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Resistance Breeding to Northern Corn Leaf Blight with Dominant Genes, Polygene, and Their Combinations—Effects to Yield Traits

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Abstract: Resistance breeding is the most economic method to control northern corn leaf blight (NCLB). The objectives of these studies were: To assess effects of dominant genes (*Ht*(s)), polygene (PG), and combinations on percent leaf area affected (PLAA), yield, kernel moisture, kernel number per ear, and 100-kernel weight; to understand genetic action of combinations; to predict losses and effects of resistant genes for yield traits with PLAA; and to assess yield under different NCLB epidemic conditions. Two experiments were conducted. E1 had 120 crosses, their parents, and ten hybrid checks, inoculated NCLB twice in 2015 and 2016; and E2 had 85 crosses and 10 hybrids, with none, one, and two inoculation treatments in 2015. E1 results showed the order of PLAA was *Ht3* ≈ *Ht2* ≈ PG*Htm1* ≈ PG*Ht1*< PG*Ht3* ≈ PG*Ht2* < PG*Htn1* < PG < *Ht1* < *Htn1* ≈ *Htm1*. The order of *Ht*(s) effects for yield was *Ht2* > *Ht3* > *Ht1* > *Htm1* > *Htn1*. Gene effects of cross ≈ gene effects of (female + male) for all five traits. Predicted losses and predicted effects of resistant genes between yield traits with PLAA were determined. E2 results indicated resistant genes increased yield more efficiently under NCLB epidemic environments.

Keywords: 100-kernel weight; corn; dominant genes; *Ht1*; *Ht2*; *Ht3*; *Htm1*; *Htn1*; kernel moisture; kernel number per ear; northern corn leaf blight; percent leaf area affected; polygene; resistance; yield

Citation: Zhu, X.; Kebede, A.; Woldemariam, T.; Wu, J.; Jindal, K.K.; Reid, L.M. Resistance Breeding to Northern Corn Leaf Blight with Dominant Genes, Polygene, and Their Combinations—Effects to Yield Traits. *Agronomy* **2023**, *13*, 1269. https://doi.org/10.3390/agronomy13051269

Academic Editors: Junhua Peng and Yong-Bao Pan

Received: 9 March 2023
Revised: 29 March 2023
Accepted: 26 April 2023
Published: 28 April 2023

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 maize (*Zea mays* L.) worldwide. In Canada, NCLB recently became an economically important foliar disease. It infected 98% of surveyed fields in 2010 in Ontario and Quebec [1], and 97% of fields in 2015 in Ontario [2]. A large epidemic area along Lake Erie, >10 km long in Ontario was found in 2022 [3]. Heavy infections of NCLB can cause grain yield losses ranging from 40 to 70% [4–7] for grain corn and up to 91% [8] for silage corn.

The most economic, sustainable, and effective way to control NCLB is to develop resistance hybrids. Seven dominant resistant genes; *Ht1* [9–12], *Ht2* [13], *Ht3* [14], *HtN* = *Htn1* [15], *Htm1* [16], *HtP* [17,18], and *HtNB* [19]; and two recessive resistant genes, *ht4* [20] and *rt* [17,18], were found. H99, a polygene resistant line, was estimated to have 2–3 genes affecting lesion number and 4–7 genes affecting percent leaf area affected [21]. *Ht1* was used extensively during the late 1960s and 1970s [20,22], and later [23–25]. *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* were used for physiological races identification recently. Twenty races (0, 1, 2, M, N, 12, 13, 23, 1M, 1N, MN, 123, 1MN, 2MN, 23M, 23N, 12MN, 23MN, 123M, and 123MN) exist in north central United States [26]; seventeen races (0, 1, 2, 3, M, N, 12, 1M, 1N, 3M, 13M, 12N, 13N, 1MN, 12MN, 13MN, and 123MN) were found in Ontario, Canada [27]. The above results indicated none of the above *Ht* genes are immune

to the *E. turcicum* population; better combinations between *Ht* genes or the polygene + *Ht* gene will be valuable. Polygenic resistant lines (Oh43, Oh45, Mo17) were combined with monogenic *Ht1*, *Ht2*, and *Htn1* for resistance study. Polygenic resistant lines combined with *Htn1* had better resistance than what *Ht2* and *Ht1* had, and monogenic combination *Ht2*/*Ht1* had better resistance than *Ht2*/*Ht2* and *Ht1*/*Ht1* [28]. More resistant sources are available. 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 27 April 2023), including 991 accessions with high to average resistance (rating 1–4) in 2023.

Grain yield per ear is a compound trait. It can be calculated with two formulas: 1. Grain yield per ear = ear length × (3.14 × kernel depth^2) × kernel density × [(100 − kernel moisture)/(100 − Standard moisture)], where kernel depth = (ear diameter − cob diameter)/2; or 2. Grain yield per ear = kernel number per ear × (100 − kernel weight/100) × [(100 − kernel moisture)/(100 − Standard moisture)], where kernel number per ear = kernel row per ear × kernel number per kernel row. More factors influence hybrid yield, such as parents' heterosis groups, plant population, plant and ear heights, ears per plant, silking and maturity days, etc. Simple and genetic correlation coefficients were used to reveal the relationship among plant and ear traits with yield. Hallauer and Miranda [29] summarized genetic correlations in the literature; ears per plant, ear length, ear diameter, kernel depth, and kernels per row had correlation coefficients that range from 0.38 to 0.51. Comparable results were found for Iowa Stiff Stalk Synthetic; ear length, ear diameter, kernel row number, kernels per row, and kernel depth had higher positive correlations that range from 0.45 to 0.84, but days to flower had higher negative correlation (−0.52) with grain yield. Plant height, ear height, leaves per plant, leaf length, leaf width, leaf area, days to silk, tassel branches, ear weight, and kernel rows had significant correlations with grain yield, ranging from 0.28 to 0.76 [30]. Plant height, ear height, ear length, and 100-kernel weight were significantly positive relationships with inbred and hybrid grain yield [31]. In a tropical maize study on the above traits, only thousand kernel weight had a significantly positive relation with grain yield, but northern corn leaf blight and common rust had negative correlation coefficients, −0.26 and −0.43, respectively [32]. NLCB infects leaves, causes a percentage of the leaf area to be affected [21], reduces photosynthesis, affects yield traits, reduces kernel weight, and reduces yield [5].

From 2006 to 2014, based on the backcross method, *Htm1* and *Htn1* were successfully introduced into a susceptible inbred CO388, and *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* were successfully introduced into a polygenic resistant inbred CO428 [33,34]. The objectives of this paper were: 1. To estimate effects of dominant genes (*Ht*(s)), polygene (PG), and their combinations on percent leaf area affected (PLAA), yield, kernel moisture, kernel number per ear (KNPE), and 100-kernel weight (100 KW); 2. To estimate effects of resistant gene in female, male, or both to the above five traits; 3. To predict losses and the effect of resistant genes with PLAA to the above four yield traits; and 4. To estimate effects of resistant genes to yield under different NCLB epidemic conditions.

2. Materials and Methods

2.1. Materials and Experiment Methods

From 2006 to 2014, after 4 generations of back cross and 4 generations of selfing under artificial inoculation of NCLB selection; BLT01 = [CO388 × A553*N*(Orange Halo)] × CO388ˆ4, BLT02 = (CO388 × A632*HtN*) × CO388ˆ4, BLT03 = (CO388 × H102) × CO388ˆ4, BLT05 = (CO428 × 73353) × CO428ˆ4, BLT06 = (CO428 × A509*N*) × CO428ˆ4, BLT07 = (CO428 × A619*Ht2*) × CO428ˆ4, BLT09 = (CO428 × A632*HtN*) × CO428ˆ4, BLT10 = (CO428 × H102) × CO428ˆ4, BLT11 = (CO428 × LH123*Ht*) × CO428ˆ4, BLT12 = (CO428 × Pa91*Ht2*) × CO428ˆ4, BLT13 = (CO428 × Pa91*Ht3*) × CO428ˆ4; a total of 11 inbred lines with different resistant genes to NCLB were successfully made [34]. CO388, a line with higher general combining ability but susceptible to NCLB, belongs to the SS-B73 heterotic group; CO428, a line with polygene (PG) resistance to NCLB, gray leaf spot, eyespot, Stewart's wilt, and

Goss's wilt [33], but lower general combining ability, belongs to the NS-Oh43 heterotic group; LH123*Ht* (PI 601079) with *Ht1*; A619*Ht2* (Ames 25220) and Pa91*Ht2* (Ames 25373) with *Ht2*; Pa91*Ht3* (Ames 25374) with *Ht3*; 73353, a line from Cornell University [35] and H102 (PI 550496) [16] with *Htm1*; A632*HtN* (Ames 23469), A509*N* (PI 406118), and A553*N* (Orange Halo) (PI 406119) with *Htn1*. In 2013–2015, stiff stalk (SS) line CO388 and its *Ht* versions BLT01, BLT02, and BLT03 were used as lines to cross with Non-stiff stalk (NS) A619, A619*Ht1*, A619*Ht2*, A619*Ht3* (Oh43 group), CL30 (early flint group), CO442,T1, T2, and T3 (Iodent group); CO388 and its *Ht* versions BLT01, BLT02, and BLT03 were also used as testers to cross with NS-Oh43 lines CO428 and its *Ht* versions BLT05-BLT13. CO428 and its *Ht* versions BLT05-BLT13 also crossed with CL30, CO442, and two SS-B14 group lines T4 and T5. To evaluate *Ht* gene effects for NCLB resistance, CO428 and its *Ht* versions BLT05-BLT13 also crossed with same group A619 and its *Ht* versions. Base screening and yield results were obtained in 2014, along with available seeds. A total of 163 genotypes were used for experiment one (E1) in 2015 and 2016; ninety-five genotypes were used for experiment two (E2) in 2015.

E1 was designed to analyze gene effects for NCLB resistant traits [34] and yield traits. A total of 163 genotypes, including 33 inbred lines and 120 crosses, and 7 susceptible and 3 resistant commercial hybrid checks, were used for NCLB artificial inoculation in 2015 and 2016. A randomized complete block with 3 replications, a one-row plot with a row distance of 0.76 m and row length of 3.5 m for twenty plants (75,188 plant per hector) was applied for this study. 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 CO388. Inbreds and hybrids were randomized in their areas. All genotypes were planted on 7 May 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 18 June in both 2015 and 2016; the second inoculation time was at the 10–12 leaf stage on 2 July and 4 July in 2015 and 2016, respectively. Two doses of diseased leaf powder (equivalent to 0.2 g) from a Bazooka (Sistrunk Inoculators, Starkville, MS 39759, USA) were injected into the whorl of each plant [36]. To make an environment that favors disease development and epidemic, 10–15 min of irrigation (equivalent to 5–8 mm rainfall) from above plants was done in the afternoon to add soil moisture and reduce air temperature from the first inoculation date, except raining days. Specific resistances and the lesion type were recorded twice, three weeks after the first inoculation and four weeks after the second inoculation. In early September, the number of lesions per leaf (NLPL) was counted, and lesion size (LS), LS (cm^2) = 0.75 × lesion length (cm) × lesion width (cm) was measured. General resistances and the disease ratings (DR) were recorded twice, in late August for early flowering genotypes or middle September for late flowering genotypes, and was about 4 weeks post silk emergence. The rating scale for general resistance has 7 ratings where: 1 = no symptoms; 2 = < 1%; 3 = 1–10%; 4 = 11–25% of leaves symptomatic; 5 = > 50% of the lower leaves are symptomatic, <25% of middle and upper leaves are symptomatic; 6 = bottom leaves are dead, >50% of the middle leaves, <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 was in a genotype, the numbers of plants with different rating scales were counted. The average DR was used to convert to percent leaf area affected (PLAA) for further studies in this paper. Based on our previous study, when DR <2.0, PLAA = DR −1; when DR ≥ 2.0, PLAA = 2.933 × DR^2 − 7.189 × DR + 4.322, (r^2 > 0.9).

