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Assessment of Elongation of the Mesocotyl-Coleoptile and Biomass in Parents and Crosses of Corn Seedlings of the High Valleys of Mexico

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Citation: Villalobos González, A.; Benítez Riquelme, I.; Castillo González, F.; Mendoza Castillo, M.d.C.; Espinosa Calderón, A. Assessment of Elongation of the Mesocotyl-Coleoptile and Biomass in Parents and Crosses of Corn Seedlings of the High Valleys of Mexico. *Seeds* **2023**, 2, 449–473. https://doi.org/10.3390/seeds2040034

Academic Editors: José Antonio Hernández Cortés, Gregorio Barba-Espín and Pedro Diaz-Vivancos

Received: 30 July 2023
Revised: 19 October 2023
Accepted: 23 October 2023
Published: 22 November 2023

Abstract: The elongation of the mesocotyl and the coleoptile and other seedling traits were analyzed from 16 hybrids of two seed sizes, five varieties and a control. Sowing was conducted in sand beds during the S-F 2020 cycle, where nine genotypes were identified that differed in the elongation of the mesocotyl: long (H-48, HS-2 and Promesa); medium (H-44-H-52 and H-70); and short (H-49 AE, H-40 and H-32). A total of 36 possible crosses were obtained between these nine parents, which were established in the S-S 2021 cycle, and on sand beds. Results show that seed size affected ($p \leq 0.05$) the speed and percentage of emergence, the elongation of mesocotyl–coleoptile, the biomass and the heterosis in parents and their crosses. The H-48 hybrid presented greater speed and percentage of emergence and elongation of the mesocotyl and the coleoptile with both seed sizes. The highest dry weight of mesocotyl, coleoptile, roots, and leaves was found in the hybrids Promesa and H-48. The crosses between parents with contrasting mesocotyl presented superior elongation and dry weight ($p \leq 0.05$) compared to their parents, with the long × long (1 × 2, 1 × 3 and 2 × 3) crosses standing out for all the traits measured. A strong positive association was obtained ($p \leq 0.01$) between the elongation of the mesocotyl–coleoptile, the percentage of emergence, and the production of total dry matter in parents and their crosses.

Keywords: stress and depth; vigor; seed size; genotype; crosses

1. Introduction

The elongation of the mesocotyl and the coleoptile plays an important role in the emergence and normal development of seedlings [1], in particular, the elongation of the mesocotyl, which is influenced by radiation, temperature and water, and exhibits variability in response to the depth of sowing, amount of water and salinity of the soil [2].

The seedlings of cereals, such as oats (*Avena sativa* L.), rice (*Oryza sativa* L.), and corn (*Zea mays* L.), are model systems for the study of coleoptile and mesocotyl growth [3]. The mesocotyl of corn is an organ without chlorophyll that develops during the seed's germination in darkness and connects the seed with the base of the coleoptile or knot of the first shoot [4].

The mesocotyl has a bark, an epidermis and a central vascular stele, with a meristem zone on its upper part [5]. The mesocotyl is important during seedling emergence because when it elongates it pushes the coleoptile through the soil [6]; in addition, in some corn lines, the adventitious roots are differentiated into the mesocotyl [7]. Therefore, varieties with long mesocotyl could be used to partially surpass the rate of establishment of those requiring direct and deep sowing [2].

The inhibition of corn seedling growth after emergence is attributed to the limited absorption of nitrogen at soil temperatures below 5 °C and phosphorus at temperatures below 12 °C [8]. In addition, these environmental conditions reduce the dry matter production of the organs that grow under the soil surface, such as mesocotyl, coleoptile and roots [8], and with this, the yield potential [9]. Consequently, frost and hail could also damage the crop during early sowing. If the producers could sow seeds deeper and earlier in the spring, the period of the sowing season could be extended and the potential of damage from frosts would be reduced because the growth point would be underground for longer [9].

Deep sowing is an agricultural practice commonly used in high altitudes or drier habitats. Its implementation is based primarily on the seed size and the capacity of the seedling to germinate from seeds [1]. The size and the shape of corn seeds are valuable characteristics for seed-producing companies and for the consumers [10]. Some varieties [11] show an inability of elongation of their mesocotyls and coleoptiles, and they fail to emerge after deep sowing.

