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Estimation of Genetic Variance Components for Corn Ear Rot in RIL Populations Derived from Three Biparental Crosses

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HIGHLIGHTS

- A new methodological proposal for estimating variance components is presented.
- Proportion of genetic variance was assessed in corn populations for corn ear rot.
- Breeding strategies could be proposed from estimations of heritabilities.
- Nested ANOVA estimates phenotypic variance components in RIL populations.

Abstract: Classical Quantitative Genetics offers statistical approaches to estimate variance components of important agronomical traits, such as diseases resistance, through different experimental designs. However, the use of RIL in these approaches is limited. As Recombinant Inbred Lines are considered important for developing special breeding projects, we propose a new methodological approach to simultaneously estimate broad and narrow sense heritabilities. This proposed model was applied to calculate heritabilities for resistance to corn ear rots caused by *Fusarium verticillioides* in three independent RIL populations analyzed by nested ANOVA. *Fusarium* Incidence (%) and *Fusarium* Severity heritabilities were significant. The proposed approach proved to be useful for estimating broad and narrow sense heritabilities in the same environment and with these genotypes which is advantageous for corn breeders.

Keywords: nested Analysis of Variance; *Zea mays*; *Fusarium verticillioides*; Quantitative Genetics; Plant Breeding.

INTRODUCTION

Classical Quantitative Genetics allows the development of different statistical models for estimating phenotypic variance components, splitting the genetic variance and the environmental variance, assuming genotype and environment factors as independent, the genotype-environment covariance becomes null [1]. Also, if populations with adequate genetic structure are available, genetic variance can be separated into

additive and non-additive components, enabling the estimation of broad sense heritability (H^2: genetic variance/phenotypic variance) and narrow sense heritability (h^2: additive genetic variance/phenotypic variance). Although both heritabilities estimations present a putative range of variation among 0 and 1, h^2 is expected to be lower or, at least, equal to H^2, since the first is a component of the latter. However, statistical approaches are not enough to simultaneously assess both heritabilities in a same population: in some studies, heritabilities estimations are calculated in different populations in the same crop or, in other studies, heritabilities are calculated on the same populations but in a different environment. For example: offspring-parent regression is a good estimator of h^2, but it is not suitable for estimating H^2 and offspring and parent respective populations are generally growth in different crop generation. Therefore, ANOVA applied to a set of pure lines allows assessing H^2 but the genetic structure of such a population is not necessarily representative of the whole genetic variability available in a given crop. As a consequence, sometimes the expected range of variation $0 \leq h^2 \leq H^2 \leq 1$ is not achieved. Mating schemes where each member of a group of parents used as males is mated to each member of another group of parents used as females have been proposed to simultaneously estimate variance components, such as North Carolina Design II and III, or Diallel designs, but assaying recombinant inbred lines (RIL) populations has presently become usual in Plant Breeding. RIL populations present various advantages because they are generally a representative sample of genetic variance in a given genotype, they are replicable in time and in space because the lack of heterozygous genotypes, and they have a low level of genetic disequilibrium, hence estimations may be skewed by linkage effects. Recombinant inbred lines, which consist of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents. The length of time needed for producing RIL populations is the major disadvantage, because usually six to eight generations are required [2]

Corn (*Zea mays*) is one of the most important crops in the world. It is widely cultivated in tropical and temperate areas for many uses such as human and animal food, forage, and biomass production for bioenergy production. Corn demand is predicted to increase in the short term hence exploitation of genetic potential of its wide biodiversity should be improved in plant breeding programs. Therefore, application of robust statistical techniques is crucial to precisely estimate the variance components and to assess heritabilities for agronomic traits such as yield, grain quality, and tolerance or resistances to biotic and abiotic stresses. Traditional corn breeding is based on phenotyping, not only because of tradition but also because of essence. A plant phenotype is the result of the interactions between the genome of a stationary plant and all the micro- and mega-environments encountered during its life span. Over the recent years, we have witnessed an explosion of technologies developed through collaborative efforts of multidisciplinary teams to assist the process of high-throughput plant phenotyping in plant breeding first only under controlled conditions and, more recently, also under real field conditions [3]. Yet, plant phenotyping is still the bottleneck for breeding and farming [4] and the average plant-breeding program has not been adopting the new developments adequately [5]. Among the solutions proposed, a major international effort is being directed towards data and protocol standardization [6].

