



Article Resistance Breeding for Northern Corn Leaf Blight with Dominant Genes, Polygene, and Their Combinations—Effects on Disease Traits

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Abstract: Resistance breeding is the most effective method to control northern corn leaf blight (NCLB). The objectives were to (1) Assess effects of dominant genes (Ht(s)), polygene (PG), and their combinations to disease rating (DR), number of lesions per leaf (NLPL), and lesion size (LS); (2) Estimate genetic components, general combining abilities (GCA), and heritability under two Line × Tester analyses; and (3) Determine gene action through mid-parent heterosis (MPH) and better-parent heterobeltiosis (BPH) analysis. A total of 163 genotypes, including 120 crosses, their parents, and 10 hybrid checks, were evaluated under two NCLB artificial inoculations in 2015 and 2016. The results indicated that PG had the best resistance to DR, NLPL, and LS in crosses, followed by PGHt(s) and single Ht(s). Ht1 had both resistant and susceptible lesions. Ht2 and Ht3 expressed more resistance to LS significantly, while Htm1 and Htn1 had more resistance to NLPL. Htm1/Ht2, PG/Htm1, and the other 11 combinations were found excellent for NCLB resistance. Line × Tester analysis showed that additive effects were more important, and GCA of Ht(s) reduced disease traits. However, lower narrow sense heritability indicated that additive effects were low. MPH and BPH results showed that dominant and over-dominant gene actions existed for DR and NLPL.

Keywords: corn; disease rating; heritability; heterosis; heterobeltiosis; lesion size; Line × Tester; northern corn leaf blight; number of lesions per leaf; resistance genes; *Ht1*; *Ht2*; *Ht3*; *Htm1*; *Htn1*; polygene

1. Introduction

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* (Passerini) Leonard and Suggs = *Setosphaeria turcica* [Luttrell] Leonard and Suggs = *Helminthosporium turcicum*, is the most common and economically significant leaf disease of corn (*Zea mays* L.) worldwide. NCLB occurs during relatively cool and wet seasons [1]. Sporulation requires a 14 h dew period and a temperature of 20–25 °C [2]. Periods of wetness that last more than 6 h at a temperature of 18–27 °C are most conducive to NCLB infection [3]. Under favorable conditions, susceptible lesions can form in 12 days [1,4]; thus, one complete disease cycle on susceptible plants takes place within 14 days, whereas it takes about 20 days on resistant plants [2,3,5]. Typically, NCLB causes 15–30% yield losses [6]. Heavy infections of NCLB can cause grain yield losses ranging from 40% [7,8] up to 70% [6] and silage losses up to 91% [9]. In Canada, NCLB became an economically important foliar disease recently. It infected 98% of surveyed fields in 2010 in Ontario and Quebec [10] and 97% of the field in 2015 in Ontario [11], and yield losses could reach up to 50% [12].

Resistance breeding is the most economical, sustainable, and effective way to control NCLB. In NCLB studies, resistance expression included lesion type [13–16], disease score = scale = rating [17,18], area under the disease progress curve (AUDPC), apparent infection rate (r) [8,19], yield loss, kernel weight loss [8], number of lesions per plant, lesion size, area of the sporulating zone, number of conidia produced on lesion segments [20],



Citation: Zhu, X.; Reid, L.M.; Woldemariam, T.; Wu, J.; Jindal, K.K.; Kebede, A. Resistance Breeding for Northern Corn Leaf Blight with Dominant Genes, Polygene, and Their Combinations—Effects on Disease Traits. *Agronomy* **2023**, *13*, 1096. https://doi.org/10.3390/ agronomy13041096

Received: 9 March 2023 Revised: 31 March 2023 Accepted: 9 April 2023 Published: 11 April 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and percent leaf area affected [21]. AUDPC and latent period were used for the partial resistance study [22].

Resistant sources of NCLB and their resistant genes were identified in corn breeding history. In 1956, 1066 accessions of corn germplasm were evaluated for resistance to NCLB [23]. Eighty-four sources had chlorotic lesion resistance [18]. There are 3286 accessions with NCLB resistance results recorded in U.S. National Plant Germplasm System (https://npgsweb.ars-grin.gov/gringlobal/descriptors.aspx, (accessed on 8 March 2023), including 991 accessions with high to average resistance (rating 1–4) in 2022. Welz and Geiger [24] reviewed all genes for resistance to NCLB in a diverse maize population. The dominant gene Ht1 was found in inbred GE440 and Ladyfinger popcorn [13–16]; Ht2 was found in NN14 [25]; *Ht3* was incorporated from *Tripsacum floridanum* into corn [26]; HtN = Htn1 was derived from Pepitilla [27]; Htm1 was derived from Mayorbela [28]; HtP was derived from an American resistant line L10 [29,30]; HtNB was derived from an Indonesian variety Bramadi [31]; and two recessive resistant genes, *ht4* and *rt*, were found in synthetic BS19 [32] and L40 [29,30], respectively. The dominant gene NN from Thailand-inbred Ki14 was also mentioned by Welz and Geiger [24]. In recent Genome-wide association studies, quantitative trait locus (QTL) for resistant genes were detected from different populations [33–39].

Ht1, *Ht2*, *Ht3*, and *Htn1* were used for physiological race identification. Based on the new system for the designation of physiological races [40], races 0, 1, 2, 23, 2 N, and 23 N existed in the United States [41–47]; 0, 1, 3, N, 12, 13, 23, 1 N, 2 N, 3 N, 12 N, 23 N, and 123 in China [48]; 23, 2 N, and 23 N in Mexico; 0, 23, and 23 N in Zambia, 0, 1, 2, 3, N, and 23 N in Uganda [49]; 0, 1 N, 12 N, and 123 N in Brazil [50]; 0 and 1 in Serbia [51]; and 0, 1, 2, 3, N, 12, 13, 23, 3 N, 123, 13 N, and 23 N in Kenya, Germany, and Austria [52]. *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* were used for physiological race identification recently. Twenty races (0, 1, 2, M, N, 12, 13, 23, 1 M, 1 N, MN, 123, 1 MN, 2 MN, 23 M, 23 N, 12 MN, 23 MN, 123 M, and 123 MN) existed in north central United States [53]; seventeen races (0, 1, 2, 3, M, N, 12, 1 M, 1 N, 3 M, 13 M, 12 N, 13 N, 1 MN, 12 MN, 13 MN, and 123 MN) were found in Ontario, Canada [54].

Ht1 was introduced into 30 public inbreds [17] and was used extensively during the late 1960s and 1970s [32] and later [42,55]. Inbred Oh43, Oh45, and Mo17 had polygenic resistance and were combined with monogenic *Ht1*, *Ht2*, and *Htn1* for resistance study; polygenic combined with *Htn1* had better resistance than with *Ht2* and *Ht1*, and monogenic combination *Ht2/Ht1* had better resistance than *Ht2/Ht2* and *Ht1/Ht1* [20]. In a study inoculated with race 0 and 1, *Ht1* + partial resistance hybrid sweet corn had the best resistance, followed by partial resistance, *Ht1*, and susceptible hybrids [56]. Similar results were found in grain corn hybrids crossed with partial resistance line H99 [57]. H99 was estimated to have 2–3 genes for lesion number and 4–7 genes for the percent leaf area affected [21].

The traditional resistance breeding methods include population improvement and backcross. If polygene resistance is the only source available, population improvement should be used to make a more resistant population. When the dominant resistant gene is available, the backcross method can incorporate a resistant gene from the donor to have higher yield materials or to have materials with polygene resistance or partial resistance for better resistance. From 2006 to 2014, based on the backcross method, *Htm1* and *Htm1* were successfully introduced into a susceptible inbred CO388, and *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htm1* were successfully introduced into a polygenic resistant inbred CO428. The objectives of this paper were to: (1) estimate single and polygene effects and better gene combinations to NCLB resistance, such as disease rating (DR), number of lesions per leaf (NLPL), and lesion size (LS); (2) estimate genetic components, general combining ability (GCA), and specific combining ability (SCA), heritability (H), and the narrow sense heritability (H(ns)) by Line × Tester analysis; and (3) estimate gene action under different genotype backgrounds, additive (A) and dominant (D) gene effects through mid-parent heterosis (MPH), and better-parent heterobeltiosis (BPH) analysis.

2. Materials and Methods

2.1. Breeding History

Based on previous corn germplasm introduced from the United States (USDA-MWA-PIRU and NCRPIS, 1305 State Avenue, Ames, IOWA 50014, United States; and Cornell University, United States), seed increasing and disease screening to NCLB resulted [58,59] in 7 accessions with HT = Ht1 gene (Ames 23458, A619HT; Ames 23468, A632HT; Ames 27065, B73Htrhm; Ames 27138, N28HT; PI 600729, LP1NRHT; PI 600755, LP1 CMS HT; and, PI 601079, LH123HT), 2 accessions with Ht1 gene (Ames 25219, A619Ht1 and Ames 25372, Pa91Ht1), 2 accessions with Ht2 gene (Ames 25220, A619Ht2 and Ames 25373, Pa91Ht2), 2 accessions with *Ht3* gene (Ames 25221, A619*Ht3* and Ames 25374, Pa91*Ht3*), 2 accessions with *Htm1* gene (PI 550496, H102) [28] and inbred 73,353 [60], and 6 accessions with *Htm1* gene (PI 406112, A214N; PI 406118, A509N; PI 406119, A553N (Orange Halo); PI 406120, A553N (Red Halo), PI 406126, A661N; and, Ames 23469, A632HtN). In total, 21 resistant sources were planted and crossed with two inbred lines, CO388 and CO442, with high general combining ability (GCA) for grain yield and lower GCA CO428 but with polygene (PG) resistance to NCLB, gray leaf spot, eyespot, Stewart's wilt, and Goss's wilt [58]. Their pedigree and heterotic groups are listed in Table 1. Most resistance sources come from the non-stiff stalk (NS) group, Oh43 subgroup, and tropical materials. However, A632*HtN* and B73Htrhm belong to the stiff stalk (SS) group, B14 and B73 subgroups, respectively. The brief breeding histories were: 20 CO388 \times R sources, 12 CO442 \times R sources, and $15 \text{ CO428} \times \text{R}$ sources crosses made in 2006; In 2007, all crosses were artificially inoculated twice [61], based on their resistance, both lesion type and DR; all susceptible crosses which only showed susceptible lesion or DR > 5.5 at the late stage were deleted, including all CO442 crosses. Only 9 CO388 \times R sources and 9 CO428 \times R sources were backcrossed with CO388 or CO428, respectively. The same inoculation and selection methods were used in 2008; 3 families of (CO388 \times R sources)CO388 were deleted. Only 6 families of (CO388 \times R sources)CO388 and all 9 families of (CO428 × R sources)CO428 were backcrossed with CO388 or CO428, respectively. Similarly, in 2009, 3 families of (CO388 \times R sources)CO388(2) were deleted. Only 3 families of (CO388 \times R sources)CO388(2) and all 9 families of $(CO428 \times R \text{ sources})CO428(2)$ were backcrossed with CO388 or CO428, respectively. In 2010, all families were backcrossed. Three families of (CO388 \times R Sources)CO388(4) were simplified and named BLT01 to BLT03, and 9 families of (CO428 \times R sources)CO428(4) were named BLT05 to BLT13. These families were ear-row selfed in 2011, 2012, and 2013 in Ottawa and 2011 winter in New Zealand. BLT08 was deleted in 2013 because the inbred yield was not as well as CO428. From 2013 to 2015, these families were crossed with tester lines. During 2014 to 2016, selfed lines were re-screened with artificial inoculation, and yield trials were tested.

Table 1. Accession #/Sources/Pedigrees, heterotic groups, assumed resistant genes, and days to silking of 35 inbreds and their disease ratings, and lesion types under an artificial epidemic of northern corn leaf blight.