The genetic improvement approach for the crops has been to select superior genotypes based on the identification of superior phenotypes. This approach was used for years to characterize different traits of the aerial system and growth habits [12]. The traits of the organs that grow underground have been used as possible selection markers [1].

In corn, the elongation of roots and of the mesocotyl is a trait of interest in the vegetative phase. These two organs with opposite growth direction seem to determine the ability of the seedlings to emerge under adverse environmental conditions, such as deep sowing, drought and cold or warm soils in the stages of crop establishment [13]. The study of the inheritance of the mesocotyl length shows that it is a useful varietal trait for the genetic improvement in corn, so planned random crosses can be designed to improve the length of the mesocotyl [14].

The aforementioned studies reveal that the mesocotyl, coleoptile, roots and dry matter of corn seedlings are useful selection criteria to evaluate stress (abiotic and biotic) and the response and tolerance to deep sowing during the early stage of seedling growth. Various genetically improved corns have been generated in Mexico for different farming regions, through improvement programs at CIMMYT and institutions such as INIFAP [15,16], the public university system (UACh, COLPOS, UAAAN and UNAM) and the private sector [17,18]. Although they have made timely contributions, the demand for corn and food production has not been met, even when some of these corns have desirable agronomic traits and high yield potential [17].

In particular, in the High Valleys of the country, there are native populations [19] and lines with desirable economic traits for deep sowing [20], such as the elongation of the mesocotyl and the coleoptile [21,22]; however, there are few studies about the genetic variability of corn genotypes for deep sowing. Therefore, in order to expand the genetic base of improvement programs, research during the early stage of corn establishment is required.

In this context, the objective of the study was to analyze the genetic variability in terms of the speed and percentage of emergence, the elongation of the mesocotyl and the coleoptile, and the production of dry matter in seedlings from two seed sizes from parents and their F_1 crosses based on samples of corns obtained from the High Valleys in Mexico.

2. Materials and Methods

2.1. Experimental Site

A series of experiments were established in sand beds with rectangular bases and triangular roofs covered with polyethylene caliber 600 in Montecillo, Texcoco, Mexico (19°21′ N, 98°55′ W; altitude 2250 m), during the summer–fall (S-F) 2020 and spring–summer (S-S) 2021 cycles. The zone has temperate sub-humid climate with summer rains, the mean annual temperature varies from 12 to 18 °C, and it receives an average annual rainfall of 645 mm [23].

2.2. First Stage

Two experiments were established in the S-F 2020 cycle, where 22 genotypes that are recommended for sowing in the High Valleys of Mexico were evaluated, which included 14 hybrids (H-47 AE, H-66, H-50, H-70, H-74, H-32, H-48, H-72, H-42, H-40, H-44, H-52, H-49 AE and H-51 AE) and three varieties (V-60, V-55 A and V-54 A) obtained from the National Institute for Forest, Agriculture and Livestock Research (INIFAP) [24], plus two hybrids (HS-2 and Promesa), one variety (CP V-20) and one experimental variety (NATI) obtained from Colegio de Postgraduados [25] and a commercial hybrid that was used as a control (Asgrow, AS7573).

The first experiment was initiated on 13 October with a large seed (0.13 ± 0.01 g) and the second on 10 November with a small seed (0.10 ± 0.02 g). In both experiments, a completely randomized block design was used with four repetitions. The genotypes were characterized, among other variables, according to the length of the mesocotyl into long (MEL), medium (MEM) and short (MES).

2.3. Second Stage

Nine genotypes were identified, and all of them were hybrids, which differed in their elongation of the mesocotyl: three with long mesocotyl (H-48, HS-2 (trilinear hybrids) and Promesa (doble hybrid)); three trilinear hybrids with medium mesocotyl (H-44, H-52 (double hybrids) and H-70 (trilinear hybrid)); and three with short l mesocotyl (H-49 AE, H-40 (trilinear hybrids) and H-32 (double hybrid)). A total of 36 direct crosses were obtained from the nine parents, whose F_1 seed was sown on June 14th in the S-S 2021 cycle in an experiment performed in sand beds using completely randomized blocks with four repetitions.