Regarding biotic stress, although, resistance to *Fusarium* ear rot is under genetic control and heritable resistance has been identified in corn, no highly resistant genotypes are known [7]. In corn, as stated by [8], there is no evidence of complete resistance to either ear rot or fumonisins contamination and resistance to initial penetration and spreading of the pathogen in host tissue are two components responsible for resistance to *Fusarium* in corn. Symptoms of corn ear rot caused by *F. verticillioides* mainly occur in individual kernels or in limited areas of the ear [9]. In some cases, various independent infections can affect the same ear. A cotton-like mycelium can be observed in infected kernels. In some cases, white lines may develop in the pericarp, this symptom is known as *starbursting*. Since fungicide treatments have random effects because of the variability in the disease development, the exploitation of genetic resistance to *F. verticillioides* becomes the more efficient way to control *Fusarium* infections. The best proposal to deal with commercial production has been the use of hybrids containing resistant alleles at *loci* underlying the response to corn- *F. verticillioides* interaction. Resistance to *Fusarium* corn ear rot is under polygenic control and it is largely affected by environment, immune genotypes having not been found [10, 11]. The complexity of this trait has caused difficulties in breeding programs because most of commercial hybrids show resistance levels lower than the desired ones [12]. There are no screening sites where natural infection is uniformly enough to achieve an efficient selection so that artificial *Fusarium* inoculation projects are necessary for breeding programs. Finally, the genetic complexity of corn ear rot resistance causes low heritabilities estimations at the individual plant level. Therefore, evaluation of family means with an adequate replication design is necessary for obtaining good estimator of heritability [13].

Both broad and narrow sense heritability estimations and empirical selection studies evidence that

artificial selection is able to increase corn ear rot resistance [14]. But frequently most germplasm sources of effective resistance are landraces or non- adapted genotypes that lack of the high agronomic performance of modern elite corn lines [9, 11]. Consequently, due to the small individual effects, breeders have the challenge of introducing resistant alleles of many polygenic *loci* that are generally linked to genes associated with low agronomic performance. Hence, yield reductions due to genetic drift are usually observed when resistance to corn ear rot levels increase. In this plant breeding context, introducing RIL populations derived from widely divergent parents regarding corn ear rot is advantageous for the estimation of variance components and broad and narrow sense heritabilities. First, selection against undesirable traits can be applied along selfing generations so that RIL may have superior agronomic performance than earlier segregating generations. Then, successive recombination cycles among parental genotyoes ensure a low level of gene disequilibrium. Moreover, as RILs are highly homozygous, replication in time and space is possible, and both disease incidence and severity can be measured at families' level. Because of the above mentioned, it is expected that statistical estimation on adequately adapted genotypes becomes more precise and accurate. Finally, since these genotypes are genetically uniform, artificial inoculations can be synchronized and additionally, selected genotypes can then be crossed to obtain superior hybrids varieties for commercial launching.

In this framework, the objectives of this research were to phenotypically characterize the agronomic performance of three independent RIL populations, artificially inoculated with *F. verticillioides* and to determine phenotypic and genetic variances from a nested ANOVA as a methodological approach for calculating broad and narrow sense heritability of *Fusarium* corn ear rot.

MATERIAL AND METHODS

Plant material, agronomic management and evaluated traits

Three independent RIL populations E02/E42, E94/E05, and H32/X28, whose levels of homozygosity corresponded to F6, F7, and F8 generations, respectively, were assayed. The respective parental crosses were E02 x E42, E94 x E05, and H32 x X28. According to previous reports [15], these lines showed a differential response to *Fusarium* spp.: E02 was susceptible, E42, resistant, E94, susceptible, E05, susceptible, H32, resistant, and X28, resistant. Hence, the first RIL population was derived from a susceptible x resistant parental cross, the second one, from a susceptible x susceptible parental cross, and the third one, from a resistant x resistant parental cross. The term Background will be, from now on, used for referring to the crosses, i.e., each RIL population constitutes a background, while the term Pedigree will be applied to each individual RIL, i.e., each background is composed by a different number of pedigrees: 40, 43, and 45 in backgrounds E02/E42, E94/E05, and H32/X28, respectively.

Analyzed data correspond to season 2013/2014. Experiments were planted in a randomized block design in Venado Tuerto (Santa Fe) in 2013. Each block consisted of 26 rows of 3 m length with an average of-15 plants by row. Rows 1, 2, 9, 10, 17, 18, 25, and 26 were fillers. Artificial inoculations were performed in blocks in the remnant rows, the odd rows were inoculated and the even rows remained non inoculated as an experimental control. Isolate 15 was utilized for *F. verticillioides* inoculation as it presented enough virulence and growing rate at lab [15]. Therefore, the pathogen's molecular identity was confirmed by PCR method at Faculty of Agriculture Sciences at National University of Rosario (Laboratory of Plant Mycology Certification). The concentration and inoculation technique are described in [15].

