closed to the combining data in parents and the hybrids detected high resistance to this disease comparing with Nubaria location. All tested maize lines (38 lines), including parents and hybrids were classified as follows, two lines were rated as 1 (highly resistant), three were rated as 2 (resistant), sixteen were rated as 3 (moderate resistant), eight were rated 4 (moderately susceptible) and nine were rated 5 (susceptible). The data explaining that the crossing between high resistant maize cultivars produced high levels of resistance to NCLB disease. Therefore, our results verified that classical breeding could efficiently increase the resistance levels of maize germplasm against NCLB disease by developing new cultivars with superior performance in terms of grain yield, disease resistance and grain quality.

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## 1. Introduction

Maize (*Zea mays* L.) is the world's most productive crop most productive crop, with 1.12 billion metric tons harvested in 2018/19 (USDA/IPAD 2020). According to projections, production will increase by more than 183 million metric tons over the next decade (OECD/FAO 2019), in order to meet rising demand for global food, feed, and fuel. Maize demand was expected to rise by 50% to over 800 million tons per year by 2020 (Pingali and Pandey 2001; Fajemisin 2003; Grote et al. 2021). Maize is an important crop for human consumption, particularly in developing countries, that can represent up to 65% of the total calories and 53% of the

protein (Atlin et al. 2011). Maize is distributed worldwide and is the world's third highest-produced cereal (Magenya et al. 2009; Sharma and Misra 2011; Wani et al. 2018).

Northern corn leaf blight (NCLB) of maize is the major problems in the late summer and is one of the important foliar diseases of maize in temperate areas of the world, including Egypt (Lawry et al. 2009). The NCLB losses can range from 15 to over 60% worldwide, especially in tropical and subtropical environments (Romero 2016; Bindhu et al. 2017). Similarly, NCLB infections can reduce silage digestibility and predispose to stalk rot, posing a significant threat to seed growers and farmers (Galiano-Carneiro et al. 2021). NCLB disease occurs sporadically in most temperate, humid areas where maize is grown (Lim 1974; Scrivener et al. 2001). The NCLB disease symptoms primarily appear on the leaves and plants might be infected at any growth stage, but usually at or after anthesis (Wilcoxson 1996; Scrivener et al. 2001; Sharma et al. 2015).

In Egypt, NCLB disease is mostly found in the northern and north-western regions of the Delta in late summer (off-season) maize planting, due to suitable weather conditions that prevail at this time of year (Pugh et al. 1980; Mellor and Boorman 1995; Sehgal et al. 2001). Moreover, sprinkler irrigation systems, which are widely used in the newly reclaimed lands, provide high air moisture in the field throughout the season, increasing the disease incidence in these areas (Raymundo et al. 1981; Elnahal et al. 2020). In Egypt, maize leaf blight caused a significant loss in grain yield, estimated at 30% or more in the Northern Delta (Barakat et al. 2009). In addition to the use of unimproved production technologies and lack of access to modern farming techniques (Elrys et al. 2019; Atallah and Yassin 2020). High temperatures and humid tropical conditions in some African regions have caused some maize diseases and pests prevalent that have a significant effect on maize yield (Fajemisin 2003; Abdelsalam et al. 2019a).

The fundamental basis of plant breeding is the selection of specific plant traits considered important by plant breeders. Classical breeding involves crosses among selected parents that have desirable traits such as high yield and/or disease resistance (Visarada et al. 2009). Selection of superior plant characteristics involves visual valuation; thus the breeder's skills lie in selecting the greatest plants with desirable recombinant characteristics from the large segregating offspring populations (Visarada et al. 2009; Ulukan 2011; Ahmed et al. 2020). Morphological markers provide an extensive data estimation of genetic diversity and the levels of genetic variation in maize to identify elite inbred lines that can be crossed to create superior hybrids (Smith and Smith 1989; Karanja et al. 2009; Atallah et al. 2021). Maize breeding relies on the available genetic diversity which can be manipulated to achieve the maximum heterosis estimation in hybrid breeding programs (Karanja et al. 2009). The main objective of this study is to assess 8 maize parents and their 28 hybrids against NCLB disease at two different locations in Egypt. The novelty of the current investigation was achieved by introducing novel maize cultivars with high quality and disease resistance traits, thus validating the importance of conventional breeding programs as the base of genetic improvement compared with the other modern techniques. Accordingly, the maize genotypes have been investigated in the current regions and as a result new sources of disease resistance along with high yield were reported.

## 2. Materials and methods

### 2.1. Experiment location and maize cultivars

The experimental research was carried out at two different location in Egypt *i.e.* Nubaria (N 31° 11' 48.0732", E 29° 53' 33.0216") and Sakha (N 31° 5' 22.7", E 30° 57' 2.8"), Agricultural

Research Station, Agricultural Research Center (ARC), Egypt, during two harvest seasons. Eight maize inbred lines were obtained from Maize Research Section, Nubaria Research Station, Field Crops Research Institute, Agricultural Research Center, Egypt were used in this study Table S1.

### 2.2. Evaluation experiments and diallel crosses

Eight maize parental inbred lines were sowing at Nubaria location to primary assessment and diallel crossing to obtaining 28 hybrids as half diallel. Two filed evaluation experiments were carried out in two different locations. The first experiment was at Nubaria location, El-Bohura governorate, Egypt and the environmental conditions of Nubaria location makes it an infected area of leaf blight so the infection is certainly for sensitive varieties. Plants were naturally infected with northern leaf blight disease. The second experiment was at Sakha location, Kafer El-Sheikh governorate, Egypt that already famous as infected area during this time (planting date from 10 June) at this location. Planting date was intentionally chosen to coincide with the time of maximum natural infection and to coincide with the ideal growth stage for infection with the most suitable environmental condition for spreading the casual fungus. Surface irrigation was applied to meet the crop requirement and to assure the proper atmospheric humidity around plants to enhance fungal infection.

Each experiment included 38 entries were as follows; 8 parents' lines; 28 F<sub>1</sub> and two check hybrids (Single cross 10 and 29) planting together in Nubaria and Sakha location, respectively. Physical, chemical and nutritional characteristics of the experimental soils are found in Table S2.

A randomized diallel block design (RCBD) with four replications was used at each location. Plot size was one row = 6 m long and 0.8 m width. Planting was done in hills spaced 25 cm apart within row and further seedling were thinned to one plant per hill at thinning each trial received 30-unit P<sub>2</sub>O<sub>5</sub>/feddan before planting, while feddan equal 4200 m<sup>2</sup>. Nitrogen fertilizer was applied in the form of Urea (46%) and ammonium nitrate (35.5%) at the rate of 120-unit N/feddan and used into three equal doses before the three irrigation times. The first irrigation was applied two weeks after planting and the following irrigations were applied every 10–12 days. All other agricultural practices were applied at the proper time. In the experiment artificially infected with northern leaf blight pathogen. However, leaf blight and other diseases were avoided through disease control practices by 2 sprays by the fungicide *Dithane M-45*. The first spray was applied after four weeks of planting and the second spray were applied after 10 days of first spray.

### 2.3. Percentage of NCLB infected plants

Infected plants with NCLB disease were recorded after 45 days of artificial infection date at the disease nursery only and estimated according to the following formula according to (Zhang et al. 2013). Data of northern leaf blight infected plants were adjusted by adding a constant number (0.5) to each value percentage and data were transformed into square roots for statistical analysis. Presentation of such data, however, will be in the original percentages.

$$\text{Diseased Plants (\%)} = \frac{\text{No of infected plants/plot}}{\text{Total no of plants/plots}} \times 100$$

### 2.4. Morphological characters

The following parameters were measured in the current study. Plant height (cm) was measured from the ground level to the base

of the flag leaf based on mean of ten guarded plants from each plot according to (Gyenes-Hegyí et al. 2002). Days to 50% silking was determined as the number of days from planting to silking of 50% of plants (Gyenes-Hegyí et al. 2002). Ear height (cm) was measured from the ground level to the upper bearing node of the same plants used in measuring plant height. A sample of ten random ears from each plot was used to determine the following trait (Woodle et al. 2008). Ear length (cm) was calculated according to (Nawar and Khamis 1983). The number of rows/ear was recorded before harvest at maturity stage, only ears containing 10 kernels or more were included in the count (Nawar and Khamis 1983) and the ear diameter (cm) was calculated as well. The number of grains/row was calculated from complete grain sample at maturity stage (Steevensz et al. 2013). The weight of 100 kernels (g) was also determined from the same sample according to (Severini et al. 2011). In addition, grain yield (ardab/feddan) was measured and adjusted to 15.5% grain moisture then converted to grain yield in ardab/feddan (ardab = 140Kg) according to (Abdelsalam et al. 2019a).