From mid-July, silking days were recorded for each plot in both 2015 and 2016. After silking, plant height, ear height, and leaf number were measured or counted from five plants in each plot in both 2015 and 2016. Ear-leaf length (cm), ear-leaf width (cm), and ear-leaf angle from the leaf of the primary ear on five plants for each plot were measured in 2016. Ear-leaf area (ELA, cm^2) = 0.75 × ear-leaf length × ear-leaf width was used for

correlation coefficients analysis. Yield was hand harvested on 5–7 October in both 2015 and 2016. Five primary ears from five continuous plants per row were measured; ear moisture (%) with modified moisture meter MT808, Kernel moisture (KM, %) = 1.1 × ear moisture [37]. All ears (including both primary and second ears) from these five plants were harvested. After measuring or counting ear length (cm), bareness-tip length (BTL, cm), ear diameter (mm), kernel rows per ear, and kernels per kernel row of 5 primary ears, the ears were shelled, and the total ear weight, total kernel weight, cob diameter (mm), and three-repeat 100 kernel weight (g) were measured for each plot (genotype). Kernel weight per plant and plant density (75,188 plant per hector) were used to calculate yield. The average 100-kernel weight of three repeats per plot was used as 100-kernel weight (100 KW, g). Yield (t h^{-1}) and 100 KW were converted to standard kernel moisture of 15.5%. Kernel number per ear (KNPE) = kernel rows per ear × kernels per kernel row and kernel depth = (ear diameter − cob diameter)/2 were used for correlation coefficients. Yield, KM, KNPE, and 100 KW were used for yield trait analysis.

E2 was designed for gene effects to yield under different infection severities through three treatments, non-inoculation check (nature infection), and once- and twice-artificial inoculation. Based on our experience of the annual corn disease survey, NCLB can spread as far as 1.8 to 2.1 km from an infection center, and cause heavy infection within 0.5 km [6]. Therefore, each treatment was separated and isolated by other crops. Treatment with no inoculation was at the bottom of a hill with clay soil, treatment with one inoculation on the top of another hill with sandy soil, and treatment with two inoculations was at a very flat field with different sandy-clay soil. Treatment with one inoculation was in the middle, and the direct distances to the other two treatments were 1.1 km. A total of ninety-five genotypes were tested, including ten commercial hybrid checks and eighty-five crosses on a randomized complete block with 3-replications, a 2-row plot with a row distance of 0.76 m and a row length of 9.0 m for fifty plants per row (73,100 plant/hector), which were applied for each treatment. All three treatments were planted on 15 May 2015. First inoculations were performed on 24 June for one and two treatment groups, and the second inoculation was performed on July 8 for the two treatment group. Inoculation and DR record methods were the same as E1, but fields without irrigation same as farm corn production. E2 was harvested with a combine harvester in the middle of October; plot grain weight (PGW, kg) and grain moisture (GM, %) were measured automatically. PGW and plot# per hectare (731 plot per hectare) were used to calculate yield. Yield was converted to a standard grain moisture of 15.5%. Because each treatment in different fields had different environmental and soil conditions, relative yield (RY, %), not real yield, was used to compare gene effects. In each replicate, the RY of each genotype was compared to the average yield of ten commercial hybrids. RY (%) = yield of each genotype/average yield of 10 commercial hybrids × 100. As with E1, DR was converted to PLAA for further analysis.

2.2. Statistic Methods for Gene Effects Comparison

Because this study focused on the effects of dominant genes, polygenes, and their combinations to yield traits, only data from 120 crosses and ten hybrid checks were analyzed for E1. Resistant genes have a direct effect on NLPL, LS, and PLAA. PLAA has direct effects on the remaining green leaf area, which affects photosynthesis. Photosynthesis will affect yield traits and will then affect yield. Therefore, PLAA only has indirect effects on yield traits. An analysis of variance (ANOVA) and further analysis of the results was performed on PLAA and four yield traits; yield, KM, KNPE, and 100 KW. PLAA and yield from E2 were used for ANOVA and further analysis. ANOVA and the least significant difference at p = 0.05 (LSD(0.05)) for single- and multi-environment trials analysis followed the methods of Gomez and Gomez [38] and IRRI [39], with two environments for E1 and three environments for E2. The calculation for heritability (H) for single- or multi-environments followed the methods of IRRI [39].

For a single environment, to test the significance between two genotype means,

$$Sd = SQRT(2 \times MS_e/r) \quad (1)$$

$$LSD(0.05) = [T.INV.2T(0.05, DF\ of\ MS_e)] \times Sd \quad (2)$$

$$H = \sigma^2{}_g/(\sigma^2{}_g + \sigma^2{}_e/r) = (MS_g - MS_e)/MS_g \quad (3)$$

where degree of freedom (DF) of $MS_e = (g - 1)(r - 1)$, $\sigma^2{}_e = MS_e$, and $\sigma^2{}_g = (MS_g - MS)/r$; MS_e and MS_g are mean squares for error and genotypes, respectively; g and r are the number of genotypes and replications in each single environment. T.INV.2T (probability, deg_freedom) is the syntax of Excel 2016 to return the two-tailed inverse of the student's t-distribution.

For combined multi-environments, to test the environment mean of all genotypes,

$$Sd1 = SQRT(2 \times MS_{RE})/(r \times g)) \quad (4)$$

$$LSD1(0.05) = [T.INV.2T(0.05, DF\ of\ MS_{RE})] \times Sd1 \quad (5)$$

To test the genotype mean of all environments,

$$Sd2 = SQRT(2 \times MS_E)/(r \times e)) \quad (6)$$

$$LSD2(0.05) = [T.INV.2T(0.05, DF\ of\ MS_E)] \times Sd2 \quad (7)$$

$$H = \sigma^2{}_G/[\sigma^2{}_G + \sigma^2{}_{GY}/e + \sigma^2{}_E/(re)] = (MS_G - MS_{GY})/MS_G \quad (8)$$

where DF of $MS_{RE} = (e - 1) \times r$, and DF of $MS_E = (e - 1) \times (r - 1) \times (g - 1)$; $\sigma^2{}_E = MS_E$, $\sigma^2{}_{GY} = (MS_{GY} - MS_E)/r$, and $\sigma^2{}_G = (MS_G - MS_{GY})/(re)$; MS_{RE}, MS_E, MS_{GE}, and M_G are mean squares for replicates within environments, pooled error, genotypes × years, and genotypes, respectively; e, r, and g are the number of environments, replications, and genotypes, respectively.

To analyze gene effects for both experiments, genotypes were grouped by: (1) same female crossed with similar male with different genes to see single gene effects of *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1*; (2) similar female with different genes crossed with same male to see gene effects of *Htm1*, *Htn1*, PG, PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1*; and (3) similar female with different genes crossed with another group of similar male with different genes to see the combination effects among susceptible (S), resistant (R), Polygenic (PG), and PG*Ht*(s) (PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, PG*Htn1*) parents.

The homoscedastic version *t*-test method [40] was used to test the significance between two similar genotypes in E1, and two treatments of the same genotype or grouped parent in E2.

$$t = \frac{m1 - m2}{\frac{s}{\sqrt{n1+n2}}} \quad (9)$$

$$s^2 = \frac{\sum_{i=1}^{n1}(X1i - m1)^2 + \sum_{i=1}^{n2}(X2i - m2)^2}{n1 + n2 - 2} = (S1^2 + S1^2)/2 \quad (10)$$

where X1i and X2i are the *i*th value of sample 1 and sample 2; m1 and m2, and S1 and S2 are the two sample means and standard deviations from samples of size n1 and n2, respectively; and S is an estimate of the standard deviation obtained from the pooled variance estimate with above formula 10. This T-test has n1 + n2 − 2 degrees of freedom.

For each genotype in E1, n1 = n2 = year number × replication number = 6. For each genotype in E2 for each genotype, n1 = n2 = replication number = 3. For grouped parents in E2, n1 = n2 = replication number × genotype number = 3 × Genotype number.

Another T-test method, the heteroskedastic version [40,41], was used for the test grouped gene and parent effects for E1.

$$t = \frac{m1 - m2}{\sqrt{\frac{s1^2}{n1} + \frac{s2^2}{n2}}} = \frac{m1 - m2}{\sqrt{A + B}} \quad (11)$$

$$df = \frac{(A + B)^2}{\frac{A^2}{(n1-1)} + \frac{B^2}{(n2-1)}} \quad (12)$$

where m1 and m2, and S1 and S2 are the two sample means and standard deviation from samples of size n1 and n2, respectively. This T-test has degrees of freedom as in formula 12. In this case, n1 and n2 = genotype number × replication number × year number = 6 × genotype number.

In the E1 study, a pair comparison was performed, with both LSD (0.05) and the above two *t*-test results. First, we sort means from biggest to smallest; second, we use LSD (0.05) to find same and different pairs; third, we use *t*-test results to find the difference between the same pair; fourth, we give same or different alphabet for each mean. For example, m1 > m2 > m3 > m4, LSD (0.05) results show that m1 and m2, m3 and m4 are not significantly different, but *t*-test results show m1 and m2 are significantly different; m2 is not significant with m3, but significant with m4, and m3 and m4 are not significantly different. The final result in this case will be m1a, m2b, m3bc, m4c.

In E2, there were some cases where S1 = 0 and/or S2 = 0, because some genotypes and their three replicates have PLAA = 0 in no inoculation treatment, and three replicates have the same PLAA in one and two inoculation treatments. If S1 = S2 = 0, and m1 $\neq$ m2, the pair comparison for m1 and m2 was considered significant. However, when compared with the third sample, if $S_1 = S_2 = 0$, $S_3 \neq 0$, and $m_1 > m_2 > m_3$, if the *t*-test between m_1 and m_3, m_2 and m_3 were not significant, then m_1 and m_2 were considered not significant.