Resistance was evaluated by Incidence assessment (percentage of infected plants/total number of plants in the row) *100, and Severity (percentage of infected ear tissue), was measured ~~in~~ considering the Reid & Hamilton scale according to [16]. Severity was measured at the individual level and then, a mean population value was obtained in grade and in percentage (%). Hence, grade 1 represents 0% of infection, grade 2:1-3% of infection, grade 3: 4-10% of infection, grade 4: 11-25% of infection, grade 5: 26-50% of infection, grade 6: 51-75% of infection, and grade 7: 76-100% of infection. *F. verticillioides* was assumed to be the predominant species causing the lesions because of the infection characteristics.

Statistical Analysis

Shapiro-Wilk's test was firstly applied to assess normal distribution of phenotypic traits. Then, frequency histograms were plotted to visualize the phenotypic distribution of I and S in each background in comparison to the respective parent mean values. Finally, nested ANOVA with random effects was used to evaluate backgrounds and pedigrees within backgrounds for both traits. Variance components, H^2, and h^2 were determined from ANOVA according to the following formulae, as a methodological approach proposed in this research:

Table of Analysis of Variance

SV	d.f.	MS	EMS
Between backgrounds	s -1	MS_B	$\sigma^2_w + k\sigma^2_{P/B} + dk\sigma^2_B$
Between pedigrees within backgrounds	s(d -1)	$MS_{P/B}$	$\sigma^2_w + k\sigma^2_{P/B}$
Within pedigrees (error)	sd(k -1)	MS_W	σ^2_w
Total	sdk - 1		

Where:
σ^2_w (Variance within pedigrees) = $\sigma^2E = MS_W$
$\sigma^2_{P/B}$ (Variance between pedigrees within backgrounds) = $\sigma^2A = (MS_{P/B} - MS_W)/k$
σ^2_B (Variance between backgrounds) = $\sigma^2NA = (MS_B - MS_{P/B})/dk$
σ^2G (Genetic Variance) = $\sigma^2A + \sigma^2$ NA
H^2 (Broad sense heritability) = σ^2G/σ^2P
h^2 (Narrow sense heritability) = σ^2A/σ^2P
s = Number of backgrounds (independent RIL populations)
d = Number of pedigrees within backgrounds (RIL from a same cross)
k= Number of replications within pedigrees (individual plants within each RIL)
σ^2E = Environmental Variance, σ^2A = Additive Genetic Variance, σ^2NA = Non Additive Genetic Variance, σ^2G = Total Genetic Variance, σ^2P = Phenotypic Variance = $\sigma^2G + \sigma^2E$
SV: Source of Variations, d.f.: degrees of freedom, MS: Mean Square, EMS: Expected Mean Square

Infostat software, developed by National University of Córdoba (Argentina) was used in all cases to accomplish the corresponding statistical analysis.

RESULTS

Phenotypic characterization of RIL populations and parental lines

Normality was observed for *Fusarium* Incidence and Severity, with W values > 0.96, n.s. in the whole data according to Shapiro-Wilk's test. Significant differences for both traits were found among inoculated and non-inoculated plants (data not shown in this communication), evidencing the importance of artificial inoculations. All subsequent analyses were done from inoculated plants.

Mean values and Standard errors of I and S for inoculated parental lines and each RIL population (background) are shown in Table 1. Frequency histograms of pedigrees by background for I and S are shown in Figures 1 and 2, respectively, together with mean values of the parents and the corresponding RIL population.

Table 1. Mean values and standard errors of Incidence and Severity in each genotype

Plant material	Incidence (in percentage)	Severity (in grade)
Line E02	88.62 ± 4.69 a	3.65 ± 0.19 a
Line B05	98.12 ± 4.68 a	2.58 ± 0.19 bc
Line E94	91.78 ± 4.18 a	3.30 ± 0.17 a
Line E42	74.85 ± 4,18 b	2.21 ± 0.17 cd
Line H32	68.09 ± 4.68 b	2.14 ± 0.19 cd
Line X28	54.58 ± 4,18 c	1.87 ± 0.17 d
Background E42/E02	88.46 ± 1.50 a	2.88 ± 0.06 b
Background E94/E05	95.22 ± 1.46 a	3.53 ± 0.06 a
Background X28/H32	71.99 ± 1.45 b	2.33 ± 0.06 c

Means with different letters present statistically significant differences ($p < 0.05$).