### 2.5. Isolation and identification of pathogenic fungi

Damped-off maize plants were collected from Nubaria location. The diseased plants were cut into small pieces of 0.5 cm, cleaned completely with tap water, surface sterilized by soaking in a 1% sodium hypochlorite solution for 2 min. then washed numerous times in sterile water and dried among sterilized filter paper. The surface sterilized pieces were plated on potato dextrose agar medium (PDA) supplemented with streptomycin sulphate at the rate of (50 µg ml<sup>-1</sup>) in petri dishes and kept at room temperature, 28C, for 5 days. The isolated fungi were purified by using single-spore isolation and hyphal-tip methods. Pure cultures were full-grown on PDA medium. The fungal isolates were categorized as *Helminthosporium turcicum*. The obtained isolates were identified in Maize Pathology Research Section, Plant Disease Research institute, Agricultural Research Center (ARC), Giza, Egypt, according to (Gillman and Gilbert 1957; Barnett and Hunter 1972).

### 2.6. The artificial infection

The artificial infection was performed to improve the natural infection, via an isolate of *Helminthosporium turcicum* that was a single spore culture grown in petri dishes including potato dextrose agar medium for ten days at 25 ± 2C°. Spore suspensions were prepared by adding sterilized distilled water over fungal growth, which was scraped off, using a sterilized needle. The suspensions were, then, strained through a sterilized cheesecloth. Spore concentration was adjusted at 2.5 × 10<sup>3</sup> spore.ml<sup>-1</sup> using sterilized distilled water. Plants were inoculated at sprayed on the leaves of 45 days old plants growth, in the evening, using a spore suspension. Severity of NCLB, as a percentage of infected plants leaf area (% average lesion size) with NCLB disease were recorded 45 days after artificial infection date at the disease nursery, shown in Table S3, only and estimated according to previous formula according to (Ellison et al. 2005).

### 2.7. Statistical analysis

A randomized complete block design (RCBD) with four replications was used to reveal the significant differences among the parents and their hybrids. The LSD (least significant differences) test was conducted to identify the significant differences among the means at 5% level of probability. Comparison of the mean values is usually calculated after an ANOVA.

## 3. Results

### 3.1. Assessment of parents' growth and yield characteristics

The analysis of variance and mean squares for the growth, yield characteristics of maize parents that have been sown in two different locations, Nubaria and Sakha, were recorded in Table 1. Nine parameters were involved, including plant height (cm), number of days to mid-silking, ear height (cm), ear length (cm), number of rows/ears, ear diameter (cm), number of grains/row, 100- grain weight (g) and Grain yield (ardb/fed). Data clearly indicated high significant variations among the eight tested parents in both locations. In addition, there were high significant variations within locations. Results showed that the mean of squares for Sakha was higher than Nubaria location. When combining between the two different locations, data showed that the parents growing in Sakha location achieved higher values for the growth characters comparing with Nubaria location. Significant effect was detected against NCLB disease between the two different locations for the tested growth parameters.

Data in Table 2 clearly indicated that high significant variations between the eight tested parents and high variation within the two different locations Nubaria and Sakha. Concerning to plant height, there were high significant variations between the tested parents in the two different locations. The maximum plant height value in Sakha was 209.5 cm in (Giza 612), whereas the minimum value was 155.75 cm in (Mo 17). On the other hand, Nubaria location showed lower values since the highest average were 152.5 cm in (Sids 7) and the lowest value was 108.75 cm in (Giza 628). When combined data of plant height with the mean NCLB score in Table 5, Giza 612 showed the highest average value of 168.5 cm with a moderate resistance (MR) reaction type against NCLB disease, whereas Sids 63 was the lowest one with an average 140.38 cm though it regarded as a highly resistant (HR) cultivar.

Data for number of days to mid silking showed high significant variations between the tested parents in both locations. Fortunately, data detected high relationship between the number of days to mid silking and the infection by NCLB disease. The maximum value in Sakha location was 69.50 days in Nubaria 39, whereas the minimum value was 58.0 days recorded by Sids 63. While date of Nubaria location showed the highest value in Giza 602 with 73.25 days comparing with the lowest value of 65.0 in Sids 63. Accordingly, the lowest value were recorded by Sids 63 that showed highly resistance toward NCLB disease.

Concerning to ear height, data indicated that the maximum value in Sakha location was 104 cm (Giza 612) and the minimum value was 80.5 cm in (Sids 63 and Gemmiza 18). Whilst, Nubaria location showed lower values regarding ear height. In addition, Giza 628 has the highest average of both locations for ear height was 85.63 cm and it was resistant to NCLB disease as shown in Table 5, while lowest average was 70.88 cm recorded by Gemmiza 18 which classified as susceptible cultivar.

Regarding data of the ear length, it was found that maize parents growing in Sakha location achieved the highest values comparing with Nubaria location. The maximum value in Sakha was 19.31 cm in Giza 602 but the minimum value was 9.43 cm in Gemmiza18. While values of Nubaria location were lower than Sakha since the highest value were 16.0 in Nubaria 39 while the lowest value was 11.63 cm by Giza 628. Besides, the highest ear length average in both locations was 16.61 cm recorded by Giza 602, which exhibited resistance against NCLB compared with Gemmiza18 that has the lowest average with 11.22 cm, which has been classified as a susceptible cultivar, as shown in Table 5.

In addition, data in Table 2 showed high significant variations between the maize parents relating to the number of rows per

**Table 1**  
Analysis of variance and mean of squares of morphological traits of different maize parents in Nubaria and Sakha locations.

Mean of Squares Source of Variation	d.f.	Plant height (cm)			No. of days to mid-silking			Ear height (cm)		
		Nubaria	Sakha	Average	Nubaria	Sakha	Average	Nubaria	Sakha	Average
Replicate	3	169.03	524.28	365.05	0.5833	4.92	3.28	152.11	456.03	214.05
Maize Parent	7	677.71**	1354.82**	789.81**	45.29**	51.86**	39.88**	101.75**	311.10**	189.07
Error	21	168.67	1129.95	177.50	2.35	1.80	2.04	80.85	110.39	90.55
Total	31									
		Ear length (cm)			Number of rows/ears			Ear diameter (cm)		
Replicate	3	2.27	2.10	2.07	0.9683	1.79	1.04	0.2395	0.1255	0.2825
Maize Parent	7	7.04**	41.50**	26.55*	10.31**	3.05**	6.24**	0.6307**	0.6333**	0.6588**
Error	21	1.57	10.74	7.09	2.87	1.52	2.05	0.3264	0.2332	0.19285
Total	31									
		No. of grains/row			100- grain weight (g)			Grain yield (ardbs/fed)		
Replicate	3	13.83	14.31	10.22	4.70	7.55	3.80	16.65	4.17	2.77
Maize Parent	7	22.74**	134.59**	66.22**	119.32**	71.87**	56.27**	5.62**	17.01**	4.91**
Error	21	12.89	20.39	5.88	20.53	32.72	13.99	2.14	1.59	0.5894
Total	31									

