Colegio de Postgraduados

Seed Quality Assessment of the Blue Corn Hybrid Vampiro H10 (*Zea mays* L.) Through Its Parental Genotypes

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ABSTRACT

Objective: To monitor the physical and physiological quality of the trilinear corn hybrid Vampiro H10 seeds through its parental genotypes and to rank the evaluated characteristics according to their importance for germination and seedling development.

Design/methodology: Using a completely randomized design with four replications (100 seeds), in parental genotypes of the hybrid Vampiro H10 seeds, it was evaluated their physical (width, thickness, length, volume, relative density, and width/length and thickness/length ratios) and physiological characteristics (normal and abnormal seedlings, inert seeds, and dry weight and length of plumule, radicle, and total). Seed germination was estimated by the proportion of normal seedlings produced. The results underwent analysis of variance, comparison of means (Tukey's test, $P \leq 0.05$), and principal components analysis.

Results: Each cross involved in the formation of Vampiro H10 made different contributions to the seed characteristics. In the single cross, the physical dimensions and dry matter of the seedling were increased, and in the trilinear cross, the formation of normal seedlings, radicle elongation and biomass were favored. This indicated that the hybridization sequence first affected the dimensions and then the physiological quality of the seed.

Limitations of the study: No limitations were presented for the present study.

Conclusions: An increase in volume, weight and length of seed was observed in the single cross and an increase in germination and seedling development in length and biomass was observed in the trilinear cross.

Keywords: Germination, heterosis, seedling development, seed dimensions.

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INTRODUCTION

Corn is currently the most widely planted species and has the highest production volume and economic value worldwide [1]. It holds this position due to its broad adaptability to various climates, soils, production technologies, and crop purposes [2], as well as the heterogeneity of its final product, whether it be ears, grain, or forage (fresh or dry).

In the extensive genetic variability of corn [3], pigmented corns stand out due to their content of carotenoids or anthocyanins [4]. These corns have functional and nutraceutical properties in themselves, meaning without the need for post-harvest treatments, and are considered biofortified foods obtained through conventional genetic improvement. They are included alongside crops that, through genetic crosses, the addition of fertilizers, or genetic modification, have been enriched with vitamins or minerals [5,6]. Anthocyanins are phenolic compounds from the flavonoid group [7] that give corn kernels their attractive and distinctive blue color, which varies in shades and intensities [8]. These pigments are located in the pericarp and/or in the aleurone layer of the caryopsis.

Blue corn is a staple ingredient in many traditional Mexican dishes. Additionally, the numerous nutraceutical and functional properties recently reported for anthocyanins (anticancer, lipid-reducing, antidiabetic, anti-inflammatory, antimicrobial, antioxidant) [7] have increased their consumption [9], leading to a current production that does not meet the high and growing demand.

Therefore, it is pertinent to develop high-yielding hybrids and improved varieties with superior agronomic qualities, among which seed quality stands out, as it is the essential input for crop establishment.

Seed quality is determined by its health attributes (presence of pests or diseases), genetic attributes (varietal purity), physical attributes (size, weight, density), and physiological attributes (germination suitability). In agronomic terms, physiological quality [10,11] is the seed's ability to germinate and produce complete, healthy, and potentially productive seedlings [12] with which the crop is established in the production field [13].

In the Central High Valleys of Mexico (Mexico State, Puebla, Tlaxcala, and Mexico City), 150,000 hectares of blue corn are planted using traditional varieties, traditional technology, and rainfed conditions, yielding 300,000 tons of grain, which only cover 65% of the demand [14,15].

Despite its agronomic significance, there is limited knowledge about the fluctuation of seed quality components throughout the sequential stages of maize hybrid formation. Therefore, the objectives of this study were: i) To monitor the physical and physiological quality of Vampiro H10 maize hybrid seeds through its sequence of parental genotypes (lines and single and trilinear crosses) and ii) To rank the evaluated characteristics according to their contribution to germination and seedling development.