A wider range of variation was observed in all cases for pedigrees when compared to their respective parents. A broadening of phenotypic variance for resistance to corn ear rot is evidenced by this result. The greater variance may be explained by recombination of positive alleles contributed by both parental lines in their advanced progenies, which increases the variation for both I and S.

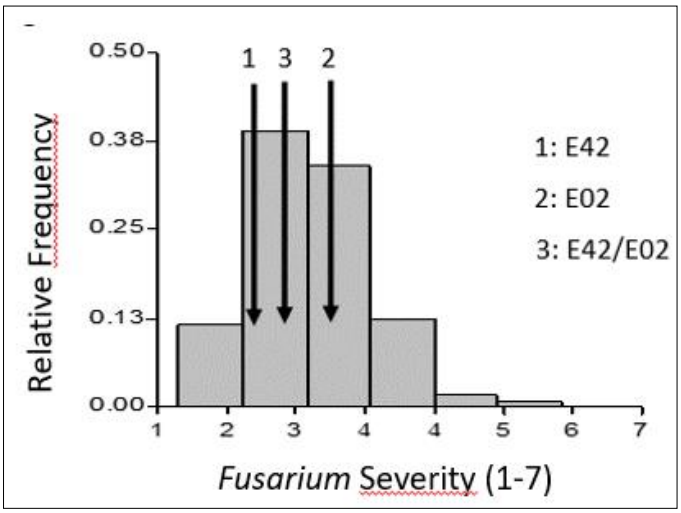

Figure 1a. Background E94/E05

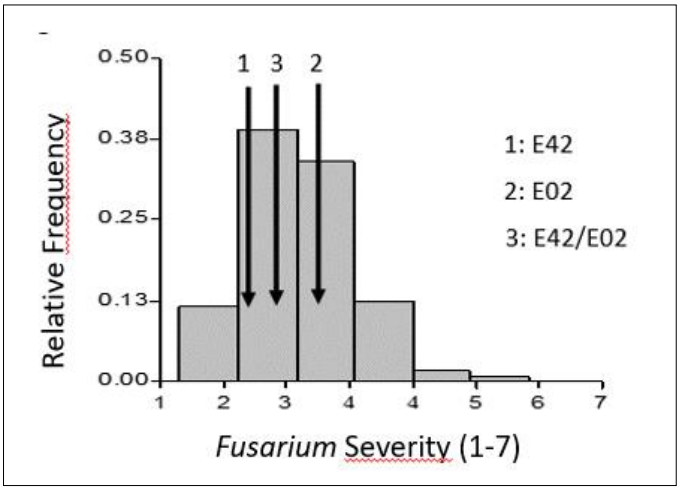

Figure 1b. Background E42/E02

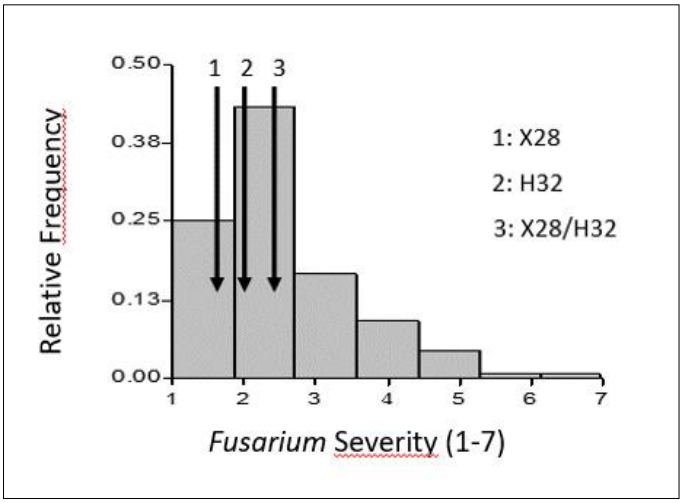

Figure 1c. Background X28/H32

Figures 1. a, b, c. Frequency distribution of pedigrees (RIL) within each background (cross) for Severity in percentage caused by *F. verticillioides*. Arrows indicate mean values of parental lines and of RIL population.