\*\* = significant at 0.05 levels of probability

**Table 2**  
Mean of morphological parameters of different maize parents in Nubaria and Sakha locations.

Maize parent	Plant height (cm)			No. of days to mid-silking			Ear height (cm)		
	Nubaria	Sakha	Average	Nubaria	Sakha	Average	Nubaria	Sakha	Average
Giza 602	130.75 <sup>b</sup>	190.25 <sup>ab</sup>	160.50	73.25 <sup>a</sup>	67.50 <sup>bc</sup>	70.38	73.00 <sup>ab</sup>	92.75 <sup>ab</sup>	82.88
Giza 612	127.50 <sup>bc</sup>	209.50 <sup>a</sup>	168.50	66.50 <sup>c</sup>	66.00 <sup>cd</sup>	66.25	65.50 <sup>ab</sup>	104.00 <sup>a</sup>	82.88
Giza 628	108.75 <sup>c</sup>	175.75 <sup>ab</sup>	142.25	65.55 <sup>c</sup>	65.50 <sup>d</sup>	65.53	68.00 <sup>ab</sup>	103.25 <sup>a</sup>	85.63
Sids 7	152.50 <sup>a</sup>	164.75 <sup>ab</sup>	158.63	72.25 <sup>a</sup>	68.00 <sup>ab</sup>	70.13	68.25 <sup>ab</sup>	89.50 <sup>ab</sup>	78.88
Sids 63	116.00 <sup>bc</sup>	164.75 <sup>ab</sup>	140.38	65.00 <sup>c</sup>	58.00 <sup>e</sup>	61.50	62.50 <sup>ab</sup>	80.50 <sup>b</sup>	71.50
Nubaria 39	134.25 <sup>ab</sup>	160.50 <sup>ab</sup>	147.38	69.25 <sup>b</sup>	69.50 <sup>a</sup>	69.38	72.75 <sup>ab</sup>	93.75 <sup>ab</sup>	83.25
Mo 17	128.50 <sup>b</sup>	155.75 <sup>b</sup>	142.13	66.25 <sup>c</sup>	64.25 <sup>d</sup>	65.25	75.00 <sup>a</sup>	90.75 <sup>ab</sup>	82.88
Gemmiza18	123.00 <sup>bc</sup>	161.50 <sup>ab</sup>	142.25	72.00 <sup>a</sup>	68.25 <sup>ab</sup>	70.13	61.25 <sup>b</sup>	80.50 <sup>b</sup>	70.88
LSD at 0.05	19.098	49.431	39.18	2.252	1.972	4.20	13.223	15.450	27.99
Coefficient Variation	10.17	19.45		13.65	2.03		9.27	11.43	
	Ear length (cm)			Number of rows/ear			Ear diameter (cm)		
Giza 602	13.90 <sup>bc</sup>	19.31 <sup>a</sup>	16.61	9.45 <sup>abc</sup>	10.38 <sup>bc</sup>	9.92	2.15 <sup>b</sup>	3.69 <sup>a</sup>	2.92
Giza 612	14.33 <sup>ab</sup>	15.15 <sup>abc</sup>	14.74	11.10 <sup>ab</sup>	11.30 <sup>abc</sup>	11.20	3.08 <sup>a</sup>	3.30 <sup>ab</sup>	3.19
Giza 628	11.63 <sup>d</sup>	11.05 <sup>cd</sup>	11.34	11.90 <sup>a</sup>	12.10 <sup>ab</sup>	12.00	2.78 <sup>ab</sup>	3.20 <sup>ab</sup>	2.99
Sids 7	13.00 <sup>bcd</sup>	15.93 <sup>ab</sup>	14.47	10.70 <sup>ab</sup>	11.50 <sup>abc</sup>	11.10	2.75 <sup>ab</sup>	3.75 <sup>a</sup>	3.25
Sids 63	13.83 <sup>bc</sup>	12.43 <sup>bcd</sup>	13.13	11.00 <sup>ab</sup>	9.75 <sup>c</sup>	10.38	3.05 <sup>a</sup>	3.79 <sup>a</sup>	3.42
Nubaria 39	16.00 <sup>a</sup>	14.10 <sup>abcd</sup>	15.05	11.60 <sup>ab</sup>	11.70 <sup>ab</sup>	11.65	3.13 <sup>a</sup>	2.69 <sup>b</sup>	2.91
Mo 17	12.45 <sup>cd</sup>	16.80 <sup>ab</sup>	14.63	9.35 <sup>bc</sup>	12.40 <sup>a</sup>	10.88	2.70 <sup>ab</sup>	3.35 <sup>ab</sup>	3.03
Gemmiza18	13.00 <sup>bcd</sup>	9.43 <sup>d</sup>	11.22	7.00 <sup>c</sup>	11.47 <sup>abc</sup>	9.24	3.50 <sup>a</sup>	2.93 <sup>b</sup>	3.22
LSD at 0.05	1.842	4.820	7.83	2.492	1.870	4.21	0.840	0.710	1.29
Coefficient Variation	23.45	22.69		16.51	11.23		13.16	14.47	
	No. of grains/row			100- grain weight (g)			Grain yield (ardb/fed)		
Giza 602	30.05 <sup>a</sup>	28.70 <sup>ab</sup>	29.38	45.00 <sup>a</sup>	21.82 <sup>bc</sup>	33.41	8.65 <sup>a</sup>	4.47 <sup>bc</sup>	6.56
Giza 612	22.93 <sup>b</sup>	22.60 <sup>bc</sup>	22.77	32.75 <sup>de</sup>	30.15 <sup>ab</sup>	31.45	6.24 <sup>b</sup>	4.07 <sup>bcd</sup>	5.16
Giza 628	27.55 <sup>ab</sup>	31.65 <sup>a</sup>	29.60	34.25 <sup>cde</sup>	30.81 <sup>a</sup>	32.53	6.19 <sup>b</sup>	4.70 <sup>b</sup>	5.45
Sids 7	26.30 <sup>ab</sup>	22.50 <sup>bc</sup>	24.40	37.50 <sup>bcd</sup>	24.99 <sup>abc</sup>	31.25	6.46 <sup>b</sup>	2.91 <sup>bcd</sup>	4.69
Sids 63	26.30 <sup>ab</sup>	25.20 <sup>abc</sup>	25.75	42.50 <sup>ab</sup>	32.70 <sup>a</sup>	37.60	6.55 <sup>ab</sup>	8.06 <sup>a</sup>	7.31
Nubaria 39	23.00 <sup>b</sup>	20.90 <sup>cd</sup>	21.95	29.25 <sup>e</sup>	29.67 <sup>abc</sup>	29.46	4.45 <sup>b</sup>	2.68 <sup>cde</sup>	3.57
Mo 17	27.65 <sup>ab</sup>	15.15 <sup>d</sup>	21.40	40.00 <sup>abc</sup>	25.88 <sup>abc</sup>	32.94	5.66 <sup>b</sup>	3.20 <sup>e</sup>	4.43
Gemmiza18	26.55 <sup>ab</sup>	15.43 <sup>d</sup>	20.99	32.50 <sup>de</sup>	21.53 <sup>c</sup>	27.02	5.67 <sup>b</sup>	2.59 <sup>de</sup>	4.13
LSD at 0.05	5.280	6.641	7.13	6.663	8.412	11.00	2.149	1.854	2.26
Coefficient Variation	16.51	19.83		16.76	16.22		2.22	3.87	

\*\* = significant at 0.05 levels of probability

ear in both location. The maximum value in Sakha was ~ 12 (Mo 17 and Giza 628) whereas the minimum value was 9.75 (Sids 63). On the other side, the Nubaria location showed the lowest values towards the number of rows per ear. When combined the average data of both locations with their reaction against NCLB disease in Table 5, Giza 628 showed the highest average with 12.0 and regarded as a resistant cultivar, meanwhile the lowest average occurred by Gemmiza18 with 9.24 cm that classified as a susceptible cultivar.

For the ear diameter, data indicated high significant variations between the tested maize parents within the two locations. The

maximum value in Sakha was 3.79 cm (Sids 63) and the minimum value was 2.93 cm. While the highest ear diameter in Nubaria location was 3.50 for Gemmiza18 and the lowest value was 2.15 cm in Giza 602. Sids 63 recorded the highest average of ear diameter in both locations with 3.42 cm, which exhibited highly resistance to NCLB whereas Nubaria 39 that was susceptible against NCLB, recording the lowest average, as showed in Table 5.

Also, the other for yield characters of maize parents involved in the number of grains per row, 100- grain weight and Grain yield have been shown in Table 2, indicating high significant variations among the tested parents and the two locations. Concerning the

number of grains per row, it was found that both of Giza 628 and Giza 602 showed the highest average for the No. of grains/row 29.60 and 29.38, respectively. In addition both of them were resistance against NCLB. Meanwhile the lowest average (20.99) was achieved by Gemmiza18, which has a susceptible reaction against NCLB. Moreover, for the 100- grain weight, the highest value was 45.00 in Nubaria location that is recorded by Giza 602 that exhibited resistance against NCLB. Also, the highest average of both locations was 37.60 indicated by Sids 63 which is highly resistant cultivar. The lowest average was 27.02 and 29.46 shown by Gemmiza18 and Nubaria 39, respectively and both were susceptible cultivars against NCLB. On the other hand, concerning grain yield, it was found that Giza 602, grown in Nubaria location, has the highest value with 8.65 while Sids 63 indicated the highest average of both location where both cultivars were resistant and highly resistant cultivars. Nubaria 39 and Gemmiza 18 showed the lowest average with 3.57 and 4.13, respectively, where both cultivars exhibited a susceptible response (S) against NCLB disease.

### 3.2. Assessment of maize hybrids growth and yield characters

The analysis of variance and mean squares for the growth characters of maize hybrids in both location were recorded in Table 3. The data clearly indicated high significant variations among maize hybrids and the two other check hybrids in the two different locations and the analysis of variance with mean squares were high significant with and within the location for the growth characters. The difference between infected and non-infected maize planted recorded in Fig. 1. For combining data in Table 3 to plant height, the average 336.58 cm was closed to the first location Nubaria with a value of 476.80 comparing with Sakha location, 2827.46. While for number of days to mid silking, the combining data, 9.53, was moderate between the two locations and we can say it's close to the Sakha location, 10.52. Finally, data showed the same trend for ear height and the combining data was much closed to Nubaria location. Analysis of variance and mean of squares for grains yield of 28 maize hybrids as affected by locations environment showed high significant variations between the different hybrids and between the different locations.