The study of the fluctuation of physical and germinative seed components, as well as their contribution to seed quality, will contribute to the understanding of how these characteristics are established through the different genotechnical stages inherent to the development of a maize hybrid.

MATERIALS Y METHODS

Genetic Material

Seeds from the parental genotypes of the blue corn trilinear hybrid Vampiro H10 (Table 1) were used. This hybrid was developed in the Blue Corn Genetic Improvement Program at the Valle de México Experimental Station of the National Institute of Forest, Agricultural, and Livestock Research (CEVAMEX, INIFAP).

Table 1. Description of the parental genotypes (treatments) of the blue corn hybrid Vampiro H10 used in this study (Valle de México Experimental Station, INIFAP, Chapingo, Mexico, 2019).

Genotype	Genealogy	Inbreeding (%)
Parental lines		
A	NXOAX-19-5-1-1-2	96.87
B	BXCC-54-11-1-1-1	96.87
C	BXCC-5-9-6	87.50
Crosses		
D	(BXCC-54-11-1-1-1)×(BXCC-5-9-6)	
E	[(BXCC-54-11-1-1-1)×(BXCC-5-9-6)]×(NXOAX-19-5-1-1-2)	

The mentioned seeds were produced in a plot that was sown annually on May 2, 2019 (spring-summer cycle), under rainfed conditions, at CEVAMEX (Chapingo, Mexico, 19° 29' N and 98° 53' W), at an altitude of 2240 m, with a temperate climate, clayey-sandy soil, and annual averages of precipitation and temperature of 643 mm and 15.1 °C, respectively [16]. The experimental plot consisted of four rows measuring 5×0.8 m, with 26 plants per row and 0.2 m spacing between plants. The population density was 65,000 plants ha^{-1}, and the fertilization dosage (kg ha^{-1}) was N (120), P (60), K (30).

Physical Characteristics of the Seeds

Seed weight (SW, mg). Seeds were weighed using an analytical balance (AE Adam P W 184, precision 0.1 mg). Seed width (SWID), thickness (ST), and length (SL) were measured (mm) with a digital caliper (Mitutoyo CD-6 CSX). The volume (SV, mm^3), relative density (SDR, g/cm^3), and the ratios SWID/SL and ST/SL of the seeds were calculated according to maize variety description guidelines [17,18,19].

Physiological Characteristics of the Seeds

Germination

The standard germination test [12] was used. Seeds were placed on moistened paper, which was then rolled up and placed vertically in plastic bags, in a germination chamber at 25±2 °C and 100% r.h. After seven days of incubation, the percentage of normal seedlings (PN) was counted, *i.e.*, those that displayed a coleoptile, plumule, seminal radicle, and at least two adventitious roots. Abnormal seedlings (PA), classified as such for lacking any of the structures, and seeds with no apparent metabolic activity (SI) [20] were also counted. Seed germination was considered equivalent to the proportion of PN produced.

Seedling Development

In normal seedlings, the lengths of the plumule (PLU), radicle (RAD), and total length (TL) were measured (mm). Additionally, these structures were dried (80 °C, 3 days) in an oven (RIOSSA H-102, USA) and their dry weight (PDW, RDW, and TDW, respectively) was determined (mg).

Statistical Analysis

The experiments were conducted using a completely randomized design with four replications of 100 seeds each. To ensure normal distribution and to homogenize the scales of the variables, percentage data were transformed using the arcsine $\left(\text{arcsine}\sqrt{x}\right)$ [21].

The significance of the treatments was tested using analysis of variance, and treatment means were compared using the Tukey test ($P \leq 0.05$). To determine the relative contribution of each response variable to germination and seedling development, the results were subjected to principal component analysis; for this, the data were standardized to mean=0 and variance=1 [22]. Statistical processing of the results was performed using the SAS program [23].