2.3. Statistic Methods for Prediction Losses and Gene Effects to Yield Traits

E1 results were also used to evaluate losses of yield traits under different PLAA. Because PLAA for each single genotype only had two years and three replicates' data, and replicate results were remarkably close within years, they could therefore not be used to evaluate yield losses. However, BLT01–BLT03 came from CO388, BLT05–BLT13 came from CO428, A619*Ht1*, A6139*Ht2*, and A619*Ht3* came from A619. By grouping crosses as (CO388, BLT01-BLT03) × (A619, A619*Ht1*-A619*Ht3*), (CO428, BLT05-BLT13) × (CO388, BLT02, BLT03), and (CO428, BLT05-BLT13) × (A619, A619*Ht1*-A619*Ht3*), these three groups plus the hybrid checks had a sample size of more than 60, and the general idea of the effects of the resistant gene can be evaluated. Simple linear regressions were found between yield, KM, KNPE, or 100 KW, and PLAA. The functional form of the linear relationship between dependent variables Y (yield, KM, KNPE, and 100 KW) and independent variable PLAA were represented by the equation:

$$Y = m + \beta \times PLAA \quad (13)$$

where m is the intercept of the line on the Y axis, and β, the linear regression coefficient, is the slope of the line. The regression parameters of m, β, and r (the correlation coefficient) were calculated following chapter 9, regression and correlation analysis [38]. More detailed results, such as standard errors for m and β, and the ANOVA table, were obtained from SigmaPlot (SigmaPlot 14.5, Systat Software, Inc. San Jose, CA 95131 USA). The method of testing *r* was the same as Chapter 12 of the Statistical Analysis Handbook [41].

$$t_{n-2} = r \times SQRT((n - 2)/(1 - r^2)) \quad (14)$$

where n is the sample number = genotype × year# × replicate# = 6 × genotype#. This *t*-test has n − 2 degrees of freedom.

$$Y_{max} = m + \beta \times PLAA_{min}, (\beta < 0) \quad (15)$$

$$Y_{mean} = m + \beta \times PLAA_{mean}, (\beta < 0) \quad (16)$$

$$Y_{min} = m + \beta \times PLAA_{max}, (\beta < 0) \quad (17)$$

$$\text{Maximum predicted loss } (PL_{max}, \%) = (m - Y_{min})/m \times 100 \quad (18)$$

$$\text{Mean predicted loss } (PL_{mean} \%) = (m - Y_{mean})/m \times 100 \quad (19)$$

$$\text{Minimum predicted loss } (PL_{mib,} \%) = (m - Y_{max})/m \times 100 \quad (20)$$

$$\text{Predicted effect of resistant genes (PERG, \%)} = (Y_{max} - Y_{min})/m \times 100 \quad (21)$$

where $PLAA_{max}$, $PLAA_{mean,}$ and $PLAA_{min}$ are the maximum, mean, and minimum PLAA in each cross group, respectively, and Y_{max}, Y_{mean}, and Y_{min} are the maximum, mean, and minimum predicted Y value for Yield, KM, KNPE, or 100 KW, respectively.

All above $PLAA_{max}$, $PLAA_{mean}$, $PLAA_{min}$ and corresponding PL_{max}, PL_{mean}, and PL_{min} were used to make regressions to predicted losses (PL, %) for Yield, KM, KNPE, or 100 KW, respectively.

$$PL\ (\%) = \beta_1 \times PLAA \quad (22)$$

where β_1 is the linear regression coefficient for PL.

PL and PERG from above regressions will give the overall relationship between yield traits losses or effects of resistant gene(s) and PLAA.

All statistics were done with Excel 2016 (Microsoft Office Professional Plus 2016, Microsoft). All regression results were checked with SigmaPlot 14.5 (Systat Software, Inc. San Jose, CA 95131 USA) to make sure they are correct.

3. Results

3.1. ANOVA Results for E1 and E2

Single year ANOVA results (data not shown) from E1 showed replicate effects were not significant for KM and KNPE in 2015, hybrid check yield, hybrid check KNPE in 2016, and hybrid check vs. cross yield in both 2015 and 2016. All other sources were significant ($p < 0.05$). Two-year combined ANOVA results (data not shown) showed that Year × Genotype effects were not significant for PLAA, yield, KM, KNPE, and 100 KW. All other sources, including hybrid checks vs. cross effects, were significant ($p < 0.05$). The coefficients of variation (CVs, %) from combined ANOVA were 39.5, 20.7, 17.7. 15.5, and 10.3 for PLAA, yield, KM, KNPE, and 100 KW, respectively. These were bigger than any single year's CVs. Four reasons causing big CV(s) were explained in another paper [34].

ANOVA results of PLAA (data not showed) for E2 each single treatment and combined treatments, e all sources were significant. ANOVA results of yield and RY (data not showed) showed that replicate effects were significant for yield, but not significant for RY with no inoculation treatments. Reverse results were obtained for one inoculation treatment. All other sources for single treatment or combined data were significant. Their CV(s) ranged from 10.6 to 15.5, heritability ranged from 0.89 to 0.91, and were both good enough under such complicated genotypes and different environments and treatments.

3.2. E1 Inbred Results

Table 1 showed PLAA, yield, KM, KNPE, and 100 KW results of thirty-three inbred lines in E1. All lines without a resistance gene had PLAA > 80%, up to 97.7%. Under A619 and Pa91 backgrounds, *Ht2* expressed the least PLAA, followed by *Ht3* and *Ht1*. However, *Htm1* and *Htn1* were affected by different genetic backgrounds. The PLAA of BLT05 was

significantly higher than what CO428 had; it indicated that one or more minor resistant genes might have been lost when *Htm1* was introduced into CO428. Inbred yield traits affected by several factors: 1, tropical lines A553*N*, H102, and the Pa91 family had lower yields because they are very late in Ottawa, and CL30 had low yield because it is one of earliest lines in Canada; 2, A619 and CO428 families had a similar problem, some plants could not silk on time, silking at the end of the pollen-shedding period; and 3, in many cases, *Ht*(s) increased KNPE, 100 KW, and yield. Maturity and higher PLAA had more effects on KM; the lower the KM, the better.

Table 1. Percent of leaf area affected (PLAA, %), Yield (t h^{-1}), kernel moisture (KM, %), kernel number per ear (KNPE), and 100-kernel weight (100 KW, g) after artificial epidemic condition of northern corn leaf blight among 33 inbred lines.

Name/Code	Purpose	Heterotic Group	Assumed Resistant Gene	PLAA (%)	Yield (t h^{-1})	KM (%)	KNPE	100 KW (g)
A619	Tester	NS-Oh43	None	84.1	3.5	40.4	194	24.0
A619*Ht1*	Tester	NS-Oh43	*Ht1Ht1*	42.1	3.2	39.4	183	24.3
A619*Ht2*	Tester and R source	NS-Oh43	*Ht2Ht2*	35.0	5.4	45.2	269	29.1
A619*Ht3*	Tester	NS-Oh43	*Ht3Ht3*	40.2	4.1	39.4	197	26.5
A632*HTN*	R Source	SS-B14	*Htn1Htn1*	46.2	5.0	31.1	252	26.2
A553N	R Source	Tropical	*Htn1Htn1*	16.5	0.7	56.9	48	23.1
73353	R Source	Tropical	*Htm1Htm1*	11.5	3.2	39.6	165	32.0
H102	R Source	Tropical	*Htm1Htm1*	8.0	3.8	55.6	205	24.2
Pa91	Inbred check	SS	None	39.2	3.8	55.6	260	24.2
Pa91*Ht1*	Inbred check	SS	*Ht1Ht1*	20.1	3.1	56.8	221	21.5
Pa91*Ht2*	R Source	SS	*Ht2Ht2*	16.0	2.6	60.3	204	23.7
Pa91*Ht3*	R Source	SS	*Ht3Ht3*	18.4	5.5	59.7	332	24.1
CO353	MR check	SS	Unknown	21.2	5.7	47.0	246	29.4
CO388	Line and tester	SS-B73	None	80.0	4.3	32.5	253	23.7
BLT01	Line and tester	SS-B73	*Htn1Htn1*	57.8	5.8	34.2	305	28.8
BLT02	Line and tester	SS-B73	*Htn1Htn1*	51.8	5.5	30.4	295	30.3
BLT03	Line and tester	SS-B73	*Htm1Htm1*	56.5	4.9	31.1	262	27.3
CO428	Line	NS-Oh43	PG PG	9.7	5.9	42.9	287	26.7
BLT05	Line	NS-Oh43	PG*Htm1* PG*Htm1*	24.2	6.8	40.0	294	27.9
BLT06	Line	NS-Oh43	PG*Htn1* PG*Htn1*	7.0	5.9	42.1	304	25.6
BLT07	Line	NS-Oh43	PG*Ht2* PG*Ht2*	11.0	6.0	42.6	304	25.4
BLT09	Line	NS-Oh43	PG*Htn1* PG*Htn1*	10.8	4.6	46.2	299	26.9
BLT10	Line	NS-Oh43	PG*Htm1* PG*Htm1*	2.7	6.1	42.4	292	29.7
BLT11	Line	NS-Oh43	PG*Ht1* PG*Ht1*	5.9	5.2	38.2	298	23.7
BLT12	Line	NS-Oh43	PG*Ht2* PG*Ht2*	2.7	6.4	42.4	302	29.8
BLT13	Line	NS-Oh43	PG*Ht3* PG*Ht3*	3.3	6.3	42.5	304	29.6
CL30	Tester	NS-Flint	None	97.7	1.3	16.2	155	16.2
CO442	Tester and S check	NS-Iodent	None	96.6	4.4	17.2	402	14.9
T1	Tester	NS-Iodent	None	97.7	3.4	15.8	203	24.6
T2	Tester	NS-Iodent	None	92.2	3.6	18.4	281	18.4
T3	Tester	NS-Iodent	Partial	69.6	4.7	23.3	280	24.8
T4	Tester	SS-B14	None	90.0	5.0	20.5	306	23.2
T5	Tester	SS-B14	None	92.7	3.6	14.5	251	18.9
CV%				26.4	15.7	12.7	12.5	8.4
LSD(0.05)				12.9	1.8	4.7	59.5	2.8
H				0.93	0.94	0.94	0.93	0.90

CV% = coefficients of variation percentages; LSD(0.05) = the least significant differences at probability level 0.05; H = heritability. SS = stiff stalk group, including B73 and B14 subgroups. NS = non-stiff stalk group, including Oh43 and Iodent subgroups. Iodent can combined well with both SS and NS groups. None = no specific gene from Female or male. PG = polygenic resistance.

3.3. Correlation Coefficients among Disease and Yield Traits of Crosses from E1

Figure 1 shows relationships and correlation coefficients among important traits in this study. Genotype (Genetics) is the center of this figure, which had the most key role for yield traits. Another key factor was photosynthesis, connected with outside environments such as climatic factors and other biotic factors, such as NCLB. NCLB affects PLAA and reduces green leaf area and shortens leaf living time, therefore reducing photosynthesis and affecting yield traits.