Data in Table 4a showed high significant variations between the tested maize hybrids in both locations. The plant height in Nubaria location was ranged from 174.25 cm in (Nb39 × Gm18) to 225 cm in (Gz612 × Sd7) by range ~ 50 cm. While, the Sakha location showed highest values in relation to plant height with the highest value were 272.75 cm (Gz602 × Sd63) comparing with the lowest

value 164.75 cm for (Nb39 × Mo17). The range between two different locations was 47 cm when comparing the highest values and was 10 cm on the lowest average. The highest average for plant height was recorded by Sd63 × Gz602 with 241.63 cm followed by Gz628 × Sd63 with 239.63 cm, which were resistant (R) and moderate resistant (MR) against NCLB, respectively, as shown in Table 5. Meanwhile, the lowest average was recorded by Nb39 × Mo17 with 191.13 cm followed by Nb39 × Sd63 with 192.50 cm and both of them were susceptible (S) hybrids against NCLB.

Concerning the number of days to mid silking, the highest average was recorded by Gz628 × Mo17 and Sd63 × Mo17 with 63.25 day and both hybrids were (MR) toward NCLB disease as shown in Table 5. However, the lowest average was recorded by Nb39 × Sd63 with 57.75 day followed by Nb39 × Gm18 with 57.88 day and both of them were (S) hybrids against NCLB disease.

For the ear height, data of Table 4a also showed high significant variations between the tested maize hybrids at the two different locations. The range between the maximum and minimum value in Nubaria location ranged from 100.5 cm in (Nb39 × Gz602) to 127 cm in (Gz612 × Sd63) by range ~ 27 cm. While the Sakha location showed higher values in relation to ear height and the highest value was 163.75 cm (Sd7 × Gm18) comparing with the lowest value of 99.5 cm for (Gz628 × Sd63). The range between both locations was ~ 26 cm when comparing the highest values and was 1 cm on the lowest average. When combined between Nubaria and Sakha location for ear height against NCLB, the highest average was detected by Gz612 × Sd63 with 137.75 cm that has susceptible reaction against NCLB, whereas the lowest average was detected by Nb39 × Sd63 with 102.38 that was susceptible to NCLB disease.

Means of ear length, number of row/ear and ear diameter of different maize hybrids in both locations were shown in Table 4b and the results showed high significant variations between the tested hybrids at both locations for these parameters. First, concerning the ear length, the maximum values were 21.75 and 22.0 cm and the minimum values were 16.1 and 12.85 cm for Nubaria and Sakha locations, respectively. Sd63 × Sd7 (HR) and Sd63 × Gz602 (R) showed the highest average with 21.88 and 21.63, respectively. While the lowest average was described by Gz612 × Sd63 and Nb39 × Gz602 with 14.48 and 18.35, respectively and both hybrids exhibited susceptibility (S) against NCLB disease. Second, data of number of rows/ear showed that the maximum value was in Sakha location was 15.90 recorded by (Gz628 × Gm18) and the minimum value was 12.30 by (Nb39 × Mo17). On the other side, Nubaria

**Table 3**  
Analysis of variance and mean of squares of morphological and yield characters of different maize hybrids as sowing in Nubaria and Sakha locations.

Mean of Squares Source of Variation	d.f.	A) Plant height (cm)			No. of days to mid-silking			Ear height (cm)		
		B) Nubaria	C) Sakha	Average	Nubaria	Sakha	Average	Nubaria	Sakha	Average
Replicates	3	213.01	478.49	122.05	14.90	4.86	8.33	222.32	112.56	99.08
Hybrids	29	476.80**	2827.46**	336.58**	11.55**	10.52**	9.53**	228.81**	958.95**	201.33**
Error	87	90.95	179.33	88.09	2.79	2.36	1.05	47.93	67.17	25.83
Total	119									
	d.f.	Ear length (cm)			Number of rows/ear			Ear diameter (cm)		
Replicates	3	3.08	3.77	2.44	0.236	0.236	0.123	0.089	0.0328	0.0152
Hybrids	29	6.73**	11.90**	6.06**	2.470**	2.470**	2.033**	0.2496**	0.2827**	0.2001**
Error	87	1.80	1.07	1.00	0.647	0.647	0.546	0.1139	0.0768	0.0369
Total	119									
	d.f.	Number of grains/row			100- grain weight (g)			Grain yield (ardbs/fed)		
Replicates	3	17.76	4.99	7.03	47.66	87.67	36.08	186.69	21.97	16.61
Hybrids	29	27.37**	31.29**	19.85**	99.98**	66.77**	43.35**	95.13**	124.06**	70.08**
Error	87	8.85	4.72	3.77	15.03	10.21	8.06	8.22	10.35	5.55
Total	119									

\*\* = significant at 0.05 levels of probability.

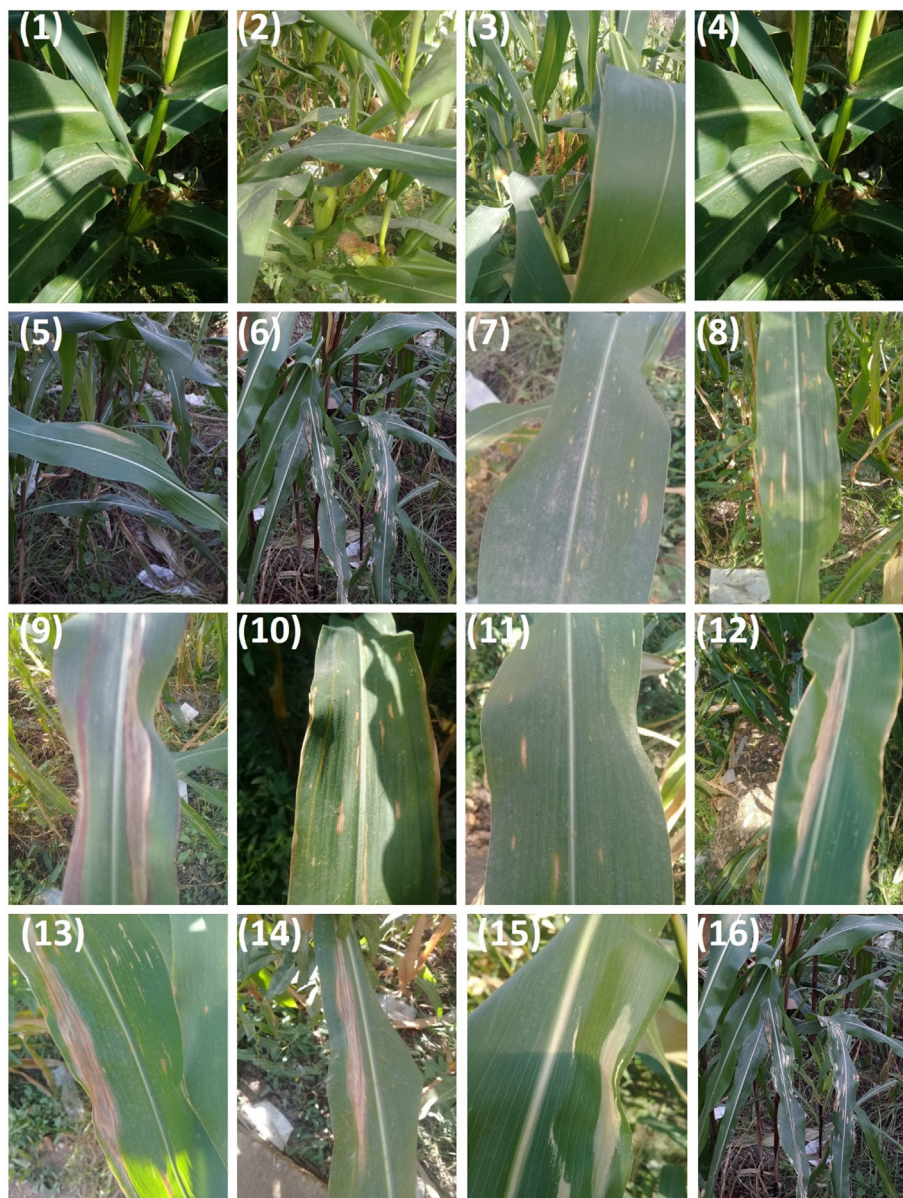


Fig. 1. Different northern leaf blight disease symptoms which detected in this current study, (1–4) healthy or control plants and (5–16) infected plants.

location showed the highest value of 15.45 by (Gz628 × Gm18) and the lowest value was recorded by (Gz612 × Sd63) with a value of 11.60. The combined data of both locations against NCLB disease showed that Gz628 × Gm18 (MR) followed by Sd63 × Gz602 (R) recorded the highest average with 15.68 and 14.40, respectively. Meanwhile, the lowest average was detected by Gz612 × Sd63 (S) and Gz612 × Gm18 (MS) with 12.25 for both hybrids. Third, the highest ear diameter was 4.9 cm in both locations recorded by Sd7 × Gm18 (MR hybrid) and the lowest value was recorded by Nb39 × Gm18 (S) and Gz612 × Gz628 (S) with 3.75 and 4.0 cm for Sakha and Nubaria locations, respectively.