Name/Code	Purpose	Accession #/ Source/Pedigree	Heterotic Group	Assumed Resistant Gene	Days to Silking	Disease Rating	Lesion Type
A619	Tester	PI 587139, USA	NS-Oh43	None	83	6.6	S
A619Ht1	Tester	Ames 25219, USA	NS-Oh43	Ht1Ht1	84	5.0	R/S
A619Ht2	Tester & R source	Ames 25220, USA	NS-Oh43	Ht2Ht2	84	4.7	MR/MS
A619Ht3	Tester	Ames 25221, USA	NS-Oh43	Ht3Ht3	85	4.9	MR/MS
A632HTN	R Source	Ames 23469, USA	SS-B14	Htn1Htn1	85	5.2	MR/MS
A509N	R Source	PI 406118, USA	Tropical	Htn1Htn1	92	5.0	R/MR
A553N	R Source	PI 406119, USA	Tropical	Htn1Htn1	92	3.6	R/MR

Table 1. Cont.

Name/Code	Purpose	Accession #/ Source/Pedigree	Heterotic Group	Assumed Resistant Gene	Days to Silking	Disease Rating	Lesion Type
73353	R Source	Cornell University, USA	Tropical	Htm1Htm1	87	3.2	R/MR
H102	R Source	PI 550496, USA	Tropical	Htm1Htm1	89	2.9	MS
LH123HT	R Source	PI 601079, USA	NS-Oh43	Ht1Ht1	96	4.0	R/S
Pa91	Inbred check	PI 587147, USA	SS	None	91	4.9	S
Pa91 <i>Ht1</i>	Inbred check	Ames 25372, USA	SS	Ht1Ht1	92	3.9	R/S
Pa91Ht2	R Source	Ames 25373, USA	SS	Ht2Ht2	94	3.6	MR/MS
Pa91Ht3	R Source	Ames 25374, USA	SS	Ht3Ht3	91	3.2	MR/MS
CO353	Inbred check	AAFC	SS	Unknown	87	3.9	MR/MS
CO388	$Line \times tester$	AAFC, (B73 × CO272) CO272	SS-B73	None	82	6.5	S
BLT01	$Line \times tester$	AAFC, (CO388 \times A553N)CO388(4)	SS-B73	Htn1Htn1	85	5.7	MR/MS
BLT02	$Line \times tester$	AAFC, (CO388 \times A632HtN)CO388(4)	SS-B73	Htn1Htn1	81	5.4	MR/MS
BLT03	$Line \times tester$	AAFC, (CO388 \times H102)CO388(4)	SS-B73	Htm1Htm1	83	5.6	MR/MS
CO428	Line	AAFC, OH43 \times H99	NS-Oh43	PGPG	84	3.1	MS
BLT05	Line	AAFC, (CO428 × 73353)CO428(4)	NS-Oh43	PGHtm1 PGHtm1	81	3.5	MR/MS
BLT06	Line	AAFC, (CO428 \times A509N)CO428(4)	NS-Oh43	PGHtn1 PGHtn1	85	2.8	R/MR
BLT07	Line	AAFC, (CO428 \times A619Ht2)CO428(4)	NS-Oh43	PGHt2 PGHt2	83	3.5	MR/MS
BLT09	Line	AAFC, (CO428 × A632HtN)CO428(4)	NS-Oh43	PGHtn1 PGHtn1	85	2.9	R/MR
BLT10	Line	AAFC, (CO428 × H102)CO428(4)	NS-Oh43	PGHtm1 PGHtm1	83	2.2	MR/MS
BLT11	Line	AAFC, (CO428 \times LH123Ht)CO428(4)	NS-Oh43	PGHt1 PGHt1	84	2.5	R/MR
BLT12	Line	AAFC, (CO428 \times Pa91Ht2)CO428(4)	NS-Oh43	PGHt2 PGHt2	85	1.7	R/MR
BLT13	Line	AAFC, (CO428 \times Pa91Ht3)CO428(4)	NS-Oh43	PGHt3 PGHt3	85	1.8	R/MR
CL30	Tester	AAFC	NS-Flint	None	70	7.0	S
CO442	Tester	AAFC	NS-Iodent	None	79	7.0	S
T1	Tester	Thurston Genetics, USA	NS-Iodent	None	82	7.0	S
T2	Tester	Thurston Genetics, USA	NS-Iodent	None	79	5.8	SMS
T3	Tester	Thurston Genetics, USA	NS-Iodent	Partial	78	6.1	S
T4	Tester	Thurston Genetics, USA	SS-B14	None	78	6.8	S
T5	Tester	MBS Genetics, USA	SS-B14	None	79	6.9	S

PI or Ames number is the number from USDA-MWA-PIRU and NCRPIS, 1305 State Avenue, Ames, Iowa 50014, United States. AAFC means Agriculture and Agri-Food Canada, Ottawa Research and Developmental Centre, Ottawa, Ontario, K1A 0C6, Canada. SS = stiff stalk group, NS = non-stiff stalk group; None = no resistant gene, PG = polygene resistance, Partial = partial resistance. A509N and LH123HT data from previous records.

2.2. Experiment Method

Based on the seeds available, a total of 163 genotypes, including 33 inbred lines and 120 crosses, and 10 commercial hybrid checks, were used for NCLB artificial inoculation in 2015 and 2016. Except for A509N and LH123HT in Table 1, all other 33 lines were included in this Study. Stiff stalk (SS) line CO388 and its *Ht* version BLT01, BLT02, and BLT03 were used as lines to cross with a non-stiff stalk (NS) A619, A619Ht1, A619Ht2, A619Ht3 (Oh43 group), CL30 (early flint group), CO442, T1, T2, and T3 (Iodent group); these were also used as testers to cross with NS-Oh43 lines CO428 and its Ht version BLT05-BLT13. CO428 and its Ht version BLT05-BLT13 also crossed with CL30, CO442, and two SS-B14 group lines, T4 and T5. To evaluate *Ht* gene effects for NCLB development, CO428 and its *Ht* version BLT05-BLT13 also crossed with the same group A619 and its *Ht* versions. In the ANOVA statistics, CO388 was only used as a tester; therefore, it had 11 lines, 14 testers, and 8 inbred checks. Ten commercial hybrids were used as hybrid checks, including 3 hybrids with the Ht gene. A randomized complete block has 3 replications, a one-row plot with a row distance of 0.76 m and a 3.5 m row length for 20 plants. To reduce the shade effects of hybrid to inbred, this randomized complete block was modified by separating inbreds and hybrids into two parts in each block by adding one row inbred; inbreds and hybrids were randomized in their parts. All genotypes were planted on May 7 at Centre Experimental Farm, Ottawa Research and Development Centre, Agriculture and Agri-Food Canada in both 2015 and 2016.

Two artificial inoculations were used to create an epidemic environment. The first inoculation time was at the 6–8 leaf stage on June 18 in both 2015 and 2016; the second inoculation time was at the 10–12 leaf stage on July 2 and July 4 for 2015 and 2016, respectively. Collected infected leaves from previous NCLB nursery and yearly corn disease survey in Ontario, Canada were ground to powder as inoculum, and two doses of powder (equivalent to 0.2 g) from a Bazooka (Sistrunk Inoculators, Starkville, MS, USA) were injected into the whorl of each plant [61]. To make an environment that favors disease development and epidemic, 10–15 min of irrigation (equivalent to 5–8 mm rainfall) from above the plants was conducted in the afternoon to add soil moisture and reduce air temperature from the first inoculation date except for rainy days. The irrigation must make the whorl have water in the morning, which is a key factor for a successful inoculation with leaf powder because only wet powder could produce spores for disease infection.

Specific resistances, such as the lesion type, were recorded twice, three weeks after the first inoculation and four weeks after the second inoculation. There are four lesion types: resistant lesion (R), stripe or narrow elliptical green-yellowish lesion; moderately resistant lesion (MR), narrow, long lesion with green-yellowish or -purplish margin and small elliptical gray center; moderately susceptible lesion (MS), long, elliptical, gray lesion with green-yellowish or -purplish margin; and susceptible lesion (S), long, elliptical, gray or tan colored lesion. General resistances and the disease ratings (DR) were recorded twice, in late August for early flowering genotypes or in middle September for late flowering genotypes, about 4 weeks post silk emergence. General resistance has 7 DRs where: 1 = nosymptoms; $2 \le 1\%$; 3 = 1-10%; 4 = 11-25% of leaves symptomatic; $5 \ge 50\%$ of the lower leaves are symptomatic and <25% of middle and upper leaves are symptomatic; 6 = bottom leaves are dead, >50% of the middle leaves, and <25% of upper leaves are symptomatic; and, 7 = plant is dead. Middle leaves refer to the four leaves near the primary ear emergence. If DR were uniform, then the DR was recorded as row base; If more than one rating scale in a genotype, the numbers of plants with different rating scales were counted. The average DR was used for further analysis.

In early September, the number of lesions was counted from the leaf above the primary ear, 5 plants for each row (genotype), and the average number of lesions per leaf (NLPL) was used for further analysis. Ten typical, single, developed lesions were measured in their lesion length (cm) and lesion width (cm) for each genotype. For genotypes with more than one kind of lesion type, each type measured up to 10 lesions depending on the number found. The average lesion length and lesion width were used to calculate lesion size (LS). LS (cm²) = $0.75 \times$ Lesion length (cm) \times Lesion width (cm). Lesion number count and lesion size measurement were labor-intensive. To optimize efficiency, each replication was performed on the same day by the same person.

Average NLPL, LS, and DR were used for further analysis, representing three resistances to invasion, extension, and explosion (epidemics) of disease development.

2.3. Statistical Methods for Gene Effect Comparison

Analysis of Variance (ANOVA) using all genotypes for multi-environment trial analysis was based on the books of Crossa [62] and Gomez and Gomez [63].

Resistant genes were grouped differently to test the effects between the two groups by combining data from 2015 to 2016. Because of the sample size difference, a different comparison method was used to test the significance of the gene effect:

$$t = \frac{m1 - m2}{\sqrt{\frac{s1^2}{n1} + \frac{s2^2}{n^2}}} = \frac{m1 - m2}{\sqrt{A + B}}$$
(1)

df =
$$\frac{(A+B)^2}{\frac{A^2}{(n1-1)} + \frac{B^2}{(n2-1)}}$$
 (2)

Formula (1) and (2) are Welch's version [64,65] of the two-sample *t*-test; it differs from Student's original version. Where m1 and m2 are the two sample means from samples of size n1 and n2, and s1 and s2 are their estimates of the standard deviation. In this case, m1 presents the group means with different resistant genes, and m2 presents the check mean, m1–m2 present the gene effect in the same group; $n1 \neq n2 =$ genotype number × replication number × year number = 6 × genotype number.

2.4. Statistical Methods for Line × Tester Analysis

To understand the genetic effects of different *Ht* genes with different backgrounds, there were two Line × Tester (L × T) experiments, CO388 and its *Ht* version lines BLT01, BLT02, and BLT03 crossed with 9 testers, and CO428 and its *Ht* version lines BLT05, BLT06, BLT07, BLT10, and BLT12 crossed with 7 testers. Two L × T experiments were analyzed separately due to different resistant backgrounds, but parent data of A619, A619Ht1, A619Ht2, and A619Ht3 were used in both L × T analysis, parent data of CO388, BLT02, and BLT03 were also used as Testers' data in CO428 related L × T analysis. The 2015 and 2016 single-year data analysis for L × T was based on the methods of Abu Assar [66] and Sharma [67]. The Analysis of Variance for two-year combined data was based on the SAS codes of Ejigu [68]. Other statistical analyses, such as GCA and SCA, the standard error for combining ability effects, and genetic components, were similar, but the mean square of pooled error of two-year results was used as M_e, and Year# (y = 2) × Replication# (r = 3) = 6 was used to replace r.