RESULTS AND DISCUSSION

The response variables used in this study were statistically significant in the analysis of variance (except for ST), showed high fits to the statistical model used, and had reduced coefficients of variation (data not shown); therefore, the experiments were relevant and robust.

The significances ($P \leq 0.05$) detected indicated the high heterogeneity of the genetic material used in the measurements and allowed for the estimation of its fluctuation through the sequential genotechnical stages of the development of the blue corn trilinear hybrid Vampiro H10.

Physical and Physiological Characterization

In the statistical comparison of mean values for the variables (Table 2), the physical descriptors of the seed —SW, SV, SL, SWID, and ST— were lower ($P \leq 0.05$) in treatment C, intermediate in A and B, and higher in D and E. In terms of physiological aspects, treatments C, D, and E were superior ($P \leq 0.05$) in RSD, SWID/SL, and ST/SL. Treatments A, B, D, and E had the lowest ($P \leq 0.05$) amount of IS, while treatment C had the highest.

In the seedling development variables, the minimum plumule length (PLU) was for C and the maximum for A ($P \leq 0.05$); while E achieved the highest results ($P \leq 0.05$) in RAD, TL, RDW, and TDW, and D in PDW. In RDW and TDW, the superiority of treatments D and E was again observed, while the highest PDW value was for D and the lowest for C. Treatments D (single cross) and E (trilinear cross) had greater physical dimensions (SW, SV, SL, SWID, and ST) and greater seedling dry matter (PDW, RDW, and TDW) ($P \leq 0.05$) compared to treatments A, B, and C (inbred lines) (Table 2).

The inferior performance of the lines compared to the crosses in the described variables is explained by the genetic effects of homozygosity or inbreeding, which lead to reductions in yield, plant height, seed size, and seed volume and weight [24], as these are dominant traits. In contrast, the crosses exhibited heterosis or hybrid vigor, a metabolic process where the hybrid offspring surpass the average of their parents in certain characteristics [25]. In this study, these characteristics were the physical measurements SW, SV, SL, SWID, and ST, and the physiological traits PDW, RDW, and TDW.

Table 2. Comparison of means (Tukey, P≤0.05) for the physical and physiological characteristics of the Vampiro H10 maize hybrid seeds and its parents (UPIBI, IPN, Mexico City, Mexico, 2020).

Variables	Treatments					HSD
	A	B	C	D	E	
SW (mg)	22.06 b	21.70 b	16.66 c	31.45 a	30.34 a	2.26
SV (mm^3)	367.29 c	353.16 c	290.17 d	433.76 b	493.96 a	55.69
SL (mm)	11.55 bc	10.92 c	9.79 d	12.15 ab	12.42 a	0.85
SWID (mm)	7.23 b	6.35 c	6.58 c	7.46 ab	7.91 a	0.55
ST (mm)	4.40 b	5.09 a	4.50 b	4.78 ab	5.03 a	0.43
RSD	1.07 b	1.06 b	1.37 a	1.37 a	1.22 ab	0.17
SWID/SL	0.63 ab	0.59 b	0.67 a	0.61 ab	0.65 ab	0.08
ST/SL	0.38 b	0.47 a	0.46 a	0.39 b	0.41 ab	0.06
NS (%)	50 ab	68 ab	45 b	60 ab	83 a	34.98
AS (%)	5 a	5 a	5 a	5 a	0 a	13.81
IS (%)	45 ab	27 ab	50 a	35 ab	17 b	31.64
PLU (mm)	9.59 a	7.45 ab	6.23 b	8.10 ab	8.38 ab	2.79
RAD (mm)	12.80 b	13.45 b	12.50 b	13.98 b	19.48 a	2.57
TL (mm)	22.39 b	20.91 b	18.73 b	22.08 b	27.87 a	4.22
PDW (mg)	0.17 ab	0.16 ab	0.10 b	0.24 a	0.20 ab	0.10
RDW (mg)	0.17 b	0.20 b	0.15 b	0.30 ab	0.40 a	0.16
TDW (mg)	0.34 bc	0.36 abc	0.26 c	0.54 ab	0.60 a	0.25