Table 4c, indicates the mean of three other growth traits including, number of grains/row, 100- grain weight and grain yield of different maize hybrids in Sakha and Nubaria locations. Results showed high significant variations between the tested maize hybrids in both locations. First, the maximum average of both locations for the number of grains/row was 46.95 and 45.10 recorded by (Sd63 × Gz602) and (Sd63 × Sd7), which were resistant and highly resistant hybrids against NCLB disease, respectively. While

the minimum average was 34.58 by (Gz612 × Sd63) followed by (Nb39 × Sd63) with 38.30 and both of them showed Susceptible response against NCLB disease. Second, results of the 100- grain weight displayed that the highest value in Nubaria location was 50.0 g by (Sd63 × Sd7) which was highly resistant hybrid against NCLB disease. Whereas, the lowest value was 25.75 g by (Gz612 × Gz628) and (Nb39 × Sd63) which were susceptible hybrids. While Sakha location showed the highest value of 40.71 g by (Sd7 × Gm18) which was moderate resistant (MR) against NCLB disease comparing with the lowest value of 23.47 g by (Nb39 × Mo17) which classified as a (S) hybrid against NCLB (Table 5). The range between two different locations was 10 g when comparing the highest values and was 2 g for the lowest ones. Third, regarding the grain yield, the maximum average was 31.60 followed by 30.75 arlbs/fed that were recorded by Sd63 × Sd7 and Sd63 × Gz602, which were highly resistant and resistant hybrids, respectively. On the other hand, the lowest average of the grain yield was detected by Gz612 × Gz628 followed by Nb39 × Mo17 with 14.99 and 15.36 arlbs/fed and both hybrids

**Table 4a**  
Mean of plant height (cm), number of silking days at 50 % and ear height (cm) of different maize hybrids in Nubaria and Sakha locations.

Maize hybrid	plant height (cm)			No. of days to mid-silking			Ear height (cm)		
	Nubaria	Sakha	Average	Nubaria	Sakha	Average	Nubaria	Sakha	Average
Gz612 × Sd7	225.00	225.00	225.00	61.25	59.00	60.13	117.25	117.25	117.25
Gz612 × Sd63	200.75	244.75	222.75	61.25	60.50	60.88	127.00	148.50	137.75
Gz612 × Gz602	207.50	237.00	222.25	61.75	61.50	61.63	111.00	126.25	118.63
Gz612 × Gz628	197.50	235.00	216.25	59.00	60.75	59.88	113.25	138.25	125.75
Gz612 × Gm18	203.00	250.25	226.63	60.50	61.00	60.75	118.25	136.50	127.38
Gz612 × Mo17	217.00	204.25	210.63	62.75	61.75	62.25	110.50	138.75	124.63
Gz612 × Nb39	212.00	249.75	230.88	57.50	61.25	59.38	115.00	137.00	126.00
Gz628 × Sd7	213.25	248.00	230.63	59.75	60.50	60.13	120.50	143.50	132.00
Gz628 × Sd63	213.25	266.00	239.63	58.50	63.50	61.00	125.75	99.50	112.63
Gz628 × Gz602	210.00	237.25	223.63	61.25	61.50	61.38	126.50	135.75	131.13
Gz628 × Gm18	210.75	244.00	227.38	58.75	60.50	59.63	123.25	139.00	131.13
Gz628 × Mo17	215.00	253.75	234.38	63.25	63.25	63.25	118.75	131.00	124.88
Gz628 × Nb39	212.75	247.50	230.13	58.50	59.75	59.13	123.75	117.25	120.50
Nb39 × Sd7	200.75	200.00	200.38	58.75	58.75	58.75	108.75	115.50	112.13
Nb39 × Sd63	192.50	192.50	192.50	57.75	57.75	57.75	103.75	101.00	102.38
Nb39 × Gz602	188.75	245.00	216.88	60.75	57.75	59.25	100.50	140.25	120.38
Nb39 × Gm18	174.25	220.75	197.50	58.25	57.50	57.88	102.00	131.00	116.50
Nb39 × Mo17	217.50	164.75	191.13	60.50	62.50	61.50	112.50	140.00	126.25
Sd63 × Sd7	200.00	251.50	225.75	61.25	62.50	61.88	115.50	142.50	129.00
Sd63 × Gz602	210.50	272.75	241.63	60.75	61.75	61.25	106.75	152.75	129.75
Sd63 × Gm18	189.50	249.50	219.50	61.50	61.75	61.63	115.00	144.75	129.88
Sd63 × Mo17	197.50	264.00	230.75	63.25	63.25	63.25	103.75	147.75	125.75
Sd7 × Gz602	196.25	244.75	220.50	60.25	60.25	60.25	113.00	113.00	113.00
Sd7 × Gm18	190.50	271.75	231.13	63.25	62.00	62.63	101.00	163.75	132.38
Sd7 × Mo17	200.75	200.75	200.75	61.25	61.25	61.25	105.55	108.75	107.15
Gz602 × Gm18	198.00	198.00	198.00	62.25	62.75	62.50	104.50	133.75	119.13
Gz602 × Mo17	195.50	251.50	223.50	63.25	62.00	62.63	113.75	138.25	126.00
Gm18 × Mo17	201.25	201.25	201.25	60.50	62.50	61.50	113.75	113.75	113.75
Sc.10	202.75	208.00	205.38	61.75	61.75	61.75	116.00	119.25	117.63
Sc.129	216.25	224.75	220.50	60.50	60.75	60.63	118.75	122.50	120.63
LSD at 0.05	13.404	18.821	27.60	2.349	2.160	3.01	9.730	11.518	14.95
Coefficient Variation	4.67	5.73		2.75	2.51		6.07	6.24	

\*\* = significant at 0.05 levels of probability

**Table 4b**  
Mean of ear length (cm), number of row/ear and ear diameter of different maize hybrids in Nubaria and Sakha locations.

Maize hybrid	Ear length (cm)			Number of rows/ear			Ear diameter (cm)		
	Nubaria	Sakha	Average	Nubaria	Sakha	Average	Nubaria	Sakha	Average
Gz612 × Sd7	19.50	19.50	19.50	14.33	13.40	13.87	4.50	4.50	4.50
Gz612 × Sd63	16.10	12.85	14.48	11.60	12.90	12.25	4.60	4.35	4.48
Gz612 × Gz602	21.00	18.50	19.75	13.70	14.20	13.95	4.30	4.45	4.38
Gz612 × Gz628	21.00	19.20	20.10	12.35	14.60	13.48	4.00	4.50	4.25
Gz612 × Gm18	20.90	20.25	20.58	11.80	12.70	12.25	4.48	4.55	4.52
Gz612 × Mo17	21.00	17.35	19.18	15.15	13.00	14.08	4.00	3.85	3.93
Gz612 × Nb39	21.10	19.70	20.40	12.75	14.00	13.38	4.23	4.45	4.34
Gz628 × Sd7	21.00	19.25	20.13	13.10	13.10	13.10	4.78	4.45	4.62
Gz628 × Sd63	21.50	21.25	21.38	14.00	14.00	14.00	4.70	4.65	4.68
Gz628 × Gz602	20.65	21.15	20.90	12.10	14.00	13.05	4.60	4.65	4.63
Gz628 × Gm18	21.00	20.50	20.75	15.45	15.90	15.68	4.10	4.75	4.43
Gz628 × Mo17	21.50	21.50	21.50	11.80	12.80	12.30	4.28	4.25	4.27
Gz628 × Nb39	19.50	19.40	19.45	12.10	12.70	12.40	4.30	4.45	4.38
Nb39 × Sd7	20.50	18.50	19.50	12.55	13.40	12.98	4.55	4.70	4.63
Nb39 × Sd63	19.15	19.65	19.40	12.75	12.75	12.75	4.20	4.20	4.20
Nb39 × Gz602	17.00	19.70	18.35	12.00	13.40	12.70	4.60	4.70	4.65
Nb39 × Gm18	18.60	18.70	18.65	13.15	13.70	13.43	4.25	3.75	4.00
Nb39 × Mo17	20.50	18.30	19.40	12.25	12.30	12.28	4.40	4.25	4.33
Sd63 × Sd7	21.75	22.00	21.88	14.20	14.20	14.20	4.80	4.45	4.63
Sd63 × Gz602	21.60	21.65	21.63	14.40	14.40	14.40	4.20	4.55	4.38
Sd63 × Gm18	20.50	20.85	20.68	11.85	13.60	12.73	4.70	4.70	4.70
Sd63 × Mo17	20.50	21.00	20.75	13.70	13.80	13.75	4.20	4.05	4.13
Sd7 × Gz602	21.10	20.30	20.70	13.30	13.30	13.30	4.20	4.20	4.20
Sd7 × Gm18	20.85	20.85	20.85	13.50	13.40	13.45	4.90	4.90	4.90
Sd7 × Mo17	21.25	21.25	21.25	13.75	13.75	13.75	4.50	4.50	4.50
Gz602 × Gm18	19.30	20.30	19.80	14.00	13.70	13.85	4.30	4.70	4.50
Gz602 × Mo17	20.50	19.70	20.10	13.80	13.10	13.45	4.10	4.20	4.15
Gm18 × Mo17	19.35	19.35	19.35	12.65	12.50	12.58	4.20	4.20	4.20
Sc.10	20.00	19.05	19.53	12.90	12.95	12.93	4.70	4.67	4.69
Sc.129	21.00	21.00	21.00	13.85	14.90	14.38	4.25	4.35	4.30
LSD at 0.05	1.886	1.456	2.941	1.084	1.131	2.173	0.4744	0.3894	0.5649
Coefficient Variation	6.60	5.24		5.85	5.94		7.67	6.25	

\*\* = significant at 0.05 levels of probability

**Table 4c**

Mean of number of grains/row, 100- grain weight (g) and grain yield (ardbs/fed.) of different maize parents in Nubaria and Sakha locations.