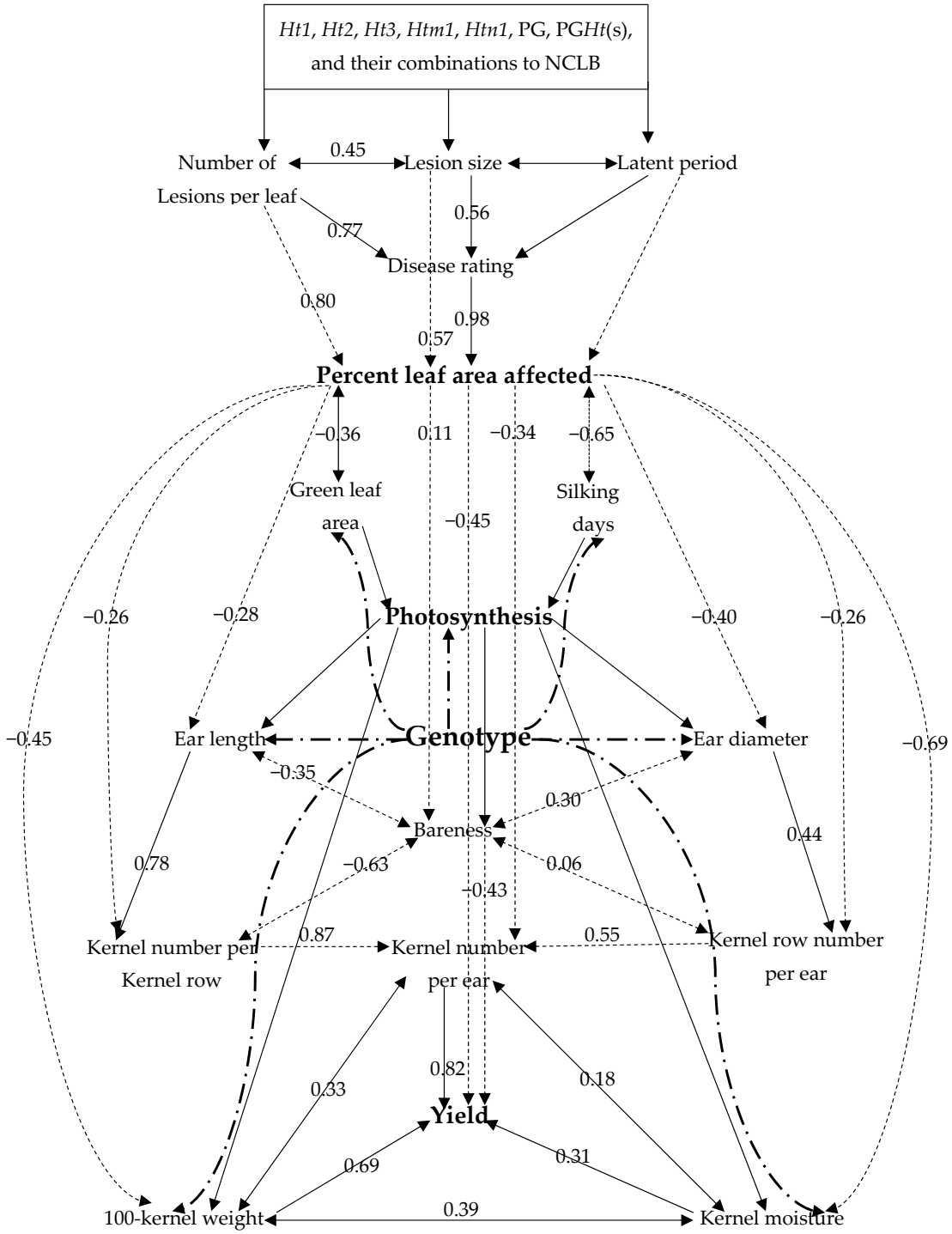

Figure 1. Relationships and their correlation coefficients among resistant genes, disease traits, and plant traits. It shows that genotype is the center for plant traits. Resistant genes only have indirect effects to yield traits. Resistant genes affect the number of lesions per leaf, lesion size, and latent

period, which determine disease rating and percent leaf area affected. Percent leaf area affected reduces green leaf area, then reduces photosynthesis, causes bareness on the ear, affects yield traits such as yield, kernel moisture, kernel number per ear, and 100-kernel weight. Solid lines present direct effects, dashed lines present indirect effects, and solid-dash dot lines present genetic effects of genotype.

The effects of resistant genes affected NLPL, LS, and the latent period, which were the three resistances to invasion, extension, and explosion (epidemics) of disease development. The latent period, the time between host infection and the onset of pathogen sporulation, was difficult to measure in this study because of four reasons: 1, the ground diseased leaf powder was used as inoculation, which included many races [27] and needed about 24 h to produce spores for invasion; 2, there were many genotypes and resistant gene combinations, which developed diverse types of lesions. After first inoculation, S lesions showed typical symptoms and sporulation in 10–14 days. However, HR and R lesions from two commercial hybrids and some crosses needed approximately 28 days to show typical symptoms and never had sporulation. MR and MS lesions showed typical symptoms and sporulation in 14–28 days; 3, when plants get older, the latent period got shorter, but sporulation got smaller as well; 4, Latent periods overlapped after second inoculation because of such complex corn populations from highly susceptible to highly resistant hybrid/crosses and inbreeds. Correlations between DR, NLPL, LS, and PLAA were 0.98, 0.80, and 0.57, respectively, all highly significant ($p < 0.01$). Correlations between PLAA and plant height, ear-leaf area, and silking days were −0.34, −0.36, and −0.65, respectively, all highly significant. Correlations between PLAA and ear length, ear diameter, kernel depth, kernel row number, kernel number per kernel row, KNPE, KM, 100 KW, and yield were −0.28, −0.40, −0.24, −0.26, −0.26, −0.34, −0.69, −0.45, and −0.45, respectively. Ear height and ear-leaf angle had low correlations with PLAA, and were not significant. Meanwhile, correlations between yield and DR, PLAA, NLPL, LS were −0.43, −0.45, −0.29, −0.16, respectively. The correlations between yield and plant height, ear height, silking days, leaf number, ear-leaf area were 0.71, 0.27, 0.47, and 0.54, all highly significant. Correlations between yield and ear length, ear diameter, kernel depth, kernel row number, kernel number per kernel row, KNPE, KM, and 100 KW were 0.81, 0.33, 0.11, 0.28, 0.81, 0.82, 0.31, and 0.69, respectively, all significant. In this study, the correlation between yield and kernel depth (0.11) was much lower than in previous studies [29]; the correlation between yield and PLAA (−0.45) was worse than in the study of Mogesse and Zeleke (−0.26) [32].

3.4. Effects of Ht1, Ht2, Ht3, Htm1, Htn1 and Partial Resistance

Yield traits were affected by genotype genetics (See Table 2 group Mean): 1. heterotic groups had the most key role, SS × NS and NS × SS had the best yield, followed by NS × Iodent, SS × Iodent, SS × Early flint, NS × NS, and NS × Early flint, which had the least yield. In most cases, when resistant genes from tropic sources were introduced into CO388 or CO428, their crosses had better yield than other temperate sources had. However, if both parents had a tropical source, it might reduce the yield. KM and 100 KW were related to their silking days; regarding lines CO388, BLT01, BLT02, BLT03, CO428, BLT05, BLT06, BLT07, BLT09, BLT10, BLT11, BLT12 and BLT13, their silking days were 82, 85, 81, 83, 84, 81, 85, 83, 84, 85, and 85, respectively; regarding tester A619, A619Ht1, A619Ht2, A619Ht3, CL30, CO442, T1, T2, T3, T4, and T5, their silking days were 83, 84, 84, 85, 70, 79, 82, 79, 78, 78, and 79, respectively [33]. All CL30 crosses were early crosses. They had the least KM and 100 KW, followed by CO442, T1, T2, T3, T4, T5, and the A619 family (including A619*Ht1*, A619*Ht2*, A619*Ht3*). CO428 family (including BLT05-BLT13) × CO388 family (including BLT01-BLT03) had the most KM and 100 KW. PLAA had less of a role than the above three factors. To reduce genotype genetic effects, by grouping genotypes with same female or same male in a similar cross, it is possible to see the effects of each gene(s) and their combinations on PLAA, yield, KM, KNPE, and 100 KW.

Table 2. Comparison of gene effects with similar backgrounds to percent leaf area affected (PLAA, %), yield (t h^{-1}), kernel moisture (KM, %), kernel number per ear (KNPE), and 100-kernel weight (100 KW, g).

Genotypes	Female/Male Genes	#	PLAA (%)	Yield (t $h^{-1)}$	KM (%)	KNPE	100KW (g)
7 susceptible hybrid checks	-/-	42	77.7a	10.7bc	20.6a	505.9bc	26.1ab
Resistant hybrid check1	*Ht*?/*Ht*?	6	35.8b	12.7ab	22.3a	590.6a	28.1ab
Resistant hybrid check2	*Ht*?/*Ht*?	6	29.8b	14.1a	20.8a	581.7ab	30.6a
Resistant hybrid check3	*Ht*?/*Ht*?	6	70.7a	9.1c	15.8b	474.7c	24.5b
Mean		60	68.0	11.1	20.3	518.8	26.6
Same female cross with different males							
CO388 × A619	-/-	6	75.8a	12.9a	29.7a	468.1a	31.7b
CO388 × A619*Ht1*	-/*Ht1*	6	39.8b	13.7a	31.6a	499.5a	33.6ab
CO388 × A619*Ht2*	-/*Ht2*	6	46.2b	13.3a	33.6a	481.2a	35.6a
CO388 × A619*Ht3*	-/*Ht3*	6	43.3b	14.0a	33.5a	492.5a	34.1ab
SS × NS Mean		24	51.3A	13.4AB	32.1B	485.3A	33.7A
(BLT01-BLT03) × A619	(*Htm1*, *Htn1*)/-	18	65.2a	13.3b	30.7b	505.6a	33.3b
(BLT01-BLT03) × A619*Ht1*	(*Htm1*, *Htn1*)/*Ht1*	18	33.9b	14.1ab	31.1b	511.8a	35.8ab
(BLT01-BLT03) × A619*Ht2*	(*Htm1*, *Htn1*)/*Ht2*	18	32.2b	14.6a	36.6a	525.7a	36.5a
(BLT01-BLT03) × A619*Ht3*	(*Htm1*, *Htn1*)/*Ht3*	18	25.4b	14.9a	35.2a	522.2a	35.9ab
SS × NS Mean		72	39.2AB	14.2A	33.4B	516.3A	35.4A
CO428 × A619	PG/-	6	50.8a	8.7a	36.5a	371.8a	28.4ab
CO428 × A619*Ht1*	PG/*Ht1*	6	24.9b	8.1a	37.3a	383.3a	26.0c
CO428 × A619*Ht2*	PG/*Ht2*	6	21.1b	9.2a	40.4a	394.2a	29.8a
CO428 × A619*Ht3*	PG/*Ht3*	6	18.3b	8.8a	38.6a	403.3a	28.1b
NS × NS Mean		24	28.8B	8.7B	38.2A	388.2B	28.1B
(BLT05-BLT13) × A619	PG*Ht*(s)/-	42	48.3a	8.6b	34.1b	375.2b	29.3b
(BLT05-BLT13) × A619*Ht1*	PG*Ht*(s)/*Ht1*	42	22.1b	9.7a	35.3ab	405.2a	30.4a
(BLT05-BLT13) × A619*Ht2*	PG*Ht*(s)/*Ht2*	42	12.7c	10.2a	37.7a	419.9a	30.5a
(BLT05-BLT13) × A619*Ht3*	PG*Ht*(s)/*Ht3*	42	15.7c	9.7a	40.0a	407.3a	30.2a
NS × NS Mean		168	24.7B	9.5B	36.8A	401.9B	30.1B
CO428 × CO388	PG/-	6	27.0a	13.5a	35.8b	503.7a	33.6a
CO428 × BLT03	PG/*Htm1*	6	18.1b	13.8a	37.3a	502.4a	33.8a
CO428 × BLT02	PG/*Htn1*	6	18.1b	12.9a	37.4a	452.7b	35.9a
NS × SS Mean		18	21.0B	13.4AB	36.8A	486.3A	34.4A
(BLT05-BLT13) × CO388	PG*Ht*(s)/-	42	25.4a	13.3a	36.7a	509.1a	33.5a
(BLT05-BLT13) × BLT03	PG*Ht*(s)/*Htm1*	30	17.3b	13.7a	36.1a	489.1a	33.8a
(BLT05-BLT13) × (BLT01, BLT02)	PG*Ht*(s)/*Htn1*	48	22.2a	13.4a	35.4a	495.5a	33.9a
NS × SS Mean		120	22.1B	13.4AB	36.0A	498.7A	33.7A
Different females crossed with the same male							
CO388 × 4 testers	-/-	24	83.9a	9.9a	23.1b	427.7a	27.6b
BLT03 × 4 testers	*Htm1*/-	24	66.2b	10.8a	25.1ab	462.2a	30.3a
(BLT01, BLT02) × 4 testers	*Htn1*/-	60	61.1b	10.7a	26.9a	454.3a	30.2a
SS × (early flint, Iodent) Mean		120	68.1A	10.5B	25.5E	449.6B	29.6D
CO388 × A619	-/-	6	75.8a	12.9a	29.7a	468.1a	31.7a
BLT03 × A619	*Htm1*/-	6	61.7b	15.0a	31.1a	570.5a	34.2a
(BLT01, BLT02) × A619	*Htn1*/-	12	67.0b	12.5a	30.5a	473.2a	32.8a
SS × NS Mean		24	67.9A	13.2A	30.5C	496.3AB	32.9BC
CO388 × A619*Ht*(s)	-/(*Ht1-Ht3*)	18	43.1a	13.6a	32.9a	491.1b	34.4b
BLT03 × A619*Ht*(s)	*Htm1*(*Ht1-Ht3*)	18	31.1b	14.7a	33.1a	525.2a	36.0a
(BLT01, BLT02) × A619*Ht*(s)	*Htn1*/(*Ht1-Ht3*)	36	30.2b	14.4a	34.9a	517.2ab	36.1a