Genetic components formulas were as follows:

$$\sigma_{\text{gca}}^2$$
 (line) = Cov. half sib (line) = $(M_l - M_{l \times t})/(\text{yrt})$ (3)

$$\sigma_{\text{gca}}^2$$
 (tester) = Cov. half sib (tester) = $(M_t - M_{l \times t})/(\text{yrl})$ (4)

for corn, $\sigma_{gca}^2 = \text{Cov. half sib (average)} = \{[(l-1)M_l + (t-1)M_t]/(l+t-2) - M_{l\times t}\}/[yr(2lt-l-t)] = \sigma_A^2/4$ (5)

as follows:

for corn,
$$\sigma^2_{\text{sca}} = (M_{1 \times t} - M_e)/(\text{yr}) = \sigma^2_D$$
 (6)

Standard error (S.E.) calculation formulas for mean effects for GCA and SCA were

S.E.(GCA for lines) =
$$[M_e/(yrt)]^{1/2}$$
 (7)

S.E.(GCA for testers) =
$$[M_e/(yrl)]^{1/2}$$
 (8)

S.E.(SCA effects) =
$$[M_e/(yr)]^{1/2}$$
 (9)

S.E.
$$(g_i - g_i)$$
line = $[2M_e/(yrt)]^{1/2}$ (10)

S.E.
$$(g_i - g_i)$$
tester = $[2M_e/(yrl)]^{1/2}$ (11)

S.E.(S_{ij}) =
$$[(l - 1)(t - 1)M_e/(yrlt)]^{1/2}$$
 (12)

S.E.
$$(S_{ij} - S_{ik}) = [(2Me/(yrl))]^{1/2}$$
 (13)

S.E.
$$(S_{ik} - S_{jk}) = [(2Me/(yrt))^{1/2}$$
 (14)

$$S.E.(S_{ii} - S_{kl}) = [2M_e/(yr)]^{1/2}$$
(15)

Formulas for heritability (H) based on genotype basis and heritability in the narrow sense (H(ns)) based on additive genetic variance were the followings:

$$\sigma^2_e = M_e \tag{16}$$

$$\sigma^2_{\rm GY} = (M_{\rm GY} - M_e)/r \tag{17}$$

$$\sigma^2_{\rm G} = (M_{\rm G} - M_{\rm GY})/(ry)$$
 (18)

for corn,
$$\sigma_A^2 = 4\sigma_{gca}^2$$
 (19)

$$H = \sigma_{G}^{2} / [\sigma_{G}^{2} + \sigma_{GY}^{2} / y + \sigma_{e}^{2} / (ry)] = (M_{G} - M_{GY}) / M_{G}$$
(20)

$$H(ns) = \sigma_A^2 / [\sigma_G^2 + \sigma_{GY}^2 / y + \sigma_e^2 / (ry)] = \sigma_A^2 / M_G$$
(21)

where M_l , M_t , $M_{l\times t}$, M_{e_r} , M_{GY} , and M_G are mean squares for lines, testers, $L \times T$, and pooled error, Genotypes \times Years, and Genotypes, respectively; y, r, l, and t are the number of years, replications, lines, and testers, respectively. σ^2_A and σ^2_D are additive and dominance variances.

2.5. Statistical Methods for MPH and BPH Analysis

The mean of parents and F1 hybrids from combined 2015 and 2016 data of two $L \times T$ were utilized for the estimation of heterosis. The mid-parent heterosis (MPH) and the better-parent heterobeltiosis (BPH) were estimated as follows [69]:

$$MPH(\%) = [(F1 - MP)/MP] \times 100$$
(22)

$$BPH(\%) = [(F1 - BP)/BP] \times 100$$
(23)

The significance of heterosis was tested using the formula as suggested by Wynne et al. [70]

't' over mid-parent heterosis, $t = (F1 - MP)/[2M_e/(yr)]^{1/2}$, with degree of freedom of M_e. (24)

't' over heterobeltiosis, t = $(F1 - BP)/[2M_e/(yr)]^{1/2}$, with degree of freedom of M_e. (25)

where F1 is the mean of the F1 hybrid; BP is the mean of the better parent for resistance; MP is the mean of the two parents; M_e is the mean square of pooled error; y and r are the numbers of years and replications, respectively.

To explain differences between 2015 and 2016, data for climatic factors, such as daily maximum and minimum temperatures (Tx and Tn) and daily rainfall, were collected from the weather station on the farm about 200–250 m far from the experimental field. Tx and Tn are used to calculate daily corn heat units (DCHU), DCHU = $[1.8 \times (Tn - 4.4) + 3.33 \times (Tx - 10) + 0.084 \times (Tx - 10)^2]/2$. If Tx > 30 °C, let Tx = 30. Accumulated CHU (ACHU) is the total DCHU from the planting date to the end date; if DCHU < 0, let DCHU = 0 [71]. The accumulated rainfall is the total rainfall from the planting date to the end date to the end date. It is possible to see the climatic differences between ACHU and accumulated rainfall between 2015 and 2016.

All statistics were conducted with Excel 2016 (Microsoft office Professional Plus 2016). However, R (R version 3.4.4) results from package "agricolae" [72] and SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA) results were used to make sure all Excel results were right for ANOVA and L \times T analysis. Only Excel results were used because they were more accurate due to more decimals involved.

3. Results and Discussions

3.1. Inbred Data

In a cold, short-season area such as Ottawa, Canada (2700 CHU \approx 90 days to relative maturity), four key factors were used to judge whether *Ht* genes were successfully back-crossed. (1) To make sure the dominant gene was introgressed. For NCLB, qualitative traits lesion type and margin color and quantitative trait DR were used to judge all new lines. These lines, except BLT10, had similar lesion types and the same margin colors as the donor had (Table 1). Line 73,353 and A509N had yellow-purplish margin, and A553N, A619Ht2,

A632*HtN*, LH123*Ht*, Pa91*Ht2*, and Pa91*Ht3* had purplish margin. H102 was without a typical margin, but when crossed with CO388, their resistant selections had a yellow-purplish margin. The purplish margin could be used as a marker to select resistant plants. (2) Their disease ratings at the late stage should be better than susceptible receptor CO388 or close to the polygene receptor CO428. For BLT10, because CO428 and H102 had similar lesions without margin color, BLT10 had a better rating (2.2) than CO428 (3.1). Table 1 shows their success. (3) Their silking or pollen-shedding date was closer to the resistant gene acceptor. Because all donors were silking 1–10 days later than CO388 and CO428, and selected lines BLT01-BLT03 were silking -1 to 3 days differently with CO388, BLT05-BLT13 were silking -3 to 1 days differently with CO428 (Table 1). (4) Selected lines kept the similar general combining ability to yield. Our results showed that under non-NCLB epidemic conditions, new lines and their crosses had similar yields; after artificially inoculating once or twice, resistant crosses had about a 4–56% yield increase. The resistant gene effects on yield traits will be reported in another paper.

All *Ht1* resistant-sources had both R and S, and other resistant sources had R and MR or MR and MS lesion types (Table 1). When *Htn1* and *Htm1* were introduced into CO388, BLT01, BLT02, and BLT03, they had the same lesion types as their resistant sources, but when *Ht2*, *Ht3*, *Htm1*, and *Htn1* were introduced into the polygene line CO428, their lesion types were better, and more R and MR lesion types appeared. Some resistant sources, such as A632*HtN*, Pa91*Ht1*, Pa91*Ht2*, Pa91*Ht3* and CO353, had purple margins. It proved three things: (1). Mixed NCLB-diseased leaf powder could be used as inoculum, which included multiple physiological races [54]. (2). Lesion type + margin color, yellow or purple, can be used similarly as a gene marker for NCLB-resistant breeding, but margin color was related to different genotype backgrounds. (3). With a PG resistance background, *Ht2*, *Ht3*, *Htm1*, and *Htn1* could improve their lesion types, which means the specific resistant lesion type can be modified by other genes.

3.2. ANOVA Results

The ANOVA results for DR, NLPL, and LS, by separate year, showed that most sources were significant, but replications and hybrids vs. inbreds in 2015 and inbred checks and testers in 2016 for LS were not significant. All CVs were in a range of 11.1–47.6%, CV for LS > NLPL > DR. The significance of replications and bigger CV indicated that NCLB developed variably, which was mainly caused by three reasons: (1) Resistance of genotypes had a bigger difference from high resistance (DR < 2) to highly susceptible (DR = 7); (2) A genotype between two different genotypes might express resistance quantitatively differently; for example, a resistant genotype between two highly susceptible genotypes rating would increase by more than 1, and a susceptible genotype between two resistant genotypes rating would reduce by 1. (3) The irrigation system could have been affected by wind; one irrigation bird can reach 34.2 m in radius at mild wind environment, but it shifts to one direction when there is stronger wind, resulting in some corner rows (genotypes) not being irrigated well and developing less disease. The combined ANOVA resulted in resistance being significantly different between years. The year 2015 had a warmer May, but 2016 had a little warmer summer; therefore, the final ACHUs were almost the same, 3337.1 and 3331.6 in 2015 and 2016, respectively (Figure 1A). It had much more rainfall in June 2015 before the first inoculation, which did not affect the disease epidemic. It rained 8 days with a total of 133.4 mm from August 11 to 21 in 2016, in which 5 days rained 2.2–74.5 mm. During the same period, it rained 54.4 mm in 5 days, but it rained 35.2 and 17.8 mm on August 11 and 20, only 1.2 mm in the middle in 2015 (Figure 1B). This difference made 2016 have heavier infections than 2015.



Figure 1. (**A**): Accumulated corn heat units (ACHU), and (**B**): accumulated rainfall (mm), from planting date (May 7th) to 30 September 2015 and 2016. Arrow 1 and arrow 2 indicate the 1st and 2nd inoculation dates. The first inoculation date was June 16th in both years; the second inoculation date was 2 July 2015 and 4 July 2016.

3.3. Effects of Resistant Genes on Disease Traits

Table 2 shows the detailed effects of different female and male resistant gene combinations on NCLB. If only females with resistant genes, all PG (CO428) background resistances were better than CO388 background resistances, better than no resistant gene crosses. With CO388 background, *Htm1* had less NLPL, but *Htn1* had a smaller LS. With CO428 background, PG had the lowest DR, NLPL, and LS, which meant none of the new inbreds had better resistance than CO428 in crosses. When combining all male resistant genes, similarly, with CO388 background, *Htm1* had less NLPL, but *Htm1* had a smaller LS; with CO428 background, PGHt1 (BLT11) had less DR and LS than PG (CO428) and others, but not statistically significant. When combining all female genes, *Htm1* (BLT03) and Htn1 (BLT01 and BLT02) had less NLPL, but Ht2 (A619Ht2) and Ht3 (A619Ht3) had a smaller LS. Overall, these four male genes had a better effect than *Ht1* (A619*Ht1*) and partial resistance (T3). Table 2 also shows that when females had no resistant gene (CO388) crossed with other *Ht* genes, *Ht1*, *Ht2*, and *Ht3* had similar resistance to DR, better than partial resistance and susceptible crosses. *Ht1* (A619*Ht1*) had less NLPL and had the best overall effects of DR; Ht2 (A619Ht2) had less NLPL and LS than Ht3 (A619Ht3); however, Ht3 had smaller DR, which meant that CO388 \times A619*Ht3* had more drying leaves caused by lesions than CO388 \times A619*Ht*2. *Ht*3 and partial had similar resistance to NLPL. These results indicated that if only one resistant gene is available, Ht1 is still a good choice to make resistant crosses.