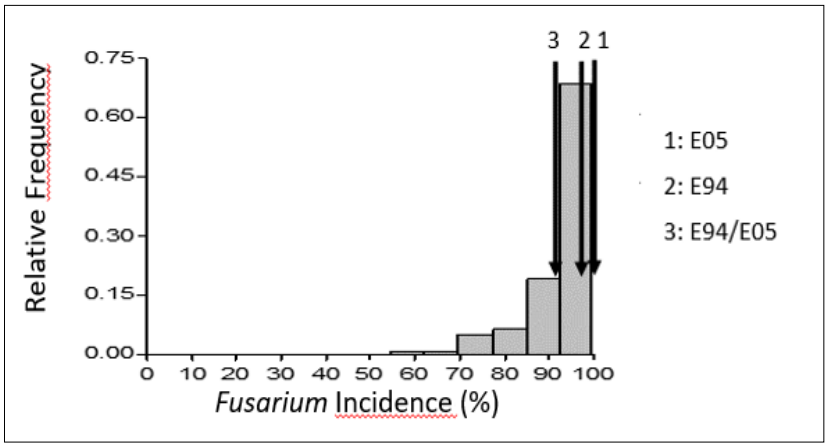

Figure 2a. Background E94/E05

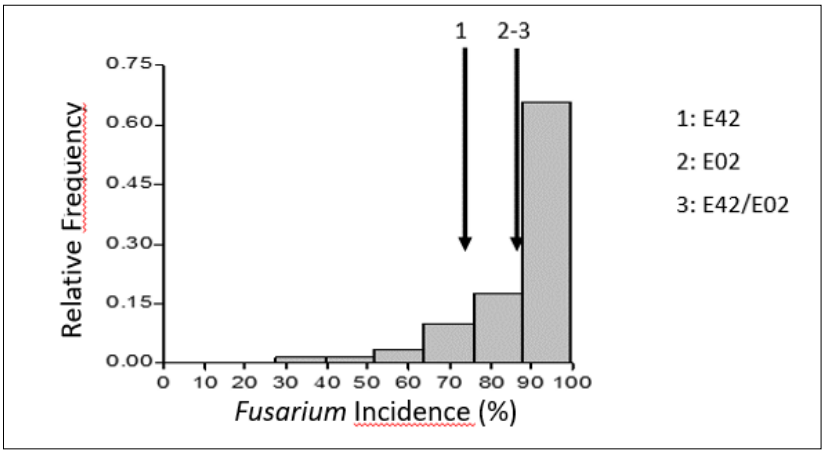

Figure 2b. Background E42/E02

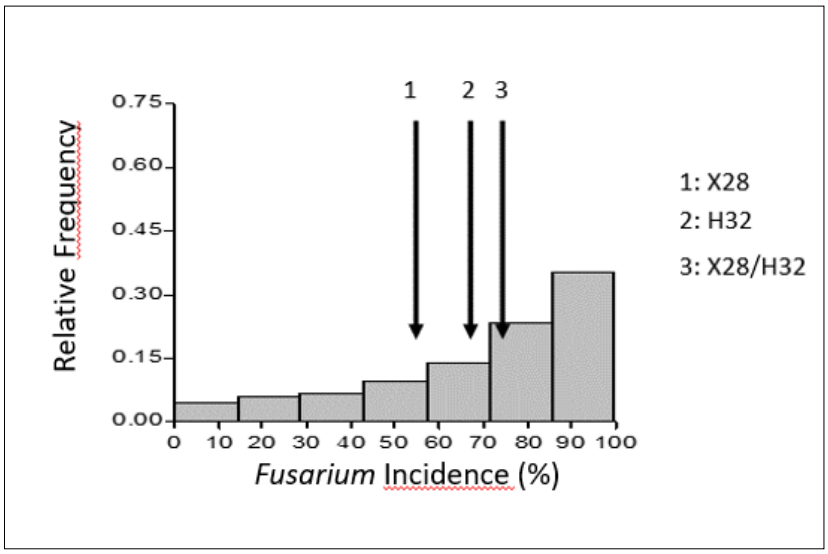

Figure 2c. Background X28/H32

Figures 2. a, b, c. Frequency distribution of pedigrees (RIL) within each background (cross) for Incidence in percentage caused by *F. verticillioides*. Arrows indicate mean values of parental lines and of RIL population.