In rows, different letters indicated significant differences. HSD=Honest Significant Difference. SW=Seed weight, SV=Seed volume, SL=Seed length, SWID=Seed width, ST=Seed thickness, RSD=Relative seed diameter, NS=Normal seedlings, AS=Abnormal seedlings, IS=Inert seeds, PLU=Plumule length, RAD=Radicle length, TL=Total length, PDW=Plumule dry weight, RDW=Radicle dry weight, TDW=Total dry weight, SWID/SL=Seed Width/Seed Length, ST/SL=Seed Thickness/Seed Length.

Hierarchization of Variables

In the principal component analysis, components 1 (CP1) and 2 (CP2) accounted for 79% of the variation in the experiment, which was an adequate proportion for this multivariate analysis procedure [22]. In CP1, the vectors with the largest positive magnitudes corresponded to the variables SV (0.31), RDW (0.29), TDW (0.30), SW (0.28), SL (0.29), NS (0.27), and TL (0.29). In CP2, the vectors were PLU (0.36), IS (0.31), and SWID (0.26). The negative eigenvectors had marginal values in PC1 but were high in CP2, corresponding to ST/SL (−0.50), ST (−0.46), and NS (−0.30).

In the graphical representation (biplot) of this method (Figure 1), treatments A, B, and C were in quadrants II and III, along with the variables RSD, SWID/SL, IS, AS, and ST/SL. In contrast, D and E were positioned in quadrants I and IV, respectively, with the remaining variables. The vectors of each variable assumed specific slopes according to their coordinates on the Cartesian plane (eigenvectors), and their graphical behavior followed the variations of CP1 and CP2.

Each cross made different contributions to the seed characteristics of Vampiro H10; in D, there was an increase in physical dimensions and seedling dry matter, while E favored the formation of normal seedlings, root elongation, and biomass. This indicated that the

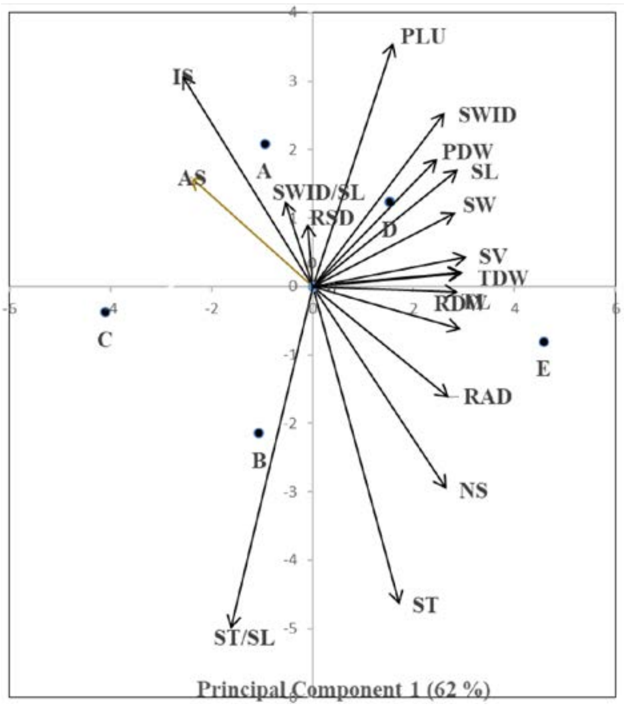

Figure 1. Biplot for the Principal Component 1 (accounts for the62% of the variation) and Principal Component 2 (accounts for the 17% of the variation) obtained for the blue maize hybrid Vampiro H10 and its parents (UPIBI, IPN. Mexico City, Mexico, 2020).
A, B, C, D, and E=Treatments (Genotypes). SW=Seed weight, SV=Seed volume, SL=Seed length, SWID=Seed width, ST=Seed thickness, RSD=Relative seed diameter, NS=Normal seedlings, AS=Abnormal seedlings, IS=Inert seeds, PLU=Plumule length, RAD=Radicle length, TL=Total length, PDW=Plumule dry weight, RDW=Radicle dry weight, TDW=Total dry weight, SWID/SL=Seed Width/Seed Length, ST/SL=Seed Thickness/Seed Length.

hybridization sequence first affected the dimensions and then the physiological quality of the seed.