Table 2. Resistant gene effects on disease rating (DR), number of lesions per leaf (NLPL), and lesion size (LS, cm²) of northern corn leaf blight compared with same female or male gene or combined all female or all male gene backgrounds.

			Gene Effects and Significance			
Assumed Gene from Female	Assumed Gene from Male	Count	DR	NLPL	LS (cm ²)	
Ef	fects of resistant gene(s) in the female of	crossed with male	e without resistant ge	ene		
-	-	30	6.5	8.1	23.8	
Htm1	-	36	-0.8 **	-2.4 **	-6.2 *	
Htn1	-	72	-0.9 **	-2.1 **	-9.9 **	
PG	-	12	-1.8 **	-4.2 **	-11.1 **	
PGHT	-	12	-1.5 **	-3.0 **	-10.5 **	
PGHt2	-	60	-1.2 **	-3.2 **	-10.4 **	
PGHt3	-	30	-1.5 **	-3.7 **	-10.8 **	
PGHtm1	-	66	-1.4 **	-3.5 **	-8.6 ""	
rgnini	-	00	-1.4	-3.9	-0.0	
	Effects of resistant gene(s) in the fer	male after combin	red all male genes	7.0	10.4	
- Utm1	All	54	0.7 **	7.0	19.4 4.7 m	
111///1 Htm1		108	-0.7	-2.0	-4.7 115	
PC-		42	_19**			
PCHT1	A11	48	_21 **	-39**	_10.7 **	
PGHt2	A11	132	-16**	-35**	_89*	
PGHt3	A11	54	-16**	-34**	-89*	
PGHtm1	All	126	-16**	-37**	-8.3 *	
PGHtn1	All	108	-1.5 **	-3.6 **	-7.3 *	
	ngle genes by being introduced into p	olvæne resistance	e after combining all	male genes		
PG	All	42	4.0	2.7	9.0	
PGHT1	All	48	-0.2 ns	0.4 ns	-0.3 ns	
PGHt2	All	132	0.3 ns	0.8 ns	1.5 ns	
PGHt3	All	54	0.3 ns	0.9 ns	1.6 ns	
PGHtm1	All	126	0.3 ns	0.7 ns	2.1 ns	
PGHtn1	All	108	0.5 ns	0.7 ns	3.1 ns	
	Effects of single genes in the male	after combining	all female genes			
All		360	5.4	5.3	15.5	
All	Ht1	72	-1.3 **	-1.6 **	-6.0 **	
All	Ht2	78	-1.7 **	-2.2 **	-9.9 **	
All	Ht3	84	-1.8 **	-2.6 **	-7.8 **	
All	Partial	24	-0.3 ns	-1.0 *	-4.7 **	
All	Htm1	42	-1.8 **	-3.6 **	-6.2 **	
All	Htn1	66	-1.7 **	-3.4 **	-6.4 **	
	Effects of single genes in the male who	en crossed with th	ne same female gene			
-	-	30	6.5	8.1	23.8	
-	Ht1	6	-1.7 **	-3.4 **	-10.8 *	
-	Ht2	6	-1.4 *	-2.9 **	-12.8 **	
-	Ht3	6	-1.5 *	-1.7 ns	-8.4 **	
	Partial	6	-0.5 ns	-1.9 *	-5.2 ns	
Htm1	-	36	5.8	6.1	17.5	
Htm1	Ht1	6	-1.4 *	-2.3 **	-10.0 **	
Htm1	Ht2	6	-1.6 *	-2.9 **	-10.6 **	
Htm1	Ht3	6	-1.5 *	-2.4 **	-8.5 **	
<i>Htm</i> 1	Partial	6	-1.0 ns	-2.3 ns	-6.4 ns	
Htn1	-	60	5.7	6.6	15.1	
Htn1	Ht1	12	-1.2 **	-1.8 **	-4.2 *	
Htn1	Ht2	12	-1.3 **	-2.2 **	-8.1 **	
Htn1	Ht3 Dortial	12	-1.9 **	-2.4 **	-7.5 **	
	Partial	12	-0.9 **	-3.0 **	-7.8 ""	
PG	-	12	4.7	4.0	12.6	
rG PC	Htl Ut2	6	-0.8 ns	-1.1 ns	-3.0 ns	
rG PC	Пt2 Ц12	6	-1.0 ns	-1.1 ns	-/.8 **	
rG PC	ПІЭ Ціт1	0	-1.1 * 1 1 *	-2.0 *	-0.9 ***	
	111///1 114	6	-1.1 11*	-2.5	-2.7 IIS	
rG	111/11	0	-1.1	-2.4	-5.2 IIS	

	Assumed Cone from Male		Gene Effects and Significance			
Assumed Gene from Female	Assumed Gene from Male	Count	DR	NLPL	LS (cm ²)	
PGHt1	-	12	5.0	5.1	13.3	
PGHt1	Ht1	6	-1.2 *	-1.7 *	-5.4 *	
PGHt1	Ht2	6	-1.7 **	-2.2 **	-10.2 **	
PGHt1	Ht3	6	-1.7 **	-2.8 **	-3.5 ns	
PGHt1	Htm1	6	-1.3 *	-3.2 **	-3.7 ns	
PGHt1	Htn1	12	-1.8 **	-3.2 **	-6.9 **	
PGHt2	-	60	5.2	4.9	16.6	
PGHt2	Ht1	12	-1.6 **	-1.5 **	-1.4 ns	
PGHt2	Ht2	12	-2.1 **	-1.9 **	-9.9 **	
PGHt2	Ht3	18	-2.0 **	-3.1 **	-7.5 **	
PGHt2	Htm1	12	-1.9 **	-3.2 **	-4.0 *	
PGHt2	Htn1	18	-1.2 **	-3.1 **	-3.4 *	
PGHt3	-	30	5.0	4.4	13.0	
PGHt3	Ht1	6	-1.1 *	-0.1 ns	-5.0 *	
PGHt3	Ht2	6	-2.0 **	-2.2 **	-8.1 **	
PGHt3	Ht3	6	-1.4 *	-2.4 **	-6.5 **	
PGHt3	Htn1	6	-1.2 *	-2.6 **	-2.5 *	
PGHtm1	-	66	5.1	4.6	15.2	
PGHtm1	Ht1	12	-1.4 **	-2.1 **	-9.3 **	
PGHtm1	Ht2	12	-1.9 **	-2.5 **	-9.1 **	
PGHtm1	Ht3	12	-1.6 **	-2.7 **	-10.1 **	
PGHtm1	Htm1	12	-1.6 **	-2.9 **	-6.5 **	
PGHtm1	Htn1	12	-1.7 **	-2.8 **	-7.4 **	
PGHtn1	-	60	5.1	4.2	14.9	
PGHtn1	Ht1	6	-1.0 ns	-0.8 ns	-5.3 **	
PGHtn1	Ht2	12	-1.8 **	-1.8 **	-10.7 **	
PGHtn1	Ht3	12	-1.8 **	-2.2 **	-6.5 **	
PGHtn1	Htm1	6	-1.4 *	-2.6 **	-5.3 **	
PGHtn1	Htn1	12	-1.1 **	-1.8 **	-2.9 ns	

Table 2. Cont.

"-" = no specific gene from female or male, "PG" = polygene resistance; Count = year # × replicate # × cross #; Gene effect = (gene mean – check mean) in the same group. "ns", "*", and "**" means significance at p > 0.05, $p \le 0.05$, and $p \le 0.01$, respectively; The row without significance markers is the group check used to compare the gene effects with its following rows.

When a female had *Htm1* (BLT03) crossed with other genes, results were similar as above, but adding the *Ht2* gene in the male had the best resistance, better than *Ht1*, *Ht3*, and partial resistance. When a female had *Htn1* (BLT01 and BLT02) crossed with other genes, adding the *Ht3* gene in the male had the best resistance, better than *Ht1*, *Ht2*, and partial resistance. *Htm1/Ht2* and *Htn1/Ht3* were two better combinations.

When a female had PG (CO428) crossed with other genes, *Ht2*, *Ht3*, *Htm1* (BLT03), and *Htn1* (BLT02) had similar resistance to DR, but *Htm1* and *Htn1* had better resistance to NLPL, while Ht2 and Ht3 had better resistance to LS. When a female had PGHt1 (BLT11) crossed with other genes, *Htm1* and *Htm1* expressed better resistance to NLPL, and *Ht2* expressed the best resistance to LS. $PGHt1 \times Htn1$ and $PGHt1 \times Ht2$ were two good resistant combinations. $PGHt1 \times Ht1$ had a rating of 3.7, and in such an artificial epidemic environment, the resistance was still very good. When a female had PGHt2 (BLT07 and BLT12) crossed with other genes, male genes Ht_2 and Ht_3 had better resistance to DR than other Ht genes. PGHt₂ × Ht₂ and $PGHt2 \times Ht3$ were two better resistant combinations. When a female had PGHt3 (BLT13) crossed with other genes, *Ht*² expressed the best resistance to DR and LS than other *Ht* genes. $PGHt3 \times Ht2$ was the best resistant combination. When a female had PGHtm1 (BLT05 and BLT10) crossed with other genes, the male gene Ht_2 expressed better resistance to DR than other *Ht* genes. PGHtm1/Ht2 and PGHtm1/Ht3 were better gene combinations. When a female had PGHtn1 (BLT06 and BLT09) crossed with other genes, Ht2 and Ht3 expressed better resistance to DR than other Ht genes. In most cases, Ht2 and Ht3 had smaller LS significantly more than other genes; meanwhile, *Htm1* and *Htm1* had less NLPL while not statistically significant. PGHtn1/Ht2 and PGHtn1/Ht3 were better combinations.

Overall, test crosses of CO388 and its Ht version BLT01, BLT02, and BLT03 showed that both parents with *Ht* genes had the best resistance, followed by only one parent with *Ht* gene; no *Ht* gene with any parent was most susceptible; all significant to DR, and LS, but not all significant with NLPL. CO428 and its Ht version BLT05, BLT06, BLT07, BLT09, BLT10, BLT11, BLT12, and BLT13 crossed with testers showed that with the same female resistant gene background, adding male *Ht* genes increased resistance. However, with the same male Ht gene background, a female with a PG background had slightly better resistance than PGHt(s) background, which meant that when the dominant Ht gene was introduced into the PG background, and PG lost one or more minor resistant gene(s). For BLT05 to BLT13, their PG backgrounds were not the same as CO428. For 10 commercial hybrid checks, 3 are with *Ht* genes, but 1 was very susceptible at the late stage and was one of the highly susceptible hybrids. It was true that *Ht1*, *Ht2*, and *Ht3* were not good enough for CO388 (SS-B73) background; only Htm1 and Htm1 in CO388 expressed tolerance to NCLB at the late stage. All five *Ht* genes were not good enough for CO442 background because they all died at the late stage. CO442 was one of the AAFC best GCA inbred, highly yielded with all other heterotic group inbreds, but was not a good *Ht* gene receptor.