Maize hybrid	No. of grains/row			100- grain weight (g)			Grain yield (ardbs/fed)		
	Nubaria	Sakha	Average	Nubaria	Sakha	Average	Nubaria	Sakha	Average
Gz612 × Sd7	44.05	38.55	41.30	34.50	34.50	34.50	25.57	14.37	19.97
Gz612 × Sd63	35.05	34.10	34.58	29.75	28.68	29.22	25.29	6.73	16.01
Gz612 × Gz602	37.80	44.55	41.18	37.50	29.22	33.36	21.06	17.57	19.32
Gz612 × Gz628	38.80	38.80	38.80	25.75	25.75	25.75	14.99	14.99	14.99
Gz612 × Gm18	41.25	41.40	41.33	35.75	31.26	33.51	25.46	15.89	20.68
Gz612 × Mo17	39.65	42.35	41.00	32.00	35.17	33.59	19.05	13.39	16.22
Gz612 × Nb39	40.60	40.60	40.60	36.25	32.82	34.54	22.68	19.19	20.94
Gz628 × Sd7	40.30	42.75	41.53	45.00	32.52	38.76	23.66	19.98	21.82
Gz628 × Sd63	45.20	43.60	44.40	40.00	40.00	40.00	25.80	21.60	23.70
Gz628 × Gz602	42.80	43.35	43.08	36.50	35.75	36.13	27.82	27.82	27.82
Gz628 × Gm18	43.80	43.80	43.80	35.75	37.48	36.62	27.95	16.06	22.01
Gz628 × Mo17	40.15	42.55	41.35	38.50	33.09	35.80	19.87	23.44	21.66
Gz628 × Nb39	44.35	45.35	44.85	38.25	36.16	37.21	29.60	17.76	23.68
Nb39 × Sd7	41.00	41.00	41.00	33.25	32.99	33.12	17.17	23.02	20.10
Nb39 × Sd63	38.30	38.30	38.30	25.75	29.81	27.78	20.63	12.65	16.64
Nb39 × Gz602	38.65	41.00	39.83	32.00	33.96	32.98	21.38	17.30	19.34
Nb39 × Gm18	39.20	39.65	39.43	30.00	32.31	31.16	23.75	12.17	17.96
Nb39 × Mo17	40.65	40.70	40.68	35.25	23.47	29.36	11.97	18.75	15.36
Sd63 × Sd7	45.30	44.90	45.10	50.00	34.79	42.40	31.60	31.60	31.60
Sd63 × Gz602	44.95	47.95	46.95	40.75	39.98	40.37	30.76	30.73	30.75
Sd63 × Gm18	42.55	42.55	42.55	33.25	37.85	35.55	25.09	22.48	23.79
Sd63 × Mo17	42.75	42.75	42.75	40.75	35.37	38.06	24.53	25.40	24.97
Sd7 × Gz602	44.15	39.80	41.98	35.00	36.09	35.55	21.66	21.63	21.65
Sd7 × Gm18	42.10	46.85	44.48	34.75	40.71	37.73	27.86	15.46	21.66
Sd7 × Mo17	40.75	43.80	42.28	37.50	37.50	37.50	20.79	21.50	21.15
Gz602 × Gm18	40.10	42.00	41.05	34.75	35.50	35.13	18.27	18.27	18.27
Gz602 × Mo17	43.20	43.40	43.30	37.50	32.90	35.20	23.74	25.92	24.83
Gm18 × Mo17	41.05	39.90	40.48	34.25	32.05	33.15	18.08	16.66	17.37
Sc.10	37.10	42.05	39.58	35.00	35.00	35.00	19.90	21.16	20.53
Sc.129	42.15	44.90	43.53	40.00	40.00	40.00	32.43	23.80	28.12
LSD at 0.05	4.180	3.054	5.710	5.449	4.491	8.349	4.030	4.521	6.929
Coefficient Variation	7.20	5.15		10.82	9.38		12.31	16.43	

\*\* = significant at 0.05 levels of probability

exhibited a susceptible response against NCLB disease. Sakha location showed the highest value of 31.60 ardb/fed by Sd63 × Sd7 (HR) and the minimum value was 6.73 ardb/fed recorded by the susceptible hybrid (Gz612 × Sd63). While the Nubaria location showed highest value of 32.43 and 31.60 ardb/fed by Sc.129 (MR) and Sd63 × Sd7 (HR) comparing with the lowest value of 11.97 ardb/fed recorded by the susceptible hybrid (Nb39 × Mo17).

### 3.3. Genetic distance and northern leaf blight disease tolerance

The morphological traits of all tested maize parents and hybrids were classified in three main clusters, (Fig. 2). Cluster I is further divided into three sub clusters Ia, Ib and Ic, cluster II is further divide into two sub cluster IIa and IIb and cluster III is further divide into three sub cluster IIIa, IIIb and IIIc. Cluster I involved in eight maize parents as follows, Sub cluster Ia contained Sids 63 (HR genotype) whereas sub cluster Ib is further sub divided into sub sub cluster that comprised two genotypes, (Giza 602 and Giza 628), and both are resistant (R) genotypes and showed close genetic relation. Sub cluster Ic has five genotypes, three of them showed close genetic relation and were moderate resistant (MR) including (Giza 602, Sids7 and Mo17), and the other two genotypes were susceptible (S) including (Gemiza18 and Nubaria 39).

Sub cluster II contained four susceptible (S) genotypes in two sub sub clusters, IIa includes (Nb39 × Gm18, Gz612 × Sd63 and Nb39 × Mo17) with a close genetic relation while IIb includes Gz612 × Gz628. On the other hand, the sub cluster III contained 26 genotypes in three sub sub clusters, IIIa contained only (Sd63 × Sd7) which is highly (HR genotype). The sub sub cluster

IIIb contained eight genotypes, one of them was resistant (R) hybrid (Sd63 × Gz602) while the other seven hybrids were moderate resistant (MR), five of them had close genetic linkage, including (Sd63 × Gm18, Sd63 × Mo17, Gz628 × Gm18, Gz628 × Gz602 and Gz602 × Mo17), the other two hybrids were (Gz628 × Sd63 and Sc.129) that had nearby genetic linkage with the resistant (R) hybrid (Sd63 × Gz602). The sub sub cluster IIIc contained 17 genotypes divided in two sub clusters, the first one contains 10 genotypes including six moderate resistant (MR) hybrids, (Sd7 × Mo17, Sd7 × Gz602, Sd7 × Gm18, Gz628 × Mo17, Gz628 × Nb39 and Gz628 × Sd7), and four moderate susceptible (MS) hybrids, (Gz602 × Gm18, Sc.10, Gz612 × Gm18 and Gz612 × Sd7). The second sub cluster of IIIc contained seven genotypes, four of them were moderate susceptible (MS) hybrids including, (Gz612 × Gz602, Gz612 × Nb39, Nb39 × Sd7 and Gz612 × Mo17) and the other three genotypes were susceptible hybrids including, (Nb39 × Gz602, Gm18 × Mo17 and Nb39 × Sd63).

Of the thirty eight lines of parents, their progeny and two check lines, (Table 5) indicated that two lines were rated 1 (highly resistant), three were rated 2 (Resistant), 16 were rated 3 (Moderately resistant), 8 were rated 4 (Moderately susceptible) and 9 were rated 5 (susceptible). In general, we could detect that crossing between the high resistant maize parents could produce the same levels of resistance, besides when crossing with moderate resistant the hybrids give a good resistance to NCLB disease. Accordingly, we can conclude that classical breeding is one of the most tools to control the NCLB disease.