Table 2. *Cont.*

Genotypes	Female/Male Genes	#	PLAA (%)	Yield (t h^{-1})	KM (%)	KNPE	100KW (g)
SS × NS Mean		72	33.7C	14.3A	33.9B	512.7A	35.6A
CO388 × T3	-/Partial	6	69.1a	9.7b	26.6a	434.1a	29.3a
BLT03 × T3	*Htm1*/Partial	6	41.7b	11.2a	27.9a	460.9a	32.6a
(BLT01, BLT02) × T3	*Htn1*/Partial	12	39.6b	10.8ab	28.1a	462.4a	31.1a
SS × Iodent Mean		24	47.5B	10.6B	27.7D	455.0AB	31.0C
CO428 × A619	PG/-	6	50.8a	8.7a	36.5a	371.8a	28.4a
BLT11 × A619	PG*Ht1*/-	6	49.0a	8.9a	34.0a	405.6a	27.6a
(BLT07, BLT12) × A619	PG*Ht2*/-	12	53.6a	8.4a	33.9a	367.5a	28.5a
BLT13 × A619	PG*Ht3*/-	6	46.5a	9.2a	33.2a	389.2a	30.2a
(BLT05, BLT10) × A619	PG*Htm1*/-	12	42.5a	8.8a	35.1a	367.1a	31.2a
BLT06 × A619	PG*Htn1*/-	6	50.5a	7.5a	33.9a	362.6a	27.7a
NS × NS Mean		48	48.6B	8.6D	34.4B	374.8D	29.2D
CO428 × A619*Ht*(s)	PG/(*Ht1-Ht3*)	18	21.5a	8.7b	38.7a	393.6b	28.0b
BLT11 × A619*Ht*(s)	PG*Ht1*/(*Ht1-Ht3*)	18	16.3b	10.0a	37.3a	437.2a	28.4ab
(BLT07, BLT12) × A619*Ht*(s)	PG*Ht2*/(*Ht1-Ht3*)	36	15.3b	9.6ab	38.6a	396.0b	30.9a
BLT13 × A619*Ht*(s)	PG*Ht3*/(*Ht1-Ht3*)	18	16.9b	10.5a	37.0a	452.4a	29.1ab
(BLT05, BLT10) × A619*Ht*(s)	PG*Htm1*/(*Ht1-Ht3*)	36	16.9b	10.0a	37.3a	405.0b	31.1a
BLT06 × A619*Ht*(s)	PG*Htn1*/(*Ht1-Ht3*)	18	17.2b	9.4ab	37.5a	384.1b	31.0a
NS × NS Mean		144	17.0E	9.7C	37.8A	408.6C	30.1CD
CO428 × CO388	PG/-	6	27.0a	13.5a	35.8a	503.7a	33.6ab
(BLT07, BLT12) × CO388	PG*Ht2*/-	12	21.3a	14.0a	39.2a	500.9a	35.2a
BLT13 × CO388	PG*Ht3*/-	6	27.6a	13.3a	37.5a	533.0a	31.6b
(BLT05, BLT10) × CO388	PG*Htm1*/-	12	23.4a	13.9a	34.9a	513.6a	35.0a
(BLT06, BLT09) × CO388	PG*Htn1*/-	12	30.5a	12.1a	35.5a	501.0a	31.2b
NS × SS Mean		48	25.6D	13.3A	36.5AB	508.5A	33.5B
CO428 × (BLT02, BLT03)	PG/(*Htm1*, *Htn1*)	12	18.1ab	13.4b	37.3ab	477.6b	34.8a
BLT11 × (BLT02, BLT03)	PG*Ht1*/(*Htm1*, *Htn1*)	18	14.4b	15.4a	39.5a	562.6a	34.6ab
(BLT07, BLT12) × (BLT02, BLT03)	PG*Ht2*/(*Htm1*, *Htn1*)	24	21.6a	13.6ab	35.6b	470.9b	33.4ab
BLT13 × (BLT02, BLT03)	PG*Ht3*/(*Htm1*, *Htn1*)	6	20.9ab	13.7ab	38.2a	527.2a	33.8ab
(BLT05, BLT10) × (BLT02, BLT03)	PG*Htm1*/(*Htm1*, *Htn1*)	18	17.0ab	13.7ab	36.3b	481.9b	35.6a
(BLT06, BLT9) × (BLT02, BLT03)	PG*Htn1*/(*Htm1*, *Htn1*)	12	20.7ab	13.1b	35.9b	497.9ab	32.3b
NS × SS Mean		90	18.6E	13.9A	37.0A	499.7AB	34.1AB
LSD(0.05)			18.4	2.7	6.4	81.6	3.7
Heritability			0.92	0.84	0.89	0.82	0.83
Combined results and their percentages shown in parentheses							
(CO388, BLT01-BLT03) × A619 (CO428, BLT05-BLT13) × A619	-	72	55.0a (100.0)	10.1b (100.0)	33.1c (100.0)	415.3b (100.0)	30.4b (100.0)
(CO388, BLT01-BLT03) × A619*Ht1* (CO428, BLT05-BLT13) × A619*Ht1*	*Ht1*	72	26.8b (47.2)	11.0a (108.7)	34.1b (102.9)	437.9ab (105.4)	31.7a (104.2)
(CO388, BLT01-BLT03) × A619*Ht2* (CO428, BLT05-BLT13) × A619*Ht2*	*Ht2*	72	21.1c (38.3)	11.5a (113.3)	37.3a (112.7)	449.3a (108.2)	32.4a (106.5)
(CO388, BLT01-BLT03) × A619*Ht3* (CO428, BLT05-BLT13) × A619*Ht3*	*Ht3*	72	20.7c (37.6)	11.3a (111.3)	38.1a (115.1)	442.8a (106.6)	31.8a (104.5)

Table 2. *Cont.*

Genotypes	Female/Male Genes	#	PLAA (%)	Yield (t h^{-1})	KM (%)	KNPE	100KW (g)
CO388 × (A619, A619*Ht*(s), 5 testers) and (CO428, BLT05-BLT13) × CO388	-	102	47.9a (100.0)	12.3b (100.0)	31.7a (100.0)	479.6b (100.0)	31.9b (100.0)
BLT03 × (A619, A619*Ht*(s), 5 testers) and (CO428, BLT05-BLT13) × BLT03	*Htm1*	90	35.2b (73.3)	13.1a (106.2)	32.3a (101.9)	498.3a (103.9)	33.2a (104.0)
(BLT01, BLT02) × (A619, A619*Ht*(s), 5 testers); and (CO428, BLT05-BLT13) × (BLT01, BLT02)	*Htn1*	162	34.4b (71.8)	12.8a (103.4)	33.0a (103.4)	489.8a (102.1)	33.4a (104.5)
CO428 × (A619, A619*Ht*(s)) and CO428 × (CO388, BLT02, BLT03)	*PG*	42	25.5a (100.0)	10.7b (100.0)	37.6a (100.0)	430.2b (100.0)	30.8c (100.0)
BLT11 × (A619, A619*Ht*(s)) and BLT11 × (CO388, BLT02, BLT03)	*PGHt1*	42	21.4a (84.1)	12.2a (114.2)	37.6a (99.9)	493.7a (114.8)	30.6c (99.5)
(BLT07, BLT12) × (A619, A619*Ht*(s)) and (BLT07, BLT12) × (CO388, BLT02, BLT03)	*PGH2*	84	23.4a (91.9)	11.2a (104.5)	37.1a (98.8)	428.3b (99.6)	31.9a (103.6)
BLT13 × (A619, A619*Ht*(s)) and BLT13 × (CO388, BLT02, BLT03)	*PGHt3*	36	23.0a (90.4)	11.8a (110.3)	36.6a (97.3)	479.8a (111.5)	31.0c (100.6)
(BLT05, BLT10) × (A619, A619*Ht*(s)) and (BLT05, BLT10) × (CO388, BLT02, BLT03)	*PGHtm1*	78	21.3a (83.6)	11.4a (106.5)	36.4a (96.7)	435.3b (101.2)	33.1a (107.6)
(BLT06, BLT09) × (A619, A619*Ht*(s)) and (BLT06, BLT09) × (CO388, BLT02, BLT03)	*PGHtn1*	60	24.3a (95.5)	10.6b (99.3)	36.4a (96.8)	435.0b (101.1)	30.7c (99.7)

From the biggest to the smallest, "a", "b", "c", "d" indicated significant difference at $p = 0.05$ for genes with a similar background. "A", "B", "C", "D", "E" indicated significant difference at $p = 0.05$ for group means. BLT01-BLT03 included BLT01, BLT02, and BLT03. BLT05-BLT13 included BLT05, BLT06, BLT07, BLT09, BLT10, BLT11, BLT12, and BLT13. A619*Ht*(S) included A619*Ht1*, A619*Ht2*, and A619*Ht3*. Testers included CO388, BLT01-BLT03, A619, A619*Ht*(s), CL30, CO442, T1, T2, and T3. "-" means no resistant gene. Tester T3 had "Partial" resistance. "PG" means parent with polygenic resistance, and PG*Ht*(s) included PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1*. # = Genotype number × replicate number × Year number.

Table 2 showed when a female had no resistant gene (CO388) and was crossed with other *Ht* genes, *Ht1* (A619*Ht1*), *Ht2* (A619*Ht2*), and *Ht3* (A619*Ht3*) had less PLAA than partial resistance (T3) and susceptible crosses (CO388 × A619). *Ht1* had the least PLAA, and *Ht3* had less PLAA than *Ht2* had. When a female had resistant gene *Htm1* (BLT03) or *Htn1* (BLT01 and BLT02), crossed with susceptible testers (A619, CL30, CO442, T1, and T2), their PLAAs were better than group susceptible checks, but not as good as *Ht1*, *Ht2*, *Ht3* were. The same conclusion can be made for partial resistance T3 when comparing CO388×T3 with the above results. If only one resistant gene was available, the resistant order from the best to worst was, *Ht1* > *Ht2* ≈ *Ht3* > *Htm1* ≈ *Htn1* ≈ Partial > S checks. These results indicate that if only one resistant gene is available, *Ht1* is still a desirable choice to make resistant crosses.