3.4. Line \times Tester Results

In L × T of CO388 and its *Ht* versions (Table 3), $\sigma^2_{gca}/\sigma^2_{sca} > 1$ for DR and NLPL, but not for LS, and $(\sigma_D^2/\sigma_A^2)^{1/2} < 1$ for all three traits, which meant additive effects were more important than dominant gene effects for DR and NLPL but both additive and dominant gene effects were not strong for LS. Based on genotype basis, H for DR, NLPL, and LS were 0.87, 0.82, and 0.57, respectively, but H(ns) for the four traits were 0.19, 0.18, 0.22, and 0.18, respectively. H(ns) were much smaller than H, which meant that additive gene effects were not strong, too. The GCA of lines showed that CO388 had all significant positive values for all traits; BLT01, BLT02, and BLT03 all reduced DR, but only BLT01 had significant effects; BLT01 also reduced NLPL and LS significantly; BLT02 only reduced LS, and BLT03 only reduced NLPL significantly. BLT01 and BLT02 both had *Htn1*, but their resistant sources were A553N and A632HtN, respectively. A553N was more resistant than A632HtN (Table 1); therefore, BLT01 was more resistant than BLT02. GCA for nine testers (Table 4) showed that A619Ht1, A619Ht2, A619Ht3, and T3 (partial resistance) all had significant negative values for all traits, implying that all resistant genes reduced NCLB development. Four genotypes without the resistant gene, A619, CL30, T1, and T2, had positive values for all four traits and increased disease development; however, CO442 reduced DR and NLPL significantly, increased LS not significantly, and expressed tolerance in the hybrid. In the SCA for 36 crosses, only 4, 4, and 6 crosses had significant positive or negative values for DR, NLPL, and LS, respectively.

Source	Resistant Gene(s)	DR	NLPL	LS (cm ²)	
	Ge	netic component and heritabil	lity		
σ^2_A		0.16	0.72	5.5	
$\sigma^2_{\rm D}$		0.04	0.16	4.6	
$(\sigma^2_{\rm D}/\sigma^2_{\rm A})^{1/2}$		2.1	2.13	1.1	
Н		0.87	0.82	0.57	
H(ns)		0.19	0.22	0.18	
Lines		Ge	eneral combining ability for li	nes	
BLT01	Htn1	-0.43 **	-0.71 **	-2.10 **	
BLT02	Htn1	-0.01 ns	0.23 ns	-2.81 **	
BLT03	Htm1	-0.12 ns	-0.77 **	0.12 ns	
CO388	-	0.57 **	1.25 **	4.78 **	
S.E(gca for lines)		0.07	0.21	0.77	

Table 3. Genetic component, heritability (H), and heritability in the narrow sense (H(ns)), and general combining abilities of CO388-related Line \times Tester analysis for disease rating (DR), number of lesions per leaf (NLPL), and lesion size (LS, cm²) under artificial inoculations of northern corn leaf blight.

Source	Resistant Gene(s)	DR	NLPL General combining ability for testers 1.40 ** -1.27 ** -1.47 ** -1.12 ** 3.55 ** -1.21 ** 0.48 ns 1.11 ** -1.47 ** 0.31	LS (cm ²)
Testers		Gei	neral combining ability for tes	ters
A619	-	0.61 **	1.40 **	3.38 **
A619Ht1	Ht1	-0.79 **	-1.27 **	-3.65 **
A619Ht2	Ht2	-0.80 **	-1.47 **	-6.30 **
A619Ht3	Ht3	-1.13 **	-1.12 **	-4.34 **
CL30	-	1.30 **	3.55 **	5.36 **
CO442	-	-0.28 **	-1.21 **	1.46 ns
T1	-	0.59 **	0.48 ns	6.52 **
T2	-	0.74 **	1.11 **	1.29 ns
T3	Partial	-0.24 *	-1.47 **	-3.72 **
S.E(gca for testers)		0.10	0.31	1.16

Table 3. Cont.

"ns", "*", and "**" mean significance at p > 0.05, $p \le 0.05$, and $p \le 0.01$, respectively; "-" = no specific gene from female or male; Partial = partial resistance.

Table 4. Genetic component, heritability (H), and heritability in the narrow sense (H(ns)), and general combining abilities of CO428-related Line \times Tester analysis for disease rating (DR), number of lesions per leaf (NLPL), and lesion size (LS, cm²) under artificial inoculations of northern corn leaf blight.

Source	Resistant Gene(s)	DR	NLPL	LS (cm ²)
Genetic component				
$\sigma^2 A$		0.08	0.33	2.09
$\sigma^2 D$		0.04	0.11	6.21
$(\sigma^2 D / \sigma^2 A)^{1/2}$		1.47	1.70	0.60
H		0.91	0.93	0.68
H(ns)		0.08	0.09	0.13
Lines				
BLT05	PGHtm1	-0.31 **	-0.71 **	-1.92 **
BLT06	PGHtn1	0.23 **	0.16 ns	1.12 *
BLT07	PGHt2	-0.04 ns	0.20 ns	-0.06 ns
BLT10	PGHtm1	0.02 ns	0.18 ns	1.00 ns
BLT12	PGHt2	-0.02 ns	0.23 ns	0.20 ns
CO428	PG	0.12 ns	-0.05 ns	-0.34 ns
S.E(gca for lines)		0.08	0.15	0.49
Testers				
A619	-	1.36 **	2.79 **	4.44 **
A619Ht1	Ht1	-0.04 ns	0.35 *	0.27 ns
A619Ht2	Ht2	-0.63 **	-0.15 ns	-5.15 **
A619Ht3	Ht3	-0.44 **	-0.79 **	-3.49 **
BLT02	Htn1	-0.03 ns	-0.77 **	-0.11 ns
BLT03	Htm1	-0.36 **	-1.10 **	-0.09 ns
CO388	-	0.13 ns	-0.34 *	4.13 **
S.E(gca for testers)		0.09	0.16	0.53

"ns", "*", and "**" = significance at p > 0.05, $p \le 0.05$, and $p \le 0.01$, respectively; "-" = no specific gene from female or male; PG = polygene resistance.

In L × T of CO428 and its *Ht* versions (Table 4), $\sigma_{gca}^2/\sigma_{sca}^2 < 1$ and $(\sigma_D^2/\sigma_A^2)^{1/2} < 1$ for DR and NLPL, which meant both additive and dominant gene effects were not strong to these traits. However, for LS, $(\sigma_D^2/\sigma_A^2)^{1/2} = 1.74$ and $\sigma_{gca}^2/\sigma_{sca}^2 = 0.08$, indicating that non-additive gene action was much more important. Based on genotype basis, H for DR, NLPL, and LS were 0.91, 0.93, and 0.68, respectively, but H(ns) for the four traits were 0.08, 0.07, 0.09, and 0.13, respectively, which meant additive gene effects were also very weak. This was very different with L × T of CO388 and its *Ht* versions, which meant gene effects depended on their background. GCA of lines reduced all traits for disease development, except for BLT05, which had significant negative values. BLT06 had significant positive values for DR and LS. All GCA values for BLT07, BLT10, BLT12, and CO428 were not significant. For the GCA of testers, for DR, both A619 and CO388 had a positive value, but only A619 was significant. All five testers with *Ht* genes had negative values, which meant they reduced disease development, but three of them, *Ht2*, *Ht3*, and *Hm1*, were significant. For NLPL, A619 had a positive value, and CO388 had a negative value; adding *Ht*(S) gene

reduced lesion number, but only *Ht3*, *Htn1*, and *Htm1* reduced significantly. Of the five Ht(s), only Ht2 and Ht3 reduced LS significantly. There were 4, 5, and 9 of 42 crosses that had significant SCA. Both L × T results showed that SCA effects were less important than GCA, but gene effects depend on genotypes.

3.5. MPH and BPH Results

Table 5 shows that MPH and BPH had similar positive or negative for DR and NLPL in most crosses; however, most MPH and BPH were positive for LS, with some BPH over 100% up to 566.3%. NCLB lesion is limited by the veins of the leaf, resulting in smaller leaves having smaller lesions. In CO388-related L \times T, tester CL30 and T1, and in CO428-related L \times T, line BLT06 had relatively smaller leaves, and their LS(s) were smaller, too. However, when crossed with normal size CO388, BLT01, BLT02, and BLT03, their F1 plants were normal; therefore, their LS(s) were normal, too. It seemed that MPH and BPH for LS were highly related to the heterosis of the leaf size. LS is not a good trait for MPH and BPH.

Table 5. The mid-parent heterosis (MPH) and the better-parent heterobeltiosis (BPH) of 36 CO388related and 42 CO428-related Line \times Tester crosses for disease rating (DR), number of lesions per leaf (NLPL), and lesion size (LS, cm²) under artificial inoculations of northern corn leaf blight.

C an alterna	Resistant Gene(S)	D	DR		NLPL		LS (cm ²)	
Genotype		MPH	BPH	MPH	BPH	MPH	BPH	
CO388 × A619	-/-	-4.5 ns	-3.8 ns	-2.0 ns	-1.6 ns	23.8 ns	28.6 ns	
CO388 × A619Ht1	-/Ht1	-16.2 **	-3.7 ns	-32.9 **	-19.4 ns	4.0 ns	22.7 ns	
$CO388 \times A619Ht2$	-/Ht2	-9.1 ns	8.4 ns	-20.2 ns	6.8 ns	1.1 ns	49.2 ns	
CO388 × A619Ht3	-/Ht3	-13.8 **	-0.2 ns	-7.6 ns	10.9 ns	36.7 ns	83.8 *	
$CO388 \times CL30$	-/-	2.6 ns	6.5 ns	38.1 **	50.7 **	73.0 **	96.2 **	
$CO388 \times CO442$	-/-	-9.9 *	-6.6 ns	-26.5 *	-24.9 *	60.4 *	65.9 **	
$CO388 \times T1$	-/-	-4.7 ns	-1.0 ns	-9.5 ns	-6.5 ns	77.6 **	91.5 **	
$CO388 \times T2$	-/-	0.7 ns	3.3 ns	17.3 ns	26.4 *	57.2 *	62.5 **	
$CO388 \times T3$	-/Partial	-4.5 ns	-1.4 ns	-11.0 ns	7.5 ns	54.0 ns	132.6 **	
$BLT01 \times A619$	Htn1/-	-6.8 ns	0.8 ns	-17.0 ns	-13.1 ns	67.5 *	215.3 **	
BLT01 \times A619 <i>Ht</i> 1	Htn1/Ht1	-22.0 **	-16.9 **	-38.0 **	-29.7 *	26.6 ns	82.9 ns	
BLT01 \times A619 <i>Ht</i> 2	Htn1/Ht2	-20.2 **	-11.9 *	-34.2 *	-17.1 ns	4.0 ns	20.4 ns	
BLT01 \times A619Ht3	Htn1/Ht3	-34.4 **	-29.6 **	-48.1 **	-41.1 **	-36.6 ns	-20.1 ns	
$BLT01 \times CL30$	Htn1/-	3.5 ns	15.7 **	16.1 ns	20.3 ns	23.7 ns	163.2 **	
$BLT01 \times CO442$	Htn1/-	-31.4 **	-23.6 **	-47.3 **	-43.2 **	45.8 ns	148.0 *	
$BLT01 \times T1$	Htn1/-	-12.3 **	-1.9 ns	-15.9 ns	-14.4 ns	122.0 **	340.6 **	
$BLT01 \times T2$	Htn1/-	-6.2 ns	3.5 ns	-20.7 ns	-18.9 ns	23.7 ns	131.5 *	
BLT01 \times T3	Htn1/Partial	-24.3 **	-21.4 **	-55.9 **	-49.7 **	23.4 ns	40.2 ns	
$BLT02 \times A619$	Htn1/-	3.0 ns	14.0 **	18.6 ns	43.0 **	84.6 *	283.5 **	
BLT02 \times A619Ht1	Htn1/Ht1	$-6.8 \mathrm{ns}$	-2.9 ns	-6.6 ns	-5.8 ns	56.3 ns	146.6 *	
$BLT02 \times A619Ht2$	Htn1/Ht2	-5.7 ns	1.8 ns	-11.3 ns	-3.4 ns	21.7 ns	52.2 ns	
BLT02 \times A619Ht3	Htn1/Ht3	-20.0 **	-16.1 **	-14.7 ns	-14.0 ns	62.8 ns	122.6 ns	
$BLT02 \times CL30$	Htn1/-	2.7 ns	17.7 **	33.0 *	46.5 **	10.4 ns	160.2 *	
$BLT02 \times CO442$	Htn1/-	-16.4 **	$-4.5 \mathrm{ns}$	-25.2 *	-7.0 ns	46.6 ns	174.0 *	
$BLT02 \times T1$	Htn1/-	-6.8 ns	6.8 ns	-6.5 ns	9.3 ns	21.3 ns	166.1 *	
$BLT02 \times T2$	Htn1/-	-7.8 ns	4.2 ns	0.5 ns	12.2 ns	8.2 ns	123.3 ns	
$BLT02 \times T3$	Htn1/Partial	-10.9 *	-5.4 ns	-25.2 ns	-25.0 ns	19.0 ns	45.7 ns	
$BLT03 \times A619$	Htm1/-	-6.9 ns	1.1 ns	-13.1 ns	3.4 ns	73.3 *	265.7 **	
BLT03 \times A619Ht1	Htm1/Ht1	-17.2 **	-12.1 *	-35.6 *	-35.4 *	12.9 ns	80.7 ns	
BLT03 \times A619 <i>Ht</i> 2	Htm1/Ht2	-18.1 **	-10.0 ns	-39.8 *	-33.6 ns	32.1 ns	67.2 ns	
BLT03 \times A619Ht3	Htm1/Ht3	-17.9 **	-12.3 *	-36.2 *	-36.0 *	52.2 ns	110.9 ns	
$BLT03 \times CL30$	Htm1/-	7.0 ns	20.1 **	59.3 **	73.3 **	116.2 **	418.1 **	
$BLT03 \times CO442$	Htm1/-	-25.3 **	-16.4 **	-64.4 **	-56.3 **	66.0 ns	215.1 **	
$BLT03 \times T1$	Htm1/-	-5.5 ns	6.1 ns	-22.7 ns	-10.8 ns	87.3 **	317.6 **	
$BLT03 \times T2$	Htm1/-	-1.3 ns	9.4 ns	-1.0 ns	9.1 ns	66.5 *	249.1 **	
$BLT03 \times T3$	Htm1/Partial	-17.3 **	-13.8 **	-34.7 *	-34.1 *	103.9 ns	152.9 *	