Data in (Fig. 3) showed the heat map of the response of eight maize cultivars and twenty-eight hybrids in addition to the two-



**Table 5**  
Mean NCLB score and reaction for the maize parents and their hybrids.

Parents and Hybrids	Mean NCLB score	NCLB reaction
Gz612	0.22	Moderately resistant
Gz628	0.09	Resistant
Nb39	0.57	Susceptible
Sd63	0.05	Highly resistant
Sd7	0.16	Moderately resistant
Gz602	0.06	Resistant
Mo17	0.17	Moderately resistant
Gm18	0.57	Susceptible
Gz612x Sd7	0.43	Moderately susceptible
Gz612x Sd63	0.56	Susceptible
Gz612x Gz602	0.44	Moderately susceptible
Gz612x Gz628	0.59	Susceptible
Gz612x Gm18	0.43	Moderately susceptible
Gz612x Mo17	0.50	Moderately susceptible
Gz612x Nb39	0.49	Moderately susceptible
Gz628x Sd7	0.20	Moderately resistant
Gz628x Sd63	0.15	Moderately resistant
Gz628x Gz602	0.17	Moderately resistant
Gz628x Gm18	0.16	Moderately resistant
Gz628x Mo17	0.20	Moderately resistant
Gz628x Nb39	0.20	Moderately resistant
Nb39 × Sd7	0.50	Moderately susceptible
Nb39 × Sd63	0.55	Susceptible
Nb39 × Gz602	0.54	Susceptible
Nb39 × Gm18	0.55	Susceptible
Nb39 × Mo17	0.57	Susceptible
Sd63 × Sd7	0.05	Highly resistant
Sd63 × Gz602	0.06	Resistant
Sd63 × Gm18	0.19	Moderately resistant
Sd63x Mo17	0.16	Moderately resistant
Sd7x Gz602	0.20	Moderately resistant
Sd7x Gm18	0.21	Moderately resistant
Sd7x Mo17	0.23	Moderately resistant
Gz602x Gm18	0.30	Moderately susceptible
Gz602x Mo17	0.16	Moderately resistant
Gm18x Mo17	0.54	Susceptible
SC.10	0.30	Moderately susceptible
Sc.129	0.14	Moderately resistant

\*<5%: Highly resistant (HR); 6–10%: Resistant (R); 11–25%: Moderately resistant (MR); 26–50%: Moderately susceptible (MS); 51–75%: Susceptible (S); greater than 75%: Highly susceptible (HS)

check lines to different levels of resistance against NCLB disease based on morphological characters. The color scale at the map on the left represents the expression level (parents group compared with their hybrids and two check lines groups), where the colors light green (highly resistant and resistant), dark green (Moderately resistant), black (Moderately susceptible), red (Susceptible). This indicates three kind of expression levels, including upregulated, down-regulated and unaltered expression.

#### 4. Discussion

Breeding for NCLB resistant maize cultivars by traditional methods is considered the most effective and feasible method to overcome yield losses due to this disease. However, the conventional breeding is laborious, time-consuming, and dependent on environmental conditions. Breeding for disease resistance in maize is an efficient control measure that is reliable and cost-effective. It is based on the identification and incorporation of major resistance genes into economically important varieties (Saxena and Hooker 1968; Wisser et al. 2006).

The most actual, environmentally safe and economical means to control NCLB of corn is the use of resistant cultivars (Geiger and Heun 1989). Qualitative resistance is normally race specific and inherited by single genes whereas quantitative resistance is race non-specific and oligogenic or polygenic (Keller et al. 2000). Both categories, qualitative and quantitative, refer to the distribution

of a trait in a population and not to its effectiveness (Abdelsalam 2014; Abdelsalam et al. 2019a; Abdelsalam et al. 2019b).

Most maize breeders therefore favor the use of quantitative NCLB resistance in their cultivar development programs (Hooker et al. 1975; Abdelsalam et al. 2018). Classical breeding involves hybridization between selected plant parents that have desirable characteristics such as high yield or disease resistance (Visarada et al. 2009; Abdelsalam 2014). Selection of superior plant traits involves visual assessment; thus the breeder's skills depend on selecting the best plants with desirable recombinant characteristics from the large segregating offspring populations (Visarada et al. 2009; Abdelsalam et al. 2019c; Abdelsalam et al. 2019d; Zhao et al. 2019).

Results of plant height indicated that the highest average of maize parents was recorded by Giza 612 (MR cultivar) while Sids 63 (HR cultivar) showed the lowest average, implying that NCLB resistance might be negatively associated with plant height, which is aligned with the results of other authors (Ojulong et al. 1995; Zwonitzer et al. 2009). However, the highest average of plant height in maize hybrids was recorded by a resistant (R) hybrid Sd63 × Gz602 and the lowest average was recorded by Nb39 × Mo17 and Nb39 × Sd63, which were susceptible (S) hybrids against NCLB, implying that NCLB resistance could be positively associated with plant height, which is matching with other findings (Welz et al. 1999; Shah et al. 2016; Razzaq et al. 2019). Concerning the number of days to mid silking, it was found that the lowest value were recorded by Sids 63 that showed highly resistance (HR), implying that the earlier silking and the early-flowering varieties could result in better protection of NCLB in maize, in accordance with (Ojulong et al. 1995). However the lowest average of 50% silking in maize hybrids was recorded by Nb39 × Sd63 and Nb39 × Gm18, which were susceptible hybrids. Accordingly, days to silking and NCLB resistance have a low positive correlation. Regarding the ear height, Giza 628 has the highest average and it was resistant (R) cultivar, while lowest average was described by Gemmiza 18 that was susceptible (S) cultivar. Besides, the lowest average in maize hybrids was detected by Nb39 × Sd63 that was also susceptible to NCLB disease. Therefore, resistance to NCLB disease could be positively associated with ear height, which is matching with other findings (Razzaq et al. 2019). However, the highest average of ear height in maize hybrids was detected by Gz612 × Sd63 that showed susceptible reaction against NCLB, in accordance with (Zwonitzer et al. 2009), suggesting a low positive association between NCLB resistance and ear height.

Results of ear length, data showed that the highest ear length average has been recorded by (Giza 602) which exhibited resistance against NCLB, whereas lowest average was recorded by (Gemmiza18), which is (S) cultivar. Also, the maize hybrids Sd63 × Sd7 (HR hybrid) and Sd63 × Gz602 (R hybrid) showed the highest average, while the lowest average was described by Gz612 × Sd63 and Nb39 × Gz602 and both were susceptible against NCLB disease. Accordingly, the ear length could be positively associated with NCLB resistance, which is aligned with other findings (Shah et al. 2016; Rehab et al. 2019). Concerning the number of rows per ear, Giza 628 showed the highest average and regarded as resistant (R) cultivar, meanwhile the lowest average occurred by Gemmiza18 (S cultivar). In addition, Gz628 × Gm18 (MR hybrid) and Sd63 × Gz602 (R hybrid) recorded the highest average among maize hybrids, meanwhile, the lowest average was detected by Gz612 × Sd63 (S hybrid) and Gz612 × Gm18 (MS hybrid). Consequently, the number of rows per ear could be positively associated with NCLB resistance, which is in accordance with other findings (Shah et al. 2016; Razzaq et al. 2019). For the ear diameter, the highest average among maize parents was recorded by (Sids 63) that is (HR) cultivar whereas the lowest aver-