Compared to group checks, the effects of *Ht1*, *Ht2*, and *Ht3* to four yield traits were similar, increased yield, KM, KNPE, and 100 KW, but was not statistically significant in most cases. The effects of *Htm1* and *Htn1* had comparable results, increased yield, KM, KNPE, and 100 KW. A total of 2/6 backgrounds, SS × (early flint, Iodent), and SS × NS for 100 KW were statistically significant ($p > 0.05$). Again, combined results showed that overall gene effects (%) of *Ht1*, *Ht2*, *Ht3*, *Htm1* and *Htn1* were 8.7, 13.3, 11.3, 6.2 and 3.4 for yield; 2.9, 12.7, 15.1, 1.9 and 3.4 for KM; 5.4, 8.2, 6.6, 3.9 and 2.1 for KNPE; and 4.2, 6.5, 4.5, 4.0 and 4.5 for 100 KW, respectively. The order of five single gene effects for yield was *Ht2* > *Ht3* > *Ht1* > *Htm1* > *Htn1*. All single genes increased yield, 100 KW, and KNPE, but it increased KM at the same time.

3.5. Effects of PG, PGHt1, PGHt2, PGHt3, PGHtm1, and PGHtn1

When comparing the PLAA result of CO428 × A619 with the CO388 × A619 family (including A619*Ht1*, A619*Ht2*, and A619*Ht3*), and (BLT01-BLT03) × A619, the effects of PG were not as good as *Ht1*, *Ht2*, and *Ht3*, but better than how *Htm1* and *Htn1* did. However, CO428 × CO388 and (BLT05-BLT13) × CO388 expressed more tolerance than all other single gene crosses did, and had much less PLAA. PG and PG*Ht*(s) (including PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1*) had the better resistance. It seems that PG can express over-dominant resistance in some specific crosses.

Table 2 showed that PG, PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1* could be compared under two NS × NS and two NS × SS backgrounds. For PLAA, though PG is not always the best, most cases of PG*Ht*(s) were not statistically better than PG. This meant that when single *Ht*(s) (including *Ht1*, *Ht2*, *Ht3*, *Htm1*, *and Htn1*) introgressed into PG, some minor genes might be lost from the original polygenes. Combined results, compared with PG, the overall gene effects (%) of PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1* were −15.9, −8.1, −9.6, −16.4, and −4.5 for PLAA, respectively. The order of PG and PG*Ht*(s) gene effects for PLAA was PG*Htm1* < PG*Ht1* < PG*Ht3* < PG*Ht2* < PG*Htn1* < PG. The overall order of all resistant genes in this study for PLAA *Ht3* ≈ *Ht2* ≈ PG*Htm1* ≈ PG*Ht1* < PG*Ht3* ≈ PG*Ht2* < PG*Htn1* < PG < *Ht1* < Partial < *Htn1* ≈ *Htm1*, and the smaller the better.

The effects of PG, PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1* on yield, KM, KNPE, and 100 KW varied, and in some cases, were statistically different. Combined results, compared with PG, the overall gene effects (%) of PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1* were 14.2, 4.5, 10.3, 6.5, and −0.7 for yield; −0.1, −1.2, −2.7, −3.3, and −3.2 for KM; 14.8, −0.4, 11.5, 1.2, and 1.1 for KNPE, and −0.5, 3.6, 0.6, 7.6, and −0.3 for 100 KW, respectively. PG*Ht1* had the best yield, mainly because BLT11 × BLT02 and BLT11 × BLT03 had specific combing ability. Both had a two-year average yield of 15.4 and 15.3 t h^{-1}. The order of these genes for yield was PG*Ht1* > PG*Ht3* > PG*Htm1* > PGHt2 > PG ≈ PG*Htn1*. PG*Ht1*, PG*Ht3*, PG*Htm1*, and PGHt2 had the potential for yield increasing.

3.6. Effects of Gene Combinations

Table 3 showed that effects of resistant genes were different, depending on their female or male backgrounds. If there was only one parent with resistance, PG/- had the best resistance, followed by PG*Ht*(s)/-, -/(*Ht1*, *Ht2*, *Ht3*), PG/-, (*Htm1*, *Htn1*)/-, (*Htm1*, *Htn1*)/-, and -/Partial, which had the least resistance. If both parents had resistance genes, PG*Ht*(s)/(*Ht1*, *Ht2*, *Ht3*) had the best resistance, followed by PG/(*Htm1*, *Htn1*) ≈ PG*Ht*(s)/(*Htm1*, *Htn1*), PG/(*Ht1*, *Ht2*, *Ht3*), (*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*), and (*Htm1*, *Htn1*)/Partial, which had the least resistance. In Table 3, when comparing female CO388 and its *Htm1* (BLT03) or *Htn1* (BLT01 and BLT02) versions, their average effects (%) were −23.1 for PLAA. When CO388 crossed with susceptible testers, compared to CO388 with resistant lines with *Ht1*, *Ht2*, *Ht3*, and Partial, their average effects (%) were −39.7 for PLAA. If both parents had resistant genes (*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*, Partial), their average effects (%) were −59.9 for PLAA. Female gene effects + male gene effects ≈ gene effects of both parents with resistant genes, which indicated that additive action was much more important for PLAA. Similarly, when compared with PG, the average female gene effect (%) for PG*Ht*(s) was −5.2 for PLAA, and the average male gene effect (%) for *Ht*(s) was −48.3 for PLAA. Female gene effects + male gene effects ≈ the gene effects (%) of PG*Ht*(s)/*Ht*(s) was −56.0 for PLAA. Again, it proves that additive action played most important role for resistance.

Table 3. Comparison of average gene effects of female, male, and their combination to percent leaf area affected (PLAA, %), yield (t h^{-1}), kernel moisture (KM, %), kernel number per ear (KNPE), and 100-kernel weight (100 KW, g).

Genotype	Female/Male Genes	#	PLAA (%)	Yield (t h^{-1})	KM (%)	KNPE	100KW (g)
Susceptible hybrid checks	-/-	42	77.7a	10.7b	20.6a	505.9b	26.1a
Resistant hybrid checks	*Ht*?/*Ht*?	18	45.5b	11.9a	19.6a	549.0a	27.7a
CO388 × 4 testers	-/-	24	83.9a	9.9b	23.1b	427.7b	27.6b
(BLT01- BLT03) × 4 testers	(*Htm1*, *Htn1*)/-	72	62.8b	10.7a	26.3a	456.9a	30.2a
CO388 × T3	-/Partial	6	69.1a	9.7b	26.6a	434.1a	29.3b
(BLT01- BLT03) × T3	(*Htm1*, *Htn1*)/Partial	18	40.3b	10.9a	28.0a	461.9a	31.6a
CO388 × A619	-/-	6	75.8a	12.9b	29.7b	468.1b	31.7b
CO388 × A619*Ht*(s)	-/(*Ht1*, *Ht2*, *Ht3*)	18	43.1c	13.6ab	32.9a	491.1ab	34.4ab
(BLT01- BLT03) × A619	(*Htm1*, *Htn1*)/-	18	65.2b	13.3b	30.7ab	505.6a	33.3ab
(BLT01- BLT03) × A619*Ht*(S)	(*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*)	54	30.5d	14.5a	34.3a	519.9a	36.1a
CO428 × A619	PG/-	6	50.8a	8.7b	36.5ab	371.8b	28.4b
CO428 × A619*Ht*(s)	PG/(*Ht1*, Ht2, *Ht3*)	18	21.5b	8.7b	38.7a	393.6ab	28.0b
(BLT05-BLT13) × A619	PG*Ht*(s)/-	42	48.3a	8.6b	34.1b	375.2ab	29.3ab
(BLT05-BLT13) × A619*Ht*(S)	PG*Ht*(s)/(*Ht1*, *Ht2*, *Ht3*)	138	16.2b	9.9a	37.7a	411.9a	30.3a
CO428 × CO388	PG/-	6	27.0a	13.5a	35.8a	503.7a	33.6a
CO428 × (BLT02, BLT03)	PG/(*Htm1*, *Htn1*)	12	18.1b	13.4a	37.3a	477.6a	34.8a
(BLT05-BLT13) × CO388	PG*Ht*(s)/-	42	25.4a	13.3a	36.7a	509.1a	33.5a
(BLT05-BLT13) × (BLT02, BLT03)	PG*Ht*(s)/(*Htm1*, *Htn1*)	72	18.7b	14.0a	36.9a	503.1a	34.0a3)
Combined results and their percentages shown in parentheses							
CO388 × (A619, CL30, CO442, T1, T2)	-/-	30	82.3a (100.0)	10.5b (100.0)	24.4b (100.0)	435.7b (100.0)	28.4b (100.0)
(BLT01- BLT03) × (A619, CL30, CO442, T1, T2)	(*Htm1*, *Htn1*)/-	90	63.3b (76.9)	11.2ab (107.5)	27.2b (111.6)	466.7b (107.1)	30.8b (108.5)
CO388 × (A619*Ht*(s), T3)	-/(*Ht1*, *Ht2*, *Ht3*, Partial)	24	49.6c (60.3)	12.7a (121.1)	31.3a (128.3)	476.8b (109.4)	33.1a (116.7)
(BLT01- BLT03) × (A619*Ht*(s), T3)	(*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*, Partial)	72	33.0d (40.1)	13.6a (130.1)	32.7a (134.2)	505.4a (116.0)	34.9a (123.0)
CO428 × (A619, CO388)	PG/-	12	38.9a (100.0)	11.1a (100.0)	36.2ab (100.0)	437.7a (100.0)	31.0a (100.0)
(BLT05–BLT13) × (A619, CO388)	PG*Ht*(s)/-	84	36.9a (94.8)	10.9a (98.7)	35.4b (97.9)	442.2a (101.0)	31.4a (101.3)
CO428 × (A619*Ht*(s), BLT02, BLT03)	PG/*Ht*(s)	30	20.1b (51.7)	10.6a (95.2)	38.2a (105.6)	427.2a (97.6)	30.7a (99.1)
(BLT05–BLT13) × (A619*Ht*(s), BLT02, BLT03)	PG*Ht*(s)/*Ht*(s)	210	17.3b (44.4)	11.2a (101.2)	37.3a (103.3)	441.9a (100.9)	31.5a (101.7)

"a", "b", "c", "d" from the biggest to the smallest, significant difference at $p = 0.05$ within a similar background. Four testers = CL30, CO442, T1, and T2 were the four testers crossed with CO388, BLT01, BLT02, and BLT03. A619*Ht*(S) included A619*Ht1*, A619*Ht2*, and A619*Ht3*; (BLT01-BLT03) included BLT01, BLT02, and BLT03; and (BLT05-BLT13) included BLT05, BLT06, BLT07, BLT09, BLT10, BLT11, BLT12, and BLT13. "-"means no resistant gene. Tester T3 had "Partial" resistance. "PG" means parent with polygenic resistance, *Ht*(s) included *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1*; and PG*Ht*(s) included PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1*. # = Genotype number × replicate number × Year number.

Table 3 showed that when compared, CO388, BLT03 or BLT01 or BLT02 crossed with susceptible or resistant testers, plus resistant gene(s) for one or both parents, yield, KM, KNPE, and 100 KW increased in most cases, but was not always significant. The average female gene effects (%) of (*Htm1*, *Htn1*) for yield, KM, KNPE, and 100 KW were 7.5, 11.6, 7.1, and 8.5, respectively; the average male gene effects (%) (*Ht1*, *Ht2*, *Ht3*, Partial) for yield, KM, KNPE, and 100 KW were 21.1, 28.3, 9.4, and 16.7, respectively. Female

gene effects + male gene effects ≈ the average gene effects (%) of (*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*) for yield, KM, KNPE, and 100 KW, which were 30.1, 34.2, 16.0, and 23.0, respectively. Additive action played the most key role for yield traits, too. However, such additive action was not found within crosses of CO428 and BLT05-BLT13 crossed with A619, A619Ht(s), CO388, and BLT01–BLT03 for their yield traits. There were eight crosses (gene combinations) which had both good PLAA (<30%) and good yield (>14.0 t/ha), which were BLT01 × A619Ht1 (*Htn1*/*Ht1*, 28.1, 14.3), BLT01 × A619*Ht2* (*Htn1*/*Ht2*, 26.2, 15.4), BLT01 × A619*Ht3* (*Htn1*/*Ht3*, 16.1, 15.3), BLT10 × BLT03 (PG*Htm1*/*Htm1*, 23.3, 14.3), BLT11 × BLT02 (PG*Ht1*/*Htn1*, 11.7, 15.4), BLT11 × BLT03 (PG*Ht1*/*Htm1*,19.8, 15.3), BLT12 × BLT01(PG*Ht2*/*Htn1*, 19.1, 15.2), and BLT12 × BLT03 (PG*Ht2*/*Htm1*, 15.5, 15.0). Some dominant effects were found within crosses of BLT10, BLT11, and BLT12 with BLT01–BLT03 for their yield traits.