 $BLT12 \times CO388$

LS (cm²)

Cenotype	Pasistant Cana(S)						cm)
Genotype	Resistant Gene(5)	MPH	BPH	MPH	BPH	MPH	BPH
CO428 × A619	PG/-	9.8 ns	73.2 **	25.3 *	293.5 **	34.4 ns	189.4 **
$CO428 \times A619Ht1$	PG/Ht1	-2.0 ns	29.3 **	-21.3 ns	89.1 *	29.1 ns	110.3 **
$CO428 \times A619Ht2$	PG/Ht2	-4.2 ns	21.4 *	-9.4 ns	89.1 *	-19.3 ns	3.8 ns
$CO428 \times A619Ht3$	PG/Ht3	-9.4 ns	18.6 ns	-45.7 **	30.4 ns	-12.3 ns	23.6 ns
$CO428 \times BLT02$	PG/Htn1	-15.0 *	18.0 ns	-56.0 **	4.3 ns	53.7 ns	57.4 ns
$CO428 \times BLT03$	PG/Htm1	-17.0 *	17.8 ns	-60.4 **	-4.3 ns	112.5 **	115.2 **
CO428 × CO388	PG/-	-14.2 *	34.1 **	-60.8 **	23.9 ns	30.6 ns	165.8 **
$BLT05 \times A619$	PGHtm1/-	-13.7 *	12.6 ns	-23.2 *	96.6 **	-11.5 ns	25.1 ns
BLT05 \times A619Ht1	PGHtm1/Ht1	-15.8 *	-6.4 ns	-39.3 **	20.3 ns	-46.8 *	-40.2 ns
BLT05 \times A619Ht2	PGHtm1/Ht2	-32.6 **	-27.6 **	-49.3 **	-11.9 ns	-35.9 ns	-31.1 ns
BLT05 \times A619Ht3	PGHtm1/Ht3	-30.5 **	-23.2 **	-64.1 **	-28.8 ns	-64.7 **	-64.6 **
$BLT05 \times BLT02$	PGHtm1/Htn1	-28.2 **	-16.4 *	-61.0 **	-23.7sn	25.6 ns	71.2 ns
$BLT05 \times BLT03$	PGHtm1/Htm1	-35.5 **	-23.4 **	-75.3 **	-50.8 ns	0.9 ns	36.9 ns
$BLT05 \times CO388$	PGHtm1/-	-28.2 **	-7.0 ns	-53.3 **	20.3 ns	22.9 ns	65.8 **
$BLT06 \times A619$	PGHtn1/-	13.3 *	91.5 **	8.4 ns	365.6 **	44.9 *	361.6 **
BLT06 \times A619Ht1	PGHtn1/Ht1	6.4 ns	49.3 **	-1.4 ns	218.8 **	44.4 ns	237.0 **
BLT06 \times A619Ht2	PGHtn1/Ht2	-10.6 ns	20.4 ns	-7.9 ns	156.3 **	-20.4 ns	41.5 ns
BLT06 \times A619Ht3	PGHtn1/Ht3	-10.7 ns	24.4 *	-32.4 *	118.8 *	52.1 ns	200.6 **
BLT06 \times BLT02	PGHtn1/Htn1	3.8 ns	53.6 **	-20.6 ns	153.1 **	146.6 **	233.2 **
BLT06 \times BLT03	PGHtn1/Hm1	-10.6 ns	35.3 **	-52.9 **	53.1 ns	155.3 **	240.4 **
BLT06 \times CO388	PGHtn1/-	$-4.5 \mathrm{ns}$	59.8 **	-45.1 **	137.5 **	122.6 **	566.3 **
$BLT07 \times A619$	PGHt2/-	8.6 ns	67.9 **	12.1 ns	175.8 **	42.3 *	130.2 **
BLT07 \times A619 <i>Ht</i> 1	PGHt2/Ht1	-1.5 ns	27.6 **	-6.3 ns	79.0 **	67.4 **	111.5 **
BLT07 \times A619 <i>Ht</i> 2	PGHt2/Ht2	-17.1 *	3.2 ns	-19.2 ns	35.5 ns	-50.4 ns	$-48.8 \mathrm{ns}$
BLT07 \times A619Ht3	PGHt2/Ht3	-18.1 *	5.3 ns	-52.7 **	-9.7 ns	-14.2 ns	-4.4 ns
BLT07 \times BLT02	PGHt2/Htn1	-14.9 *	15.8 ns	-41.9 **	9.7 ns	64.1 *	97.9 *
BLT07 \times BLT03	PGHt2/Htm1	-23.4 **	6.6 ns	-53.8 **	-11.3 ns	88.3 **	129.9 **
BLT07 \times CO388	PGHt2/-	-19.2 **	23.9 *	-53.1 **	16.1 ns	-58.2 **	-35.7 ns
$BLT10 \times A619$	PGHtm1/-	12.3 ns	88.4 **	19.9 ns	462.1 **	47.1 *	205.9 **
BLT10 \times A619 <i>Ht</i> 1	PGHtm1/Ht1	-5.7 ns	31.5 **	-12.7 ns	206.9 **	23.3 ns	94.8 *
BLT10 \times A619 <i>Ht</i> 2	PGHtm1/Ht2	-13.4 ns	15.8 ns	-28.0 ns	117.2 *	-24.3 ns	-5.2 ns
BLT10 \times A619 <i>Ht3</i>	PGHtm1/Ht3	-4.0 ns	6.6 ns	-28.4 ns	151.7 **	5.0 ns	43.8 ns
$BLT10 \times BLT02$	PGHtm1/Htn1	-18.3 *	31.5 **	-40.3 *	106.9 ns	41.6 ns	41.8 ns
$BLT10 \times BLT03$	PGHtm1/Htm1	-8.0 ns	15.8 ns	-30.7 ns	144.8 *	126.9 **	129.1 **
$BLT10 \times CO388$	PGHtm1/-	-12.8 ns	44.9 **	-31.4 **	224.1 **	100.5 **	294.3 **
$BLT12 \times A619$	PGHt2/-	18.2 **	32.9 **	50.9 **	411.9 **	39.1 *	125.5 **
BLT12 \times A619Ht1	PGHt2/Ht1	-15.4 ns	44.9 **	-11.5 ns	128.6 **	11.5 ns	41.0 ns
BLT12 \times A619 <i>Ht</i> 2	PGHt2/Ht2	-23.0 **	20.1 ns	5.3 ns	135.7 **	-53.0 *	-51.5 ns
BLT12 \times A619Ht3	PGHt2/Ht3	-15.7 *	38.4 **	-53.9 **	19.0 ns	-41.4 ns	-34.5 ns
$BLT12 \times BLT02$	PGHt2/Htn1	13.0 ns	62.1 **	-50.5 **	26.2 ns	140.3 **	189.6 *
$BLT12 \times BLT03$	PGHt2/Htm1	-21.4 **	15.4 ns	-56.0 **	14.3 ns	33.8 ns	63.1 ns

-21.7 **

DR

NLPL

Table 5. Cont.

PGHt2/-

"ns", "*", and "**" means significance at p > 0.05, $p \le 0.05$, and $p \le 0.01$, respectively; "-" = no specific gene from female or male, Partial = partial resistance, PG = polygene resistance.

-56.8 **

47.6 ns

18.6 ns

82.7 **

26.9*

For resistance, negative MPH and BPH were better than positive MPH and BPH; the smaller, the better, which means that the F1 hybrid had resistance close to or better than the more resistant parent, and dominant gene action was present. In CO388-related L \times T of Table 5 for DR, line BLT01 had the smallest MPH, followed by BLT03 and BLT02; CO388 had the biggest MPH; for NLPL, line BLT01 had the smallest MPH, followed by BLT03 and CO388; BLT02 had the biggest MPH. It proved that both *Htn1* and *Htm1* genes increased resistance. In CO388-related L \times T, testers with *Ht1*, *Ht2*, *Ht3*, and partial resistance all had negative MDP, 13 out of 16 MDPs were significant, implying the presence of dominant gene action. Some BPH(s) were significantly negative in BLT01, BLT02, and BLT03 for DR and NLPL, implying that the presence of over-dominant gene action also existed, but not often. Testers without resistant genes responded differently for DR and NLPL; CL30 had all positive values; CO442 had all negative values, and A619, T1, and T2 had both positive and negative values for DR and NLPL. As a male, CO442 expressed significant tolerance to DR and NLPL.

In CO428-related L \times T of Table 5 for DR, line BLT05 had the smallest MPH, followed by BLT07, BLT12, BLT10, and CO428; BLT06 had the biggest MPH. For NLPL, line BLT05 had the smallest MPH, followed by CO428, BLT07, BLT12, and BLT06; BLT10 had the biggest MPH. Overall, BLT05 had the best resistance, and BLT06 had the least resistance, which was similar to the GCA results. For testers, A619 crossed with CO428, BLT06, BLT07, BLT10, and BLT12 had positive MPH for DR and NLPL; meanwhile, CO388 crossed with them had significant negative values to these two traits; it proved that CO388 expressed tolerance in hybrids. For others that were crossed with five testers with *Ht* genes, except BLT06 × A619*Ht1* and BLT06 × BLT02, their MPH all were negative values, meaning dominant gene action was present. Only BLT05 crossed with five *Ht* gene lines (A619*Ht1*, A619*Ht2*, A619*Ht3*, BLT02, and BLT03) showed negative BPH to DR; some were significant, implying that over-dominant gene action existed.