H^2 and h^2 estimates. F. verticillioides Incidence and Severity in RIL populations

Tables 2 and 3 show the existence of significant differences for I and S, respectively, among backgrounds and among pedigrees within background. In other words, significant genetic variance was detected for both traits related to resistance to corn ear rot in this experiment. According to the theory of genetic variance redistribution along successive selfing cycles from biparental cross, variance between RIL derived from the same parents is additive genetic variance. Hence, then remnant genetic variance observed

among different crosses (background) corresponds to non-additive genetic variance, since the additive variance component was discounted in the nested factor "pedigrees within background". As a consequence, both heritabilities (H^2 y h^2) could be simultaneously assessed, their values and intermediate computations being presented as footnotes in Tables 2 and 3.

Table 2. Nested ANOVA of Incidence (in percentage), estimation of variance components and assessment of broad sense heritability (H^2) and narrow sense heritability (h^2)

SV	d.f.	MS	EMS
Between backgrounds	s – 1	19850.26**	$\sigma^2_W + k\sigma^2_{P/B} + dk\varsigma^2_B$
Between pedigrees within background	s (d - 1)	539.75*	$\sigma^2_W + k\sigma^2_{P/B}$
Within pedigrees (Error)	sd(k - 1)	251.79	σ^2_W

** significant at 1%, * significant at 5%
s = number of backgrounds = 3
d = amalgamated number of pedigrees within backgrounds = 43
k = amalgamated number of individual plants within pedigrees = 29.2
Estimation of variance components and computation of heritabilities
$\sigma^2_W = \sigma^2E = CM_W = 251.79$
$\sigma^2_{P/B} = \sigma^2A = (CM_{P/B} - CM_W)/k = (539.75 - 251.79)/2.93 = 98.27$
$\sigma^2_B = \sigma^2NA = (CM_B – CM_{P/B})/dk = (19850.26 - 539.75/(43 \times 2.93) = 153.27$
$\sigma^2G = \sigma^2A + \sigma^2NA = 153.27 + 98.27 = 251.54$
$\sigma^2P = \sigma^2G + \sigma^2E = 251.54 + 251.79 = 503.33$
$H^2 = \sigma^2G/\sigma^2P = 251.54/503.33 = 0.49$
$h^2 = \sigma^2A/\sigma^2P = 98.27/503.33 = 0.19$
SV: sources of variation, d.f.: degrees of freedom, MS: mean squares, EMS: expected mean squares

Table 3. Nested ANOVA of Severity (in grade), estimation of variance components and assessment of broad sense heritability (H^2) and narrow sense heritability (h^2)

SV	d.f.	MS	EMS
Between backgrounds	s – 1	52518.93**	$\sigma^2_W + k\sigma^2_{P/B} + dk\varsigma^2_B$
Between pedigrees within background	s (d - 1)	3173.71*	$\sigma^2_W + k\sigma^2_{P/B}$
Within pedigríes (Error)	sd(k - 1)	362.16	σ^2_W

** significant at 1%, * significant at 5%
s = number of backgrounds = 3
d = amalgamated number of pedigrees within backgrounds = 43
k = amalgamated number of individual plants within pedigrees = 29.2
Estimation of variance components and computation of heritabilities
$\sigma^2_W = \sigma^2E = CM_W = 362.16$
$\sigma^2_{P/B} = \sigma^2A = (CM_{P/B} - CM_W)/k = (3173.71 - 362.16)/29.20 = 96.28$
$\sigma^2_B = \sigma^2NA = (CM_B – CM_{P/B})/dk = (52518.93 - 3173.71)/(43 \times 29.2) = 39.30$
$\sigma^2G = \sigma^2A + \sigma^2NA = 96.28 + 39.30 = 135.35$
$\sigma^2P = \sigma^2G + \sigma^2E = 135.55 + 362.62 = 498.17$
$H^2 = \sigma^2G/\sigma^2P = 135.35/498.17 = 0.27$
$h^2 = \sigma^2A/\sigma^2P = 96.28/498.17 = 0.19$
SV: sources of variation, d.f.: degrees of freedom, MS: mean squares, EMS: expected mean squares

DISCUSSION

Plant breeding is a decisive component of the integrated production process from farm to industry [17]. The system corn- *Fusarium* spp. is complex but some general trends can be detected. Such trends are useful for planning strategies to get a better profit of genetic variance in breeding programs tending to improve resistance to corn ear rots. Many species of *Fusarium* genus simultaneously appear around the world, the structure and distribution of these species basically depends on the environmental conditions. *F. graminearum* (*Gibberella zeae*) and *F. verticillioides* (*G. fujikuroi*) are two of the most important *Fusarium* species causing yield losses in corn. Some studies indicate that a common mechanism resistance could exist for both pathogens in corn. Following this observation, it might be proposed that studies about genetic resistance should synergize plant breeding for both pathologies from advances in basic knowledge.