3.7. Predict Losses of Yield Traits with PLAA and Effects of Resistant Genes to Yield Traits

PLAA and four yield traits from 10 hybrid checks, 16 crosses of CO388 × A619 family, 26 crosses of CO428 × CO388 family, 34 crosses of CO428 × A619 family, 2 year, and 3 replicates were used to make regressions and losses prediction (Table 4). PLAA, yield, KM, KNPE, and 100 KW and their predicted losses varied among and within families. The maximum predicted losses (PL, %) ranged from 32.9 to 51.4 for yield, 27.9 to 40.0 for KM, 23.7 to 39.8 for KNPE, and 12.3 to 34.7 for 100 KW, respectively. The predicted effects of resistant genes (PERG, %) ranged from 32.1 to 50.3 for yield, 27.2 to 39.5 for KM, 23.1 to 37.6 for KNPE, and 12.0 to 21.4 for 100 KW, respectively. Aside from maximum PL of 100 KW for hybrid checks, all other maximum, mean, and minimum predicted yield losses > KM > KNPE > 100 KW. The overall regressions were PL_{Yield} = 0.5042 × PLAA, PL_{KM} = 0.3634 × PLAA, PL_{KNPE} = 0.3483 × PLAA, and $PL_{100\,KW}$ = 0.2721 × PLAA, and the maximum predicted losses (%) were 50.4, 36.3, 34.8, and 27.2 for yield, KM, KNPE, and 100 KW, respectively. In general, when PLAA = 5, 10, 20, 40, 60, 80, and 100, their corresponded yield losses = 2.5, 5, 10, 20, 30, 40, and 50, respectively.

Table 4. Regression relationships and predicted losses (PL, %), and predicted effects of resistant genes (PERG, %) on percent leaf area affected (PLAA, %) and yield (t h^{-1}), kernel moisture (KM, %), kernel number per ear (KNPE), and 100-kernel weight (100 KW, g).

Genotypes	Traits	#	Trait Range	Regression			PL (%)	PERG (%)
			Max/Mean/Min	m	β	r	Max/Mean/Min	
7 susceptible and 3 resistant hybrid checks	PLAA	60	97.7/68.0/9.2					
	Yield	60	17.8/11.1/6.0	16.106	−0.074	−0.713 **	44.9/31.3/4.2	40.7
	KM	60	35.3/20.3/10.8	26.034	−0.084	−0.423 **	31.4/21.9/2.9	28.5
	KNPE	60	710/518/325	631.23	−1.652	−0.550 **	25.6/17.8/2.4	23.1
	100 KW	60	36.9/26.6/16.1	35.021	−0.124	−0.670 **	34.7/24.2/3.3	31.4
(CO388, BLT01, BLT02, BLT03) × (A619, A619Ht1, A619Ht2, A619Ht3)	PLAA	96	97.7/42.2/5.5					
	Yield	96	19.7/14.0/5.5	18.017	−0.095	−0.697 **	51.4/22.2/2.9	48.5
	KM	96	51.7/33.1/25.6	38.561	−0.130	−0.637 **	32.9/14.2/1.8	31.1
	KNPE	96	709/509/253	614.27	−2.503	−0.649 **	39.8/17.2/2.2	37.6
	100 KW	96	40.8/35.0/25.9	38.869	−0.093	−0.722 **	23.3/10.1/1.3	32.0
(CO428, BLT05, BLT06, BLT07, BLT09, BLT10, BLT11, BLT12, BLT13) × (CO388, BLT01, BLT02, BLT03)	PLAA	156	64.1/21.1/1.6					
	Yield	156	19.7/13.7/5.4	15.318	−0.079	−0.485 **	32.9/10.8/0.8	32.1
	KM	156	60.2/36.5/25.7	40.201	−0.175	−0.424 **	27.9/9.2/0.7	27.2
	KNPE	156	772/505/354	547.36	−2.026	−0.444 **	23.7/7.8/0.6	23.1
	100 KW	156	41.0/33.9/24.0	35.348	−0.068	−0.331 **	12.3/4.1/0.3	12.0

Table 4. *Cont.*

Genotypes	Traits	#	Trait Range	Regression			PL (%)	PERG (%)
			Max/Mean/Min	m	β	r	Max/Mean/Min	
(CO428, BLT05, BLT06,	PLAA	204	94.3/24.3/1.2					
BLT07, BLT09, BLT10,	Yield	204	13.3/9.5/4.1	10.886	−0.059	−0.591 **	50.9/13.1/0.6	50.3
BLT11, BLT12, BLT13)	KM	204	64.6/37.0/23.4	41.282	−0.175	−0.509 **	40.0/10.3/0.5	39.5
× (A619, A619Ht1,	KNPE	204	590/402/238	444.47	−1.765	−0.526 **	37.5/9.7/0.5	37.0
A619Ht2, A619Ht3)	100 KW	204	36.8/29.8/20.1	31.529	−0.071	−0.481 **	21.3/5.5/0.3	21.0
Overall regression models for predicted losses (PL) based on above results	Yield	PL (%) for yield = (0.5042)PLAA, r = 0.995 **						
	KM	PL (%) for KM = (0.3634)PLAA, r = 0.979 **						
	KNPE	PL (%) for KNPE = (0.3483)PLAA, r = 0.959 **						
	100 KW	PL (%) for 100 KW = (0.2721)PLAA, r = 0.943 **						

** significant difference at $p = 0.01$ for regression coefficient. # = Genotype number × replicate number × Year number. Max = maximum, Mean = average, and Min = Minimum. "m", "β", and "r" are the parameters of regression formula Y = m + β × PLAA, and its regression coefficient, respectively.

3.8. E2 Results

PLAA results for all genotypes from E2 (data not showed) indicated that PLAA of twice > once > none, all significant ($p < 0.05$) with both *t*-test and/or LSD methods. Without inoculation, all PLAA were extremely low (≤ 0.7), and its heritability only 0.19. Meanwhile, for groups inoculated once or twice, PLAA ranged from 1.0 to 77.2 or 6.4 to 97.8, with an average of 18.2 and 37.1, respectively; its heritability increased from 0.92 to 0.94 (Table 5), respectively. PLAA of -/- > -/Partial > (*Htm1*, *Htn1*)/- > -/(*Ht1*, *Ht2*, *Ht3*) > PG*Ht*(s)/- > PG/- > (*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*) > PG*Ht*(s)/(*Htm1*, *Htn1*) > PG*Ht*(s)/(*Ht1*, *Ht2*, *Ht3*), and these results were similar to E1 (Table 3) results.

Because mean yield of ten hybrid checks of E2 were 10.7, 11.1, and 12.6 t h^{-1} for none, one, and two inoculation treatments, respectively (Table 5), RY—not yield—was used to compare the effects of different gene combinations under different NCLB epidemic conditions. RY results for all genotypes from E2 (data not shown) were similar as E1; the CO388 family × A619 family had the best RY, followed by the CO428 family × CO388 family, CO388 family × CO442, CO428 family × (CO442, T4, or T5), CO388 family × (T1 or T2), CO428 family × A619 family, CO388 family × CL30, and CO428 family × CL30, which had the least RY. In most cases, resistant crosses had higher RY under two inoculation treatments ($p < 0.05$), more RY had no significant difference between none and one inoculation.

Combined RY results from E2 (Table 5) showed that six combinations had no statistical difference among three treatments, including both S and R checks, -/-, (*Htm1*, *Htn1)*/-, and two partial resistance combinations. All (*Ht1*, *Ht2*, *Ht3*) combinations increased RY (%) >10 and >30 under one or two treatments, respectively. (*Htm1*, *Htn1*) combinations had unstable RY results, and did not increase when cross with early testers (CL30, CO442, T1, T2, and T3), but increased >10 to 20 under one or two treatments when crossed with late parent A619 or CO428, BLT06, and BLT10. It seems that *Htm1* and *Htn1* had better effects within later or stay-green crosses. RY of PG/- and PG*Ht*(s)/- increased >5 to 20 when crossed with bigger plant tester CO388, but was unstable when crossed with smaller plant tester A619 under one or two treatments. The grant mean RY for none, once, and twice were 89.1, 93.4, and 103.1, which meant it had 4.3 to 14.0 RY increasing, respectively; two inoculations increased RY statistically significantly than in none and one inoculations by both *t*-test and LSD2(0.05) methods. -/(*Ht1*, *Ht2*, *Ht3*), (*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*), and PG*Ht*(s)/(*Ht1*, *Ht2*, *Ht3*) had RY increasing > 30 under two inoculations compared to no inoculation environments; and maximum RY increased up to 56.2, coming from cross BLT01 × A619Ht3. When comparing within the same treatment, resistant crosses increased RY not statistically differently by the LSD(0.05) method, but had a >12% RY increase in the CO388 family crossed with the A619 family, and in early testers under the two inoculation environment. Not much RY advantage showed in CO428 family-related crosses. The above

results indicated that resistant genes increased yield more efficiently under NCLB epidemic environments, but hybrids with highly resistant PG resistance have more stable yield at all environments in this study.

Table 5. Comparisons of percent of leaf area affected (PLAA) and relative yield (RY, %) changes among none-, once-, and twice-inoculation treatments by grouping resistant (R) and susceptible (S) parents.