3.6. Discussion

This study again proved the NCLB disease cycle difference between susceptible and resistant genotypes [1–5], especially the difference in the latent period. (1) The ground diseased leaf powder needed about 24 h in a wet whorl to produce spores for invasion. (2) This study had complicated genotypes with many resistant gene combinations and a complicated disease population with many races [54], which developed different types of lesions. After the first inoculation, S lesions showed typical symptoms and sporulation approximately in 10–14 days. MS and MR lesions showed typical symptoms and sporulation in approximately 14–28 days. HR and R lesions from some crosses needed approximately 28 days to show typical symptoms and had sporulation at the late stage. Two commercial hybrids never had sporulation. (3) After the second inoculation, the latent period overlapped. (4) When plants got older, the latent period got shorter, but sporulation got smaller, too.

As discussed earlier, NCLB development was affected by genotypes, pathogen pathogenicity, and climatic and irrigation conditions. NCLB epidemic was also affected by temporal- [42] and spatial- [73] systems. In this study, 130 crosses and hybrid checks had silking days of 66–89 days, on average 77 days, and had a plant height of 183–326 cm, on average 254 cm. For early mature genotypes, their plants developed earlier and suffered longer under disease epidemic environments than later maturity genotypes. The concentrations of spores were different in different spatial heights; the top parts of taller genotypes suffered less concentration than lower parts and shorter genotypes. These might be reasons why more tolerant genotypes were screened from later and taller genotypes; even with the same resistant genes, taller hybrids were more resistant than shorter inbreds. The area under the disease progress curve and apparent infection rate [8,19] have some functions of the temporal system. More spatial- and temporal-system studies are needed for disease development.

NCLB caused dead leaf areas by diseased lesions and drying leaf edges or tips. In this study, ear-leaf length, ear-leaf width, and ear-leaf area ranged from 42–104 cm, 6–12 cm, and 222–843 cm², on average 79 cm, 9.4 cm, and 565 cm², respectively. Few lesions on smaller leaf plants caused a bigger percentage of diseased leaves. All CL30 crosses were susceptible, DR > 5.5, because they are shorter and smaller than others. CO428 family × CO388 family, CO388 family × CO442, and CO4288 family × CO442 all had long and wide leaves, which showed tolerance to NCLB, though CO442 was highly susceptible. This kind of tolerance was caused by plant architecture and could not be explained by additive or dominant gene effects.

Average gene effects can be expressed by percentage instead of real data. When resistant genes were in females, *Htm1*, *Htn1*, and PG effects (%) to DR were –13.9, –15.3, and –18.2, to NLPL were –29.4, –32.3, and –38.2, to LS were –26.5, –42.0, and –44.5, respectively. When resistant genes were in males, *Ht1*, *Ht2*, *Ht3*, *Htm1*, *Htm1*, and partial effects (%) to DR were –24.8, –28.5, –29.9, –12.6, –8.6, and –7.2, to NLPL were –42.0, –45.6, –47.6, –29.5, –20.7, and –23.6, to LS were –32.8, –57.8, –43.7, –23.8, –30.9, and –31.3, respectively. Using these percentage data to make MPH and BPH comparisons, it may be possible to resolve the problem in Table 5.

This study included five *Ht genes*, PG, PG*Ht*(s), and their combinations, and its results can be used for better selections. If only a single dominant gene is available, *Ht1* is still a good choice. However, if the pathogen population can overcome *Ht1*, other genes should be selected. CO428, with greener leaves and less sensitive reactions to NCLB, common rust, eyespot, and other leaf diseases, is a better PG choice than its resistant source H99. Compared to single dominant genes, PG and PG*Ht*(s) had better resistance to DR, NLPL, and LS.

4. Conclusions

After 10 years of resistant breeding, Htm1 and Htn1 were successfully introduced into a high-yield GCA inbred CO388, and Ht1, Ht2, Ht3, Htm1, and Htm1 were successfully introduced into a polygene-resistant inbred CO428. If only one parent was with resistance, PG had the best resistance, followed by *Ht*1, which meant if no PG parent was available, the Ht1 parent was still the best choice. The resistance of PGHt(s) was not always better than PG, meaning that one or more minor resistant gene(s) might have been lost when Ht(s) was introduced into PG. If both parents were with resistance, Ht_2 and Ht_3 reduced more LS, and *Htm1* and *Htm1* reduced more NLPL. PG and its *Ht* version crossed with *Ht2*, *Ht3*, *Htm1*, and *Htm1* had good resistance effects. In this study, *Htm1/Ht2* and *Htm1/Ht3*, PG/Htm1, PG/Htn1, PGHt1/Htn1, PGHt1/Ht2, PGHt1/Ht3, PGHt2/Ht2, PGHt2/Ht3, PGHt3/Ht2, PGHtm1/Ht2, PGHtm1/Ht2, and PGHtm1/Ht3 were better combinations. Two $L \times T$ analyses showed that GCA effects were bigger than SCA effects for DR and NLPL in CO388-related L \times T but inverse in CO428-related L \times T; only strong dominant effects were found for LS in CO428-related L \times T. Heritability based on genotype base was high, but heritability in the narrow sense was low in both $L \times T$, which meant that these resistances were heritable, but additive genetic effects were low. Line BLT01 had the best GCA in CO388-related L \times T, and line BLT05 had the best GCA in CO428-related L \times T. MPH and BPH results showed that dominant and over-dominant gene action existed for DR and NLPL. LS was not a good trait for MPH and BPH analysis because it was affected by the leaf size. PG and PGHt(s) are not only good choices for NCLB resistance breeding, reducing risks of different races of the pathogen, but also good choices for multiple-resistance breeding because PG is resistant to multiple leaf diseases.

Author Contributions: Conceptualization, X.Z. and L.M.R.; methodology, X.Z. and L.M.R.; software, X.Z.; validation, X.Z. and Aida Kebede; formal analysis, X.Z.; investigation, X.Z., T.W., K.K.J. and J.W.; data curation, X.Z.; writing—original draft preparation, X.Z.; writing—review and editing, A.K. and X.Z.; supervision, L.M.R. and Aida Kebede; project administration, A.K.; funding acquisition, L.M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by the Agriculture and Agri-Food Canada Growing Forward Partnership with the Canadian Field Crop Research Alliance and Grain Farmers of Ontario, which obtained funding through "Growing Forward 2" (GF2), a federal-provincial-territorial initiative. The Agricultural Adaptation Council assisted in the delivery of GF2 in Ontario.

Data Availability Statement: Data available by contacting correspondence author.

Acknowledgments: We Thank USDA-ARS and Cornell University, USA, for providing resistant sources; MBS Genetics, L.L.C., and Thurston Genetics for providing us tester seeds; and AgReliant Genetics Inc., Westfield, Indiana; Maizex Seeds Inc., Tilbury, ON, Canada; Pioneer Hi-Bred Ltd., Des Moines, IA, USA; and Deklab (Monsanto Canada Inc., Ottawa, ON, Canada) for providing check hybrid seeds. We also thank international students Shiyuan Lu and Qing Zhao from Yangzhou University, China, for field work and data collection. Thank Reza Jamaly for helping work on references with EndNote.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Wise, K. Northern Corn Leaf Blight-Purdue Extension; Purdue University: West Lafayette, IN, USA, 2011; p. 3.
- 2. Levy, Y.; Pataky, J. Epidemiology of northern leaf blight on sweet corn. Phytoparasitica 1992, 20, 53-66. [CrossRef]
- 3. Smith, C.W.; Betrán, J.; Runge, E.C. *Corn: Origin, History, Technology, and Production*; John Wiley & Sons: Hoboken, NJ, USA, 2004; Volume 4.
- 4. Mueller, D.S.; Wise, K.A.; Sisson, A.J.; Allen, T.W.; Bergstrom, G.C.; Bosley, D.B.; Bradley, C.A.; Broders, K.D.; Byamukama, E.; Chilvers, M. Corn yield loss estimates due to diseases in the United States and Ontario, Canada from 2012 to 2015. *Plant Health Prog.* 2016, *17*, 211–222. [CrossRef]
- 5. Salgado, J.D.; Schoenhals, J.; Pierce, A.P. Northern Corn Leaf Blight; Ohio State University Extension: Columbus, OH, USA, 2016.
- 6. Kloppers, R.; Tweer, S. Northern Corn Leaf Blight Fact Sheet; PANNA Seed (Pty) Ltd.: Greytown, South Africa, 2009.
- Nwanosike, M.; Mabagala, R.; Kusolwa, P. Effect of northern leaf blight (*Exserohilum turcicum*) severity on yield of maize (*Zea mays* L.) in Morogoro, Tanzania. *Int. J. Sci. Res.* 2015, 4, 466–475.
- 8. Raymundo, A.; Hooker, A. Measuring the relationship between northern corn leaf blight and yield losses. *Plant Dis.* **1981**, *65*, 325–327. [CrossRef]
- 9. Wang, P.; Souma, K.; Kobayashi, Y.; Iwabuchi, K.; Sato, C.; Masuko, T. Influences of northern leaf blight on corn silage fermentation quality, nutritive value and feed intake by sheep. *Anim. Sci. J.* **2010**, *81*, 487–493. [CrossRef]
- 10. Zhu, X.; Reid, L.M.; Woldemariam, T.; Tenuta, A.U. Survey of corn diseases and pests in Ontario and Québec in 2010. *Can. Plant Dis. Surv.* 2011, *91*, 77–80.
- 11. Jindal, K.K.; Reid, L.M.; Tenuta, A.U.; Woldemariam, T.; Zhu, X. Status of corn diseases in Ontario, 2015 crop season. *Can. Plant Dis. Surv.* 2016, *96*, 102–108.
- 12. Zhu, X.; Reid, L.M.; Presello, D.; Woldmariam, T. Survey of corn pests in Ontario and Quebec in 2000. *Can. Plant Dis. Surv.* 2001, *81*, 48–50.
- 13. Hooker, A. A new type of resistance in corn to Helminthosporium turcicum. Plant Dis. Report. 1961, 45, 780–781.
- 14. Hooker, A. Inheritance of chlorotic-lesion resistance to Helminthosporium turcicum in seedling corn. Phytopathology 1963, 53, 660.
- 15. Hooker, A. Monogenic resistance in Zea mays L. to Helminthosporium turcicum. Crop Sci. 1963, 3, 381–383. [CrossRef]
- 16. Ullstrup, A. Sources of resistance to northern corn leaf blight. *Plant Dis. Rep* 1963, 47, 107–108.
- 17. Hooker, A.; Kim, S.K. Monogenic and multigenic resistance to Helminthosporium turcicum in corn. Plant Dis. Rep. 1973, 57, 586.
- 18. Perkins, J.; Hooker, A. Reactions of eighty-four sources of chlorotic lesion resistance in corn to three biotypes of *Helminthosporium turcicum* [northern corn leaf blight, breeding for disease resistance]. *Plant Dis.* **1981**, *65*, 502–504. [CrossRef]
- 19. Leath, S.; Pedersen, W. Effects of the *Ht*, *Ht*2, and/or *Ht*3 genes in three maize inbreds on quantitative resistance to *Exserohilum turcicum* race 2. *Plant Dis.* **1986**, *70*, *529–531*. [CrossRef]
- Raymundo, A.; Hooker, A. Single and combined effects of monogenic and polygenic resistance on certain components of northern corn leaf blight development. *Phytopathology* 1982, 72, 99–103. [CrossRef]
- 21. Hakiza, J.; Lipps, P.; Martin, S.S.; Pratt, R. Heritability and number of genes controlling partial resistance to *Exserohilum turcicum* in maize inbred H99. *Maydica* 2004, 49, 173–182.
- 22. Carson, M. Response of a maize synthetic to selection for components of partial resistance to *Exserohilum turcicum*. *Plant Dis*. **2006**, *90*, 910–914. [CrossRef]
- 23. Kramer, H.; Ullstrup, A. Preliminary evaluation of exotic maize germ plasm. Agron. J. 1959, 51, 687–689. [CrossRef]
- 24. Welz, H.; Geiger, H. Genes for resistance to northern corn leaf blight in diverse maize populations. *Plant Breed.* **2000**, *119*, 1–14. [CrossRef]
- 25. Hooker, A. A second major gene locus in corn for chlorotic-lesion resistance to *Helminthosporium turicum*. *Crop Sci.* **1977**, *17*, 132–135. [CrossRef]
- 26. Hooker, A. Resistance to *Helminthosporium turcicum* from tripsacum floridanum incorporated into corn. *Maize Genet. Coop. Newslett.* **1981**, *55*, 87–88.
- 27. Gevers, H. A new major gene for resistance to *Helminthosporium turcicum* leaf blight of maize [breeding, fungus diseases]. *Plant Dis. Rep.* **1975**, *59*, 296–300.
- 28. Robbins, W.; Warren, H. Inheritance of resistance to *Exserobilum turcicum* in pi 20935, «mayorbela» variety of maize. *Maydica* **1993**, *38*, 209–213.
- 29. Ogliari, J.B.; Guimarães, M.A.; Camargo, L.E.A. Chromosomal locations of the maize (*Zea mays* L.) *Htp* and *rt* genes that confer resistance to *Exserohilum turcicum*. *Genet*. *Mol. Biol.* **2007**, *30*, 630–634. [CrossRef]
- 30. Ogliari, J.B.; Guimarães, M.A.; Geraldi, I.O.; Camargo, L.E.A. New resistance genes in the Zea mays: Exserohilum turcicum pathosystem. Genet. Mol. Biol. 2005, 28, 435–439. [CrossRef]
- Wang, H.; Xiao, Z.; Wang, F.; Xiao, Y.; Zhao, J.; Zheng, Y.; Qiu, F. Mapping of htnb, a gene conferring non-lesion resistance before heading to *Exserohilum turcicum* (pass.), in a maize inbred line derived from the indonesian variety bramadi. *Genet. Mol. Res.* 2012, 11, 2523–2533. [CrossRef]
- 32. Carson, M. A new gene in maize conferring the "chlorotic halo" reaction to infection by exserohilum turcicum. *Plant Dis.* **1995**, *79*, 717–720. [CrossRef]
- 33. Chung, C.-L.; Jamann, T.; Longfellow, J.; Nelson, R. Characterization and fine-mapping of a resistance locus for northern leaf blight in maize bin 8.06. *Theor. Appl. Genet.* **2010**, *121*, 205–227. [CrossRef]