It is feasible that lines B and C had phylogenetically related genetic sequences for germination, which is why there was only marginal heterosis in these events in the single cross; in contrast, the involvement of line A in the trilinear cross had a notable and favorable impact on these aspects. This can be attributed to the fact that line A was derived from a collection conducted in a different agroclimatic region (Oaxaca, Mexico) compared to the rest of the genotypes in the study (Central Mexican Highlands) (Table 1), meaning that its genetic background may have been distinct and recombined favorably [26,27], exhibiting high heterosis in essential traits for seed physiological quality, such as NS, RAD, RDW, and TDW. This strategy of using two germplasm sources to develop trilinear maize hybrids has been reported to exploit heterosis for seed production in maize [25, 28, 29].

Variable Hierarchization

In the principal component analysis, fluctuations in variables and genotypes (treatments) in response to the variation of CP1 and CP2 were visualized (Figure 1). CP1, which contributed the majority to the experimental results (62%), was characterized by seed physical dimensions (SV, SW, and SL), seedling biomass (RDW and TDW), and the formation and total elongation of normal seedlings (TL and NS). Consistent with previous observations, seed weight and size attributes were associated with the formation and elongation of normal seedlings, as well as with the biomass accumulated in them.

In summary, the variables were predominant for the germination of the Vampiro H10 seeds and the development of seedlings. CP2, which accounted for only 17% of the results, was composed of the elongation of the plumule (PLU), seed width (SWID), and inert seeds (IS). Notably, it also included normal seedlings (NS) with a proportionally high and negative value.

The placement of A, B, and C in quadrants II and III of the Cartesian plane indicated the poor seed quality of these inbred lines, as they were positioned alongside unfavorable physiological variables such as abnormal seedlings (AS) and inert seeds (IS).

This was logical, given that the initial goal of plant breeding is to identify parental lines with desirable agronomic traits and, in later stages, to focus on seed quality. In contrast, cross D was located in quadrant I and cross E in quadrant IV, alongside traits favorable for seed physiological performance such as plumule length (PLU), dry weight of plumule (PDW), seed weight (SW), total dry weight (TDW), dry weight of radicle (RDW), and especially close to normal seedlings (NS); *i.e.*, through hybridization, the aforementioned seed quality attributes were optimized in the single cross, and this effect was even more pronounced in the trilinear cross with the involvement of line A.

The variables located in quadrant I, already referred to as favorable for germination and seedling development, responded directly to the variation in both principal components. In contrast, those placed in quadrant IV reacted directly to CP1 but inversely to CP2, such as normal seedlings (NS) and radicle length (RAD and TL) and dry weight of radicle (RDW). This indicates an interaction among all the analyzed variables, with the resulting effect being specific to each genotype.

The previous arguments indicated that in the genotechnical phases of the Vampiro H10 development, a series of interdependent events occurred. This process began with the single cross between lines A and B, which increased the physical capacity of the seeds to store reserves (SV, SW, and SL), and continued with the trilinear cross involving line A, which enhanced the genetic and physiological potential for germination (NS) and seedling development in terms of length (TL) and biomass accumulation (RDW and TDW).

CONCLUSIONS

The establishment of seed quality for Vampiro H10 began with the increase in physical dimensions (volume, weight, and length of the seed) in the single cross (between lines B and C). This process continued in the trilinear cross with the effects of line A, which enhanced the genetic and physiological potential for germination (normal seedlings) and seedling development in terms of length and biomass accumulation.

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