Genotype	Assumed Resistant Genes	PLAA			RY (%)		
		None	Once	Twice	None	Once	Twice
Grand mean		0.1 a	18.2 b	37.1 c	89.1 a	93.4 b	103.1 c
Check mean		0.2 a	36.3 b	54.3 c	100.0 a	100.0 a	100.0 a
S checks	-/-	0.2 a	44.4 b	66.2 c	95.9 a	94.2 a	95.5 a
R checks	*Ht?/Ht?*	0.2 a	17.3 b	26.7 b	109.5 a	113.4 a	110.6 a
Cross mean		0.1 a	16.1 b	35.1 c	87.8 a	92.6 b	103.4 c
S × S cross	-/-	0.2 a	39.7 b	70.0 c	99.5 a	94.1 a	93.0 a
S × R cross	-/(*Ht1*, *Ht2*, *Ht3*)	0.1 a	13.6 b	25.9 c	103.7 a	118.7 b	134.8 c
S × Partial cross	-/Partial	0.3 a	23.0 b	51.8 c	91.6 a	91.7 a	96.6 a
R × S cross	(*Htm1*, *Htn1*)/-	0.2 a	23.1 b	50.6 c	102.4 a	103.8 a	101.8 a
R × R cross	(*Htm1*, *Htn1*)/(*Ht1*, *Ht2*, *Ht3*)	0.0 a	11.6 b	14.9 c	101.4 a	109.9 b	135.9 c
R × Partial cross	(*Htm1*, *Htn1*)/Partial	0.1 a	8.8 b	31.8 c	100.8 a	102.4 a	98.8 a
PG × S cross	PG/-	0.1 a	7.9 b	25.7 c	87.6 ab	82.2 a	99.5 b
PG × R cross	PG/*Ht*(s)	0.0 a	5.0 b	9.5 c	67.8 a	92.4 b	94.8 b
PG*Ht*(s) × S cross	PG*Ht*(s)/-	0.1 a	19.6 b	46.5 c	84.3 a	87.1 a	99.2 b
PG*Ht*(s) × R cross	PG*Ht*(s)/*Ht*(s)	0.1 a	3.9 b	11.0 c	69.1 a	77.2 b	96.1 c
Real grand mean		0.1 a	18.2 b	37.1 c	9.5 a	10.4 b	13.0 c
Real check mean		0.2 a	36.3 b	54.3 c	10.7 a	11.1 ab	12.6 b
Real cross mean		0.1 a	16.2 b	35.3 c	9.3 a	10.3 b	13.0 c
CV(%)		199.8	40.4	30.3	12.6	14.6	10.6
LSD(0.05)		0.4	11.8	18.1	18.2	22.0	17.6
LSD(0.05) among treatments			4.4			6.3	
Heritability		0.19	0.92	0.94	0.90	0.89	0.91

"a", "b", "c" from smallest to biggest, significant difference at $p = 0.05$ for comparing three treatment effects among none-, once-, and twice- inoculation treatments. "-"means no resistant gene from females or males. S = susceptible parent without resistant gene; R = Resistant parent with dominant gene *Ht1*, *Ht2*, *Ht3*, *Htm1*, or *Htn1*; PG = parent with polygene resistance; PG*Ht*(s) = parent with in PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, or PG*Htn1*. LSD (0.05) is the least significant difference at $p = 0.05$.

3.9. Discussion

The correlation coefficient between PLAA and BTL was 0.11, lower than expected, because bareness was found not only on the tip, but also at side or bottom of an ear. Bareness should be used for future studies.

To understand inbred yield losses, yields from E1 for tester lines were measured and compared with lines in breeding or isolated nurseries (data not shown) in 2015. Yield losses (%) were 67.0, 48.2, 33.4, 26.8, 80.3, 87.5, 33.6, 57.0, 71.7, 46.8, 27.8, 64.5, 36.0, 28.6, and 50.5 for CO388, BLT01, BLT02, BLT03, A619, A619*Ht1*, A619*Ht2*, A619*Ht3*, CL30, CO442, T1, T2, T3, T4, and T5, respectively. PG line CO428 only had a yield loss of 16.4. A619 and A619*Ht1* seemed have yield losses > 80; however, they pollen-shed 5–7 days earlier than silking emergence, and a one-row plot was not enough for a good kernel-setting, their real yield losses caused by NCLB should be smaller. Inbred had yield losses up to 71.7 as CL30 was more reliable, which is similar to Kloppers and Tweer's report [7].

Under the NCLB epidemic environment, the mean yield for 10 hybrid checks had a mean yield of 11.1 t h^{-1} and KM 20.3% (Table 4). Considering predicted losses, the mean yield and KM could be 16.1 t h^{-1} and 26.0%, which was close to the OCC (Ontario Corn Committee) hybrid corn trial (41 hybrids) at the same farm, which had a mean yield of 15.9 t h^{-1} and KM 23.5% [42]. Its indicated yield traits and prediction results were reliable.

In 2015, one fact was observed was that crosses with bigger plants, especially those with longer and wider leaves, were more resistant or tolerant to NCLB. Therefore, three leaf traits, ear-leaf length, ear-leaf width, and ear-leaf angle were measured in 2016. Its results supported the 2015 observation. In three hybrid checks with resistant gene(s), their ELA (cm^2) were 544.7, 621.1, and 428.2, and responded with PLAA 58.0, 50.5, and 84.0, respectively. Four crosses (CO388, BLT01, BLT02, and BLT03) × CO442 had an average ELA and PLAA of 647.4 and 59.8. Crosses with CL30 had an ELA and PLAA of 480.8 and 89.0, respectively; similar results were found with 7 crosses with the same female with PG*Ht*(s); (BLT05 to BLT13) × CO442 had an ELA and PLAA of 566.7 and 42.9, and crosses with CL30 had an ELA and PLAA of 303.8 and 86.1. All CO428 × CO388 related had an ELA and PLAA of 685.0 and 28.1, meanwhile, all CO428 × A619 related had an ELA and PLAA of 488.7 and 32.9. The correlation coefficients between PLAA and ear-leaf length, ear-leaf width, ear-leaf area, and ear-leaf angle were −0.24, −0.45, −0.36, and −0.15, respectively. Crosses with bigger leaves, especially wider leaves, had less PLAA, which is logical because PLAA includes two parts, the diseased area and dried area caused by merged lesions. Only a few susceptible merged lesions could easily cause leaf tip or edge dried on narrower leaves, such as plants of CL30 crosses. This kind of tolerance or susceptibility caused by plant architecture could not be explained with additive or dominant gene effects. Yield losses and gene effects related to CO442 and CL30 crosses could not be predicted in this study, and all correlation coefficients were not significant (data not shown). This indicates that to improve NCLB resistance, introgression *Ht* genes to the larger plant parent as a male will be more effective. More studies are needed to understand the roles of leaf traits to disease development.

E2 results indicated that inoculation effects for PLAA increased 18.1 and 37.0 for one and two inoculations (Table 5), respectively. Irrigation effects for PLAA could be estimated with E2 two-inoculation results and E1 results (Table 3). Irrigation increased PLAA range from 4.8 to 21.9, depending on their resistant combinations; however, both had similar heritability. In a favorite environment for NCLB development such as Ottawa, it rained every 5.2 days in 2015 and 4.8 days in 2016 after first inoculation. The year 2016 had a prolonged period rainfall (rained 133.4 mm in 8 days) in the middle of August, which increased average PLAA 18.7, and maximum PLAA increased up to 52.6. It indicated that artificial inoculation and a favored environment are two key factors for NCLB resistant gene studies.

CVs (%) of E2 were 199.8, 40.4, 30.0, and 51.4 for none, once, twice, and combined treatments (Table 5), respectively. For the same reasons as E1, there were high CVs; an extra high CV for non-inoculation checks meant that natural infection results were not reliable.

In resistan t breeding, resistance, maturity, and yield are not the only things that need to be considered, but also other problems, such as some BLT11 crosses that are sensitive to some herbicides; some BLT12 and BLT13 crosses are also not tolerant to hot temperatures (>37 °C). Thus, only BLT01, BLT02, and BLT03 from the CO388 family, and BLT07, BLT09, and BLT10 from the CO428 family were released as CO468 to CO473 in 2018 [43]. When they crossed with A679 (PI 587142), A681 (Ames 23504), and Wil903 (PI 601686), more higher yield crosses (>18 t h^{-1}) were found in 2018, and their seeds production and yield trials will be done in the coming years.

4. Conclusions

After 10 years of resistant breeding, the *Htm1* and *Htn1* lines were obtained from a high yield GCA inbred, CO388, and the PG*Ht1*, PG*Ht2*, PG*Ht3*, PG*Htm1*, and PG*Htn1* lines were obtained from a PG inbred CO428. Depending on their pedigrees, these lines cross each other, and crossed with *Ht1*, *Ht2*, and *Ht3* lines from A619, partial resistant tester T3, and other testers without resistant genes CO442, CL30, T1, T2, T4, and T5. E1 results showed *Ht1*, *Ht2*, and *Ht3* reduced PLAA effectively (>30%) more than *Htm1* and *Htn1* (>20%) did. If there is only one parent with resistance, *Ht1* had the best resistance, followed by *Ht2* ≈ *Ht3* ≈ PG, and *Htm1* ≈ *Htn1* ≈ Partial. In some cases, the resistance of PG*Ht*(s) was not

statistically better than PG, meaning that one or more minor resistant genes might have been lost when *Ht*(s) introgressed into PG. The overall order of all resistant genes in this study for PLAA is *Ht2* ≈ *Ht3* ≈ PG*Htm1* ≈ PG*Ht1* < PG*Ht3* ≈ PG*Ht2* < PG*Htn1* < PG < *Ht1* < Partial < *Htn1* ≈ *Htm1*, the smaller the better. If both parents have resistant genes, hybrid gene effects ≈ female + male gene effects, (*Htm1*, *Htn1)*/(*Ht1*, *Ht2*, *Ht3*, Partial) reduced PLAA > 50%, and PG*Ht*(s)/*Ht*(s) reduced PLAA > 39%. All single genes and partial resistances increased yield, KM, and KNPE, but 100 KW reduced in some cases. The order of five single gene effects for yield was *Ht2* > *Ht3* > *Ht1* > *Htm1* > *Htn1*. When *Htm1* and *Htn1* are females, their average effects to increased yield were 7.5%; When *Ht1*, *Ht2*, *Ht3*, and Partial are males, their average effects to increased yield were 21.1%; (*Htm1*, *Htn1)*/(*Ht1*, *Ht2*, *Ht3*, Partial) increased yield by 30.1%. Additive action played a major factor for PLAA reduction and increasing yield, KM, KNPE, and 100 KW under NCLB epidemic environments in SS × NS, SS × Iodent, and SS × early flint crosses. PG*Ht1*, PG*Ht3*, PG*Htm1*, and PG*Ht2* had the potential for increasing yield, KNPE, and 100 KW. Additive action was not significant for yield traits related to the CO428 family; specific combing ability was found in some cases. Linear regressions were found between predicted losses of four yield traits: yield, KM, KNPE, 100 KW and PLAA. Predicted losses (%) = (0.5042)PLAA, = (0.3634)PLAA, = (0.3483)PLAA, = (0.2721)PLAA for yield, KM, KNPE, and 100 KW, respectively. When PLAA = 100, maximum yield losses ≈ 50% for crosses. Predicted effects of resistant genes range from 32.1 to 50.3%, 27.2 to 39.5, 23.1 to 37.6, and 12.0 to 31.4 for yield, KM, KNPE, and 100 KW, respectively. However, tolerant CO442 crosses and early CL30 crosses, their losses, and gene effects could not be predicted for yield traits. E2 results indicated that resistant crosses showed significant yield advantages under two inoculations as opposed to none- and one- inoculation environments. When comparing within the same treatment, resistant crosses increased RY > 12% in the CO388 family crossed with the A619 family and early testers under two-inoculation environments. Meanwhile, highly resistant CO428-related crosses have stabler yields at none-, one-, and two-inoculation environments. A resistant gene is better introduced into large parents with a tall plant and long and wide leaves as a male. More studies are needed for polygene resistance and leaf-trait-related tolerance.

Author Contributions: Conceptualization, X.Z. and L.M.R.; methodology, X.Z. and L.M.R.; software, X.Z.; validation, X.Z. and A.K.; 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 A.K.; 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, who obtained funding through "Growing Forward 2" (GF2), a federal-provincial-territorial initiative. The Agricultural Adaptation Council assists in the delivery of GF2 in Ontario.

Data Availability Statement: Data available by contacting correspondence: xiaoyang.zhu@agr.gc.ca.

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, AgReliant Genetics Inc., Maizex Seeds Inc., Pioneer Hi-Bred Ltd., and Deklab (Monsanto Canada Inc.) 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. We thank Reza Jamaly for helping work on references with EndNote.

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

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