- Ding, J.; Ali, F.; Chen, G.; Li, H.; Mahuku, G.; Yang, N.; Narro, L.; Magorokosho, C.; Makumbi, D.; Yan, J. Genome-wide association mapping reveals novel sources of resistance to northern corn leaf blight in maize. *BMC Plant Biol.* 2015, 15, 206. [CrossRef]
- 35. Dingerdissen, A.; Geiger, H.; Lee, M.; Schechert, A.; Welz, H. Interval mapping of genes for quantitative resistance of maize to setosphaeria turcica, cause of northern leaf blight, in a tropical environment. *Mol. Breed.* **1996**, *2*, 143–156. [CrossRef]
- Freymark, P.J.; Lee, M.; Martinson, C.; Woodman, W. Molecular-marker-facilitated investigation of host-plant response to Exserohilum turcicum in maize (Zea mays L.): Components of resistance. Theor. Appl. Genet. 1994, 88, 305–313. [CrossRef]
- Poland, J.A.; Bradbury, P.J.; Buckler, E.S.; Nelson, R.J. Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proc. Natl. Acad. Sci. USA* 2011, 108, 6893–6898. [CrossRef]
- 38. Welz, H.; Schechert, A.; Geiger, H. Dynamic gene action at qtls for resistance to setosphaeria turcica in maize. *Theor. Genet.* **1999**, *98*, 1036–1045. [CrossRef]
- 39. Wang, J.; Xu, Z.; Yang, J.; Lu, X.; Zhou, Z.; Zhang, C.; Zheng, L.; Tian, R.; Hao, Z.; Yong, H. *qNCLB7.02*, a novel qtl for resistance to northern corn leaf blight in maize. *Mol. Breed.* **2018**, *38*, 54. [CrossRef]
- 40. Leonard, K.; Levy, Y.; Smith, D. Proposed nomenclature for pathogenic races of Exserohilum turcicum on corn. Plant Dis. 1989, 73, 776–777.
- Moghaddam, P.F.; Pataky, J.K. Reactions of isolates from matings of races 1 and 23n of *Exserohilum turcicum*. *Plant Dis.* 1994, 78, 767–771. [CrossRef]
- Ferguson, L.; Carson, M. Temporal variation in setosphaeria turcica between 1974 and 1994 and origin of races 1, 23, and 23n in the United States. *Phytopathology* 2007, 97, 1501–1511. [CrossRef] [PubMed]
- 43. Simcox, K.; Pedersen, W.; Nickrent, D. Isozyme diversity in Setosphaeria turcica. Can. J. Plant Pathol. 1993, 15, 91–96. [CrossRef]
- 44. Smith, D.; Kinsey, J. Further physiologic specialization in Helminthosporium turcicum. Plant Dis. 1980, 64, 779–781. [CrossRef]
- 45. Thakur, R.; Leonard, K.; Jones, R. Characterization of a new race of *Exserohilum turcicum* virulent on corn with resistance gene htn. *Plant Dis.* **1989**, *73*, 151–155. [CrossRef]
- 46. Thakur, R.; Leath, S.; Leonard, K. Resistance effects of temperature and light on virulence of *Exserohilum turcicum* on corn. *Phytopathology* **1989**, *79*, 631–635. [CrossRef]
- 47. Turner, M.; Johnson, E. Race of *Helminthosporium turcicum* not controlled by *Ht* genetic resistance in corn in the american corn belt. *Plant Dis.* **1980**, *64*, 216–217. [CrossRef]
- Dong, J.; Fan, Y.; Gui, X.; An, X.; Ma, J.; Dong, Z. Geographic distribution and genetic analysis of physiological racesof *Setosphaeria turcica* in Northern China. *Am. J. Agric. Biol. Sci.* 2008, *3*, 389–398. [CrossRef]
- Welz, H.; Wagner, R.; Geiger, H. Virulence variation in *Setosphaeria turcica* populations collected from maize in China, Mexico, Uganda, and Zambia. *Phytopathology* 1993, 83, 1356.
- 50. Gianasi, L.; de Castro, H.; de Silva, H. Raças fisiológicas de *Exserohilum turcicum* identificadas em regiões produtoras de milho no Brasil, Safra 93/94. *Summa Phytopathol* **1996**, *22*, 214–217.
- 51. Lević, J.; Stanković, S.; Petrović, T. The determination of *Exserohilum turcicum* virulence factors in Serbia. *Genetika* 2008, 40, 271–281. [CrossRef]
- 52. Muiru, W.; Koopmann, B.; Tiedemann, A.V.; Mutitu, E.; Kimenju, J. Race typing and evaluation of aggressiveness of *Exserohilum turcicum* isolates of Kenyan, German and Austrian origin. *World J. Agric. Sci.* **2010**, *6*, 277–284.
- 53. Weems, J.D.; Bradley, C.A. *Exserohilum turcicum* race population distribution in the north central United States. *Plant Dis.* **2018**, 102, 292–299. [CrossRef]
- 54. Jindal, K.K.; Tenuta, A.U.; Woldemariam, T.; Zhu, X.; Hooker, D.C.; Reid, L.M. Occurrence and distribution of physiological races of *Exserohilum turcicum* in Ontario, Canada. *Plant Dis.* **2019**, *103*, 1450–1457. [CrossRef]
- 55. Jordan, E.G.; Perkins, J.M.; Schall, R.; Pedersen, W. Occurrence of race 2 of *Exserohilum turcicum* on corn in the central and eastern United States. *Plant Dis.* **1983**, *67*, 1163–1165. [CrossRef]
- 56. Pataky, J. Effects of races 0 and 1 of *Exserohilum turcicum* on sweet corn hybrids differing for Ht-and partial resistance to northern leaf blight. *Plant Dis.* **1994**, *78*, 1189–1193. [CrossRef]
- 57. Lipps, P.; Pratt, R.; Hakiza, J. Interaction of Ht and partial resistance to *Exserohilum turcicum* in maize. *Plant Dis.* **1997**, *81*, 277–282. [CrossRef] [PubMed]
- 58. Jindal, K.K.; Zhu, X.; Tenuta, A.; Javed, N.; Daayf, F.; Reid, L.R. Maize inbreds for multiple resistance breeding against major foliar, ear and stalk rot diseases. *Maydica* 2019, *64*, 22.
- 59. Zhu, X.; Reid, L.; Woldemariam, T.; Voloaca, C.; Wu, J. Response of different *Ht* genes to northern corn leaf blight in Ottawa, Ontario. *Maize Genet. Coop. Newsl.* **2010**, *84*, 31.
- 60. Zhu, X.; Reid, L.; Smith, M. A gene for resistance to northern leaf blight is inhibited by co325. Maize Genet. Coop. Newsl. 2002, 76, 53.
- 61. Reid, L.M.; Zhu, X. Screening Corn for Resistance to Common Diseases in Canada; AAFC Technical Bulletin; Agriculture and Agri-Food Canada: Ottawa, ON, Canada, 2003; pp. 19–21.
- 62. Crossa, J. Statistical analyses of multilocation trials. Adv. Agron. 1990, 44, 55–85.
- 63. Gomez, K.A.; Gomez, A.A. Statistical Procedures for Agricultural Research; John Wiley & Sons: New York, NY, USA, 1984.
- 64. Welch, B.L. The generalization of 'student's' problem when several different population varlances are involved. *Biometrika* **1947**, 34, 28–35. [CrossRef]
- 65. De Smith, M. A Comprehensive Handbook of Statistical Concept, Techniques and Software Tools; The Winchelsea Press, Drumlin Security Ltd.: Edinburgh, UK, 2018.

- 66. Assar, A.H.A. Genetic Analysis of *Striga asiatlca* (L.) Kuntze Resistance in Line × Tester Crosses of Sorghum. Master's Thesis, University of Gezira, Wad Medani, Sudan, 1996.
- 67. Sharma, J.R. Statistical and Biometrical Techniques in Plant Breeding; New Age International: New Delhi, India, 2006.
- 68. Ejigu, Y.G.; Tongoona, P.B.; Ifie, B.E. General and specific combining ability studies of selected tropical white maize inbred lines for yield and yield related traits. *Int. J. Agric. Sci. Res.* **2017**, *7*, 381–396.
- 69. Thirumalai, R.; Palaniraja, K.; Vennila, S. Assessment of heterotic effects in rice (*Oraza sativa* L.) caostal saline environment. *Plant Arch.* 2017, *17*, 1501–1504.
- 70. Wynne, J.; Emery, D.; Rice, P. Combining ability estimates in *Arachis hypogaea* L. II. field performance of F₁ hybrids. *Crop Sci.* **1970**, 10, 713–715.
- 71. Brown, D.M. *Heat Units for Corn in Southern Ontario;* Factsheet, No. 78-063, Agdex111-31; Ontario Ministry of Agriculture and Food: Guelph, ON, Canada, 1978; p. 4.
- 72. De Mendiburu, F. Agricolae Tutorial; (Version 1.3-5); Universidad Nacional Agraria: La Molina, Peru, 2021.
- Ferguson, L.M.; Carson, M. Spatial diversity of *Setosphaeria turcica* sampled from the eastern United States. *Phytopathology* 2004, 94, 892–900. [CrossRef] [PubMed]

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