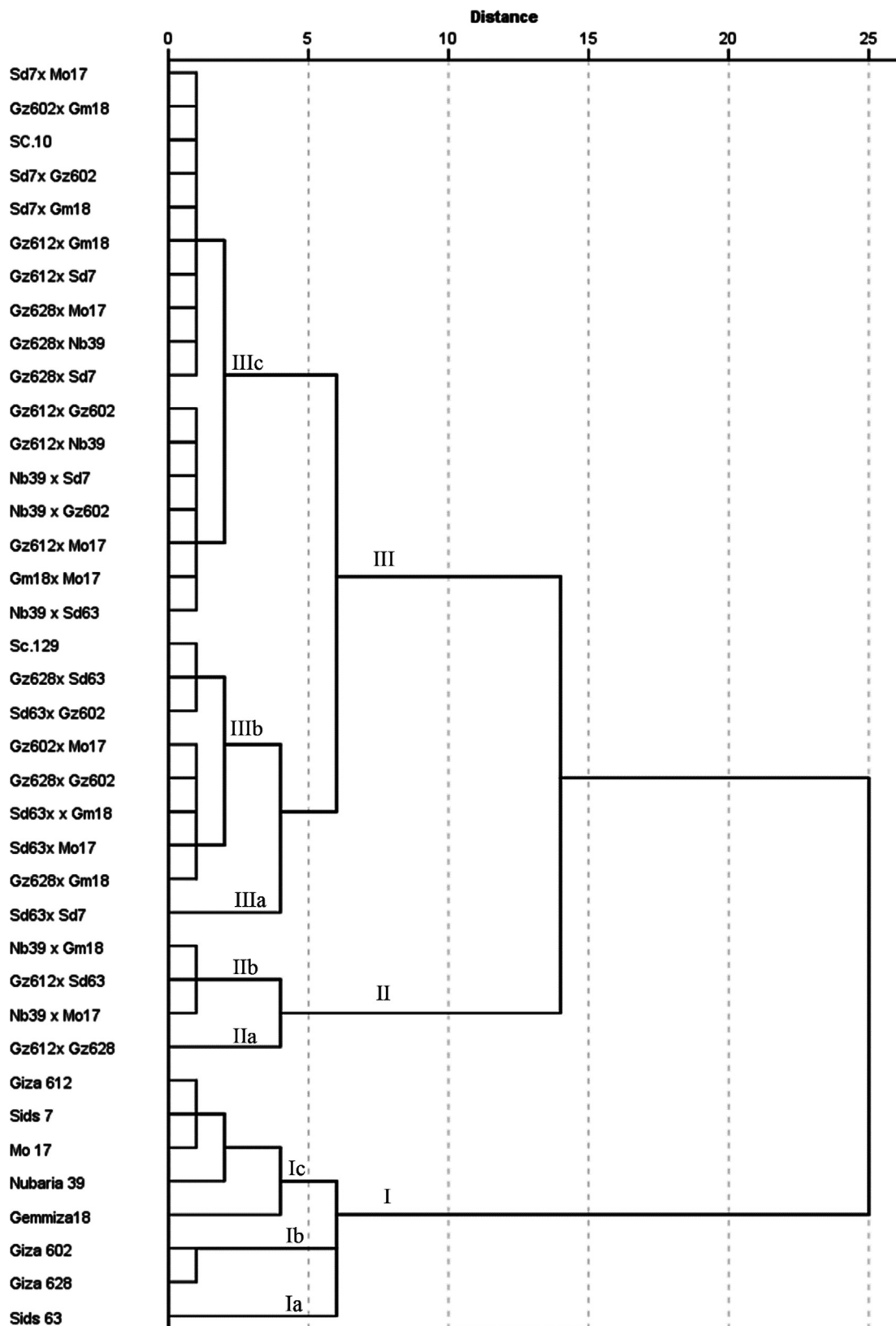
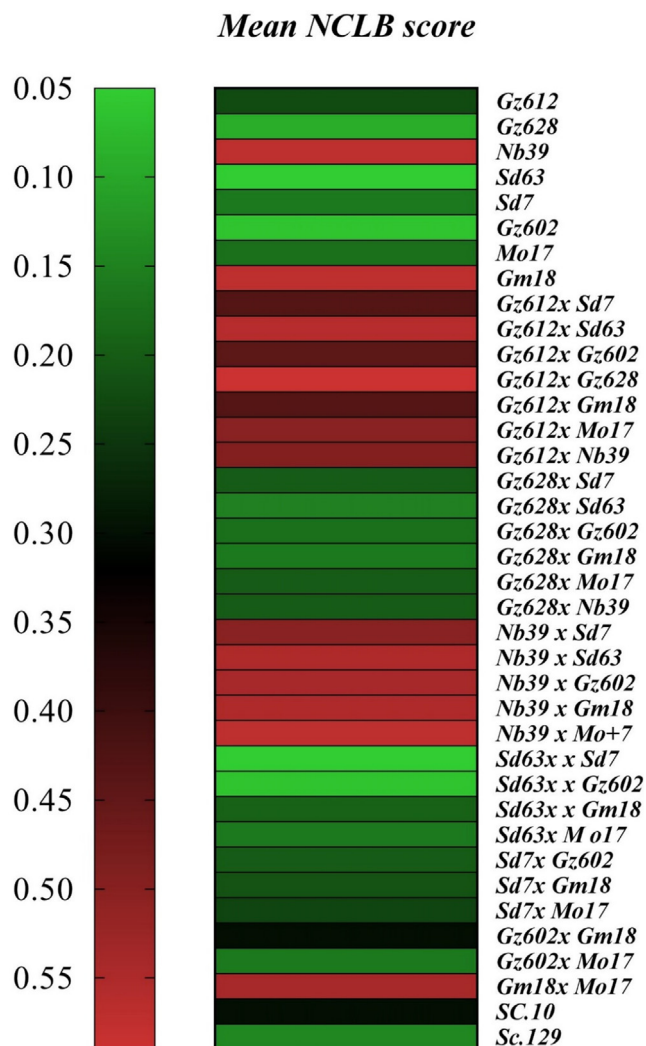


Fig. 2. Cluster analysis for maize hybrids showed the different levels of resistance against northern leaf blight disease based on morphological characters and resistant parents.



**Fig. 3.** Heat map representing the different categories of maize parents and their hybrids to different levels of resistance against northern leaf blight disease based on morphological characters. The color scale at the left of the heat map represents the expression level (parents group compared with their hybrids and two cheek lines groups), where the colors light green (highly resistant and resistant), dark green (Moderately resistant), black (Moderately susceptible), red (Susceptible) indicate upregulated, down-regulated and unaltered expression, respectively.

age was indicated by (Nubaria 39) that was (S hybrid) against NCLB. Besides, the highest ear diameter among maize hybrids was recorded by Sd7 × Gm18 (MR hybrid) and the lowest value was recorded by Nb39 × Gm18 (S hybrid) and Gz612 × Gz628 (S hybrid). Accordingly, it was suggested that the ear diameter could be positively correlated with NCLB resistance, which is in accordance with what was reported by (Razzaq et al. 2019).

Results of the number of grains per row, the 100- grain weight and grain yield indicated that the highly resistant and resistant maize lines recorded the maximum values, while the lowest values were reported by the susceptible and moderate susceptible lines, suggesting that the above-mentioned traits could be positively related with maize resistance against NCLB disease. In addition, our results confirmed a significant role in increasing grain yield with relation to NCLB resistance and other yield related attributes. The current study supports the finding of other authors (Barakat et al. 2009; Zwonitzer et al. 2009; Shah et al. 2016; Razzaq et al. 2019; Rehab et al. 2019). Some reports indicated the predominance of non-additive gene actions over additive gene for the inheritance

of some growth traits, including 50% silk emergence, plant height and ear length (Alam et al. 2008; Ohunakin et al. 2020). However, ear weight, NCLB score and grain yield revealed the predominance of additive gene effects in controlling inheritance of these traits (Ojo et al. 2007; Ohunakin et al. 2020).

For the genetic distance of all tested maize genotypes, our results displayed that resistant varieties falls in same cluster whereas susceptible ones into another separate cluster. While moderately resistant and some moderately susceptible genotypes create separate cluster indicated close genetic characteristics. Some other moderately susceptible genotypes and susceptible ones fall in same cluster, indicating that they are of different genetic nature from other groups of genotypes by falling into separate sub cluster. Results are in accordance with (Hassan 2015).

According to NCLB disease incidence and severity, results showed significant variability among all tested maize lines which might be due to presence of inoculum potential, the favorable condition for the pathogen and the genetic characteristics of the maize genotype and the plant immune system (Mubeen et al. 2017; Desoky et al. 2020; Elrys et al. 2020). The increase of diseases incidence and severity resulted in the more susceptibility of maize lines toward NCLB disease as supported by (Ali and Yan 2012). The rating scale of NCLB was as highly resistant, resistant, moderately resistant, moderately susceptible and susceptible categories. Twenty-eight crosses were performed among eight parental lines and the hybridization outcomes were (1 HR hybrid, 1 R hybrid, 12 MR hybrids, 7 MS hybrids and 7 S hybrids). Results indicated that resistant genotypes yield the similar levels of resistance. In addition, when crosses were carried out with moderate resistant, the hybrids showed a good resistance to NCLB. Consequently, hybridization outcomes thus led to improvement of the yield and yield related traits, which was supported by other authors' findings (Harlapur 2005; Razzaq et al. 2019; Ohunakin et al. 2020). Accordingly, we recommended classical breeding as one of the best management methods to control NCLB disease. Similarly, Razzaq et al. (Razzaq et al. 2019) noted that recombination of maize heterotic groups into a single training set is a meaningful approach for improvement of NCLB resistance. Moreover, this result mached with most results reported by other scientists who noted that the newly generated maize genotypes have better tolerance to disease stress (Beyene et al. 2013; Ertiro et al. 2017; Ohunakin et al. 2020).

### 5. Conclusion

In the current study, we evaluated the efficacy of screening and selection of maize genotypes under NCLB stress for the yield and yield related traits in two different location in Egypt. Our findings revealed a greater role of classical genetic methods to evaluate 38 maize genotypes, including 8 parental lines, 28 F<sub>1</sub> and 2 check hybrids against NCLB disease in two different location in Egypt. The analysis of variance and mean squares for maize parents and their hybrids showed high significant variations between the maize parents and their hybrids for all studied parameters. In general, highly resistant, resistant and moderately resistant genotypes were greater in number than susceptible and moderate susceptible lines, showing an improvement in yield and yield components, thus they could be used as an improved germplasm which would plays a key role in increasing maize production in Egypt. The current study introduce newly identified sources of NCLB resistance that will be helpful for their deployment in breeding program. Finally, we concluded that the classical breeding considers one of the best tool to manage the NCLB disease by generating new maize hybrids and cultivars.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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