Moreover, other important factor for determining a successful breeding strategy for corn ear rot resistance is the availability of statistical approaches for truly detecting different genotypes´ performance. In

the present study, a considerable amount of genetic variation was detected through nested ANOVA on properly developed corn genotypes, so that variations in additive and non-additive effects contributed to the expression of corn ear rot Incidence and Severity (Tables 2 and 3). These results imply that genotypes reacted differently to *F. verticillioides* inoculation. Some previous experiments had also confirmed the presence of genetic variation for this disease [18] but in this research, the calculation of broad and narrow sense heritabilities could be accomplished based on the phenotypic distributions of three independent RIL populations derived from parents with different reaction to *F*. *verticillioides* (Figures 1 and 2). RIL population derived from background X28/H32 presented the wider range of phenotypic variation for both Incidence and Severity, but all RIL populations, independently of being derived from a resistant x resistant, resistant x susceptible or susceptible x susceptible cross showed a broad range of phenotypic variation when compared to parental lines. This fact indicates that a considerable amount of this phenotypic variation is due to genetic variation.

Although heritabilities estimations were, in general, low to intermediate (0.27 for Severity and 0.49 for Incidence in the case of broad sense heritability and just 0.19 for both traits; in the case of narrow sense heritability, Tables 2 and 3), they were statistically significant. Moreover, these values are in accordance with the findings of previous reports which mention that resistance to *F. verticillioides* is a complex trait with polygenic inheritance and a great environment influence. A significant effect in artificial selection can be yet obtained by exploiting the additive component of genetic variance, but the non-additive component points out that heterosis is an advantageous way to improve resistance traits in corn ear rot. However, these results contrast to those reported by [19], who concluded that high levels of resistance are only observed in hybrids whose both parents are resistant. Hence, in that research, the effect of non-additive variance was not measured, the focus was on additive effects. Although in the present research, a cryptic or potential non-additive genetic variance was evaluated, because no heterozygous genotype was included in the experimental design, the results indicate that after obtaining superior genotypes with artificial selection, the selected homozygote genotypes should be crossed in order to also exploit the considerable variation for non-additive genetic effects. In fact, as mentioned in the Introduction, the exposed results pointed out that the best cultivar, the one having high levels of resistance to corn ear rot, would be a hybrid genotype.

Additionally, in this study susceptible RIL from resistant x resistant parental cross or resistant RIL from susceptible x susceptible parental cross were detected. These results agree with [14], who reported that the hybrid phenotype cannot be precisely predicted from its parents' phenotype due to the existence of non-additive gene effects. Also, this fact is common in polygenic inheritance because both parents could carry positive or negative alleles of minor effects, independently of their own phenotypes. Through selfing cycles and recombination, accumulation of positive or negative alleles from parents in some segregant individuals cause that their phenotypes exceed those of their parents. This process originates transgressive segregation, which is a well-known phenomenon in plant breeding. Although none statistically significant transgressive segregation was observed among RIL populations present in this study, as [14] reported, the number of different pedigrees should be increased to get more probabilities of detecting such a variation. As [20] reported, breeding for ear rot diseases involves two important steps: the identification and use of native genetic resources. In these steps, cryptic variation and transgressive segregations are commonly observed.

Finally, it is worth to note that inoculations accomplished in this research imply a hard work but also, this method allows the identification of genetic variance for disease resistance traits. The complexity of gene effect involved in phenotypic expression of the traits and the great environment effect impose difficulties for precisely detecting differences among genotypes. Non-significant differences were found among genotypes in the non-inoculated rows (data not shown). Hence, the benefit of artificial inoculations for obtaining accuracy and consistency among evaluated populations was evidenced.

CONCLUSION

Phenotypic characterization represents an original statistical approach and it is a proposal carried out in this research including three independent RIL populations (X28/H32, E42/E02, and E94/E05) inoculated with an isolate of *F. verticillioides*. This experiment comprised the estimation of variance components and heritabilities calculation for *Fusarium* ear rot incidence and severity. Both broad and narrow sense heritabilities were low to intermediate. From these values, designing breeding strategies is possible because artificial selection can leverage the additive genetic variance component. Then, the non-additive genetic variance component could be capitalized by obtaining superior hybrids from heterotic crosses among the best genotypes obtained by artificial selection.

Conflicts of Interest: Authors declare no conflict of interest.

REFERENCES

1. Kearsey MJ, Pooni HS. The genetical analysis of quantitative traits. London: Chapman and Hall, 1996. 382p
2. Collard BCY, Jahufer MZZ, Brouwer JB. Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica. 2005 Jan;142(1): 169-96. doi:10.1007/s10681-005-1681-5.
3. Lawrence-Dill CJ, Schnable, PS, Springer, NM. Idea factory: the maize genomes to fields initiative. Crop Sci. 2019 Jul-Aug;59(4), 1406-10. doi: 10.2135/cropsci2019.02.0071.
4. Chawade A, van Ham J, Blomquist H, Bagge O, Alexandersson E, Ortiz R. High-Throughput field-phenotyping tools for plant breeding and precision agriculture. Agronomy. 2019 May;9(5), 258. doi: 10.3390/agronomy9050258.
5. Awada L, Phillips PWB, Smyth SJ. The adoption of automated phenotyping by plant breeders. Euphytica. 2018 Aug:214(8),148. doi: 10.1007/s10681-018-2226-z.
6. Pieruschka R, Schurr U. Plant phenotyping: past, present, and future. Plant Phenomics. 2019 Mar;6. doi: 10.1155/2019/7507131.
7. Afolabi CG, Ojiambo PS, Ekpo EJA, Menkir A, Bandyopadhyay R. Evaluation of maize inbred lines for resistance to fusarium ear rot and fumonisin accumulation in grain in tropical Africa. Plant Dis. 2007 Feb;91(3):279-86.
8. Alessandra L, Luca P, Adriano M. Differential gene expression in kernels and silks of maize lines with contrasting levels of ear rot resistance after *Fusarium verticillioides* infection. J. Plant Physiol. 2010 Jul;167(16):1398-406.
9. Eller MS, Payne GA, Holland JB. Selection for reduced *Fusarium* ear Rot and Fumonisin content in advanced backcross maize lines and their topcross hybrids. Crop Sci. 2010 Nov-Dec;50(5):2249-60.
10. Nankam C, Pataky JK. Resistance to kernel infection by *Fusarium moniliforme* in the sweet corn inbred IL125b. Plant Dis.1996 May; 80:593-98.
11. Clements MJ, Maragos CM, Pataky JK, White DG. Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopathology.2007 Feb;94:251-60.
12. Bush BJ, Carson ML, Cubeta MA, Hagler WM, Payne, GA Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. Phytopathology. 2004 Jan;94(1):88-93
13. Bolduan C, Miedaner T, Schipprack W, Dhillon BS, Melchinger AE: Genetic variation for resistance to ear rots and mycotoxins contamination in early European maize inbred lines. Crop Sci. 2009 Nov;49(6):2019-28.
14. Robertson-Hoyt LA, Kleinschmidt CE, White DG, Payne, GA, Maragos CM, Holland JB. Heritabilities and correlations of *Fusarium* ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Sci 2006 Jan;46(1):353-61.
15. Belich YE. Caracterización fenotípica y molecular asociada a la resistencia de tres poblaciones de RILs de maíz a *Fusarium verticillioides* [dissertation]. Zavalla, Universidad Nacional de Rosario, Argentina, Facultad de Ciencias Agrarias. 2015. 122p.
16. Reid LM, Hamilton RI, Mather DE. Screening maize for resistance to *Gibberella* ear eot. Agriculture and Agri-Food Canada Tech Bull Publ 1996-5E. 1996, Ottawa, ON. 44p.
17. Van der Fels-Klerx HJ, Dekkers S, Kandhai, MC, Jeurissen SMF, Booij, CJH,.de Heer C. Indicators for early identification of re-emerging mycotoxins. NJAS. 2010 Jun;57(2):133-9.
18. Santiago R, Reid LM, Zhu X, Bruton A, Malvar RA. Gibberella stalk rot (*Fusarium graminearum*) resistance of maize inbreds and their F1 hybrids and their potential for use in resistance breeding programs. Plant Breed., 2010 Aug;129(4):454-6.
19. Mesterházy Á, Lemmens M, Reid LM. Breeding for resistance to ear rots caused by *Fusarium* spp. in maize—a review. Plant Breed. 2012 Dec;131(1):1–19.
20. Munkvold GP. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur. J. Plant Path. 2007 Jan;109(1):705-13.