2.4. Conditions of the Experiments

In each experiment, river sand was used as a substrate, previously sifted with a mesh with perforations of 2×2 mm; the dimensions of the bed were 6 m $\times$ 2.5 m in width and 0.20 m in height. Sowing was conducted by placing the seeds on the bed base, at a distance of 5 cm between plants and 8 cm between furrows; all of the seeds were placed with the radicle facing down, and they were covered with a sand layer of 20 cm. The experimental unit consisted of 25 seeds per repetition.

The agronomic management of the experiments was homogeneous, without temperature control; after the first irrigation, saturation was carried out immediately after sowing, and sufficient irrigation was provided to keep the substrate moist. The frequency of emergence in each experimental unit was recorded daily, based on the appearance of the first seedling on the surface. The extraction of seedlings to record the variables was performed at 10 days after sowing, and all seedlings from the experimental unit were recorded.

2.5. Variables and Statistical Analysis

The percentage of emergence (PE) was determined by counting the number of seedlings that reached the emergence at the end of the trial with respect to the number of seeds sown in the experimental unit [26].

The following variables were recorded for each seedling and reported based on the average per seedling: the length of the mesocotyl (LOM, cm) was measured from the union with the seed to the base of the coleoptile, and the length of the coleoptile (LOC, cm) from the base of the coleoptile to its apex, with a metric ruler. The dry weight of the mesocotyl (DWM, cm), of the coleoptile (transparent leaf) (DWC, mg), of the root (DWR, mg) and of the aerial part (green leaves in development) (DWAP, mg) was obtained by separating each structure and drying them on a stove (Blue M Electric Company, Illinois, USA) at 70 °C for 72 h. The speed of emergence (SE) was calculated by counting the seedlings that emerged daily in the subsequent days after the appearance of the first seedling. The unit of measurement was seedlings/day (sd-1) [27].

$$\text{Percentage of emergence (PE)} = [(\text{Number of seedlings emerged})/25)] \times 100 \quad (1)$$

$$\text{Speed of emergence (SE)} = (\text{number of normal plants/days of the first count}) + \ldots + (\text{number of normal plants/days of the final count}) \quad (2)$$

The data of maximum and minimum temperature (°C) of the air and soil, during the period when the experiments were conducted, were recorded daily, with a digital thermometer (Steren®). To obtain the air temperature, the thermometer was placed at a height of 1.2 m over the respective sand beds, and the soil temperature was determined at a depth of 20 cm using a soil thermometer (SMART®). The individual and combined analyses of variance were carried out with the statistical software SAS®, v. 9.0, for Windows [28]. The means comparison was performed using Tukey's test ($p \leq 0.05$). Later, a multiple correlation analysis was conducted to determine relationships between the variables. The average heterosis was calculated as the difference, expressed in percentage, between the cross and the average of the parents. The relative heterosis (MP) was determined and expressed as a percentage [29]. The heterosis was calculated using the differences between the mean of F_1 crosses and the mid-parental value for a given characteristic.

$$\text{Mid-parent heterosis} = [(F_1 - MP)/MP] \times 100 \text{ (relative heterosis)} \quad (3)$$

where F_1 is the mean value of the F_1, and MP is the mean value of the parents involved in F_1, i.e., (P1 + P2)/2.

3. Results and Discussion

3.1. Climatic Conditions during the Experiments

In the S-F 2020 experiments, the maximum average air temperature ranged between 11 and 17 °C and the minimum fluctuated from 0 to 8 °C, while the maximum average soil temperature ranged between 21.2 and 24.6 °C and the minimum fluctuated from 8.5 to 12.9 °C. In S-S 2021, the temperatures were warmer since the maximum air temperature varied between 15 and 25 °C and the minimum from 10 to 13 °C, while the maximum average soil temperature fluctuated from 22 to 25 °C and the minimum from 17 to 21 °C. Figure 1 shows that on average the lowest temperature was seen during sunrise (7:00 a.m.) and the highest during the afternoon (7:00 p.m.) for both temperatures and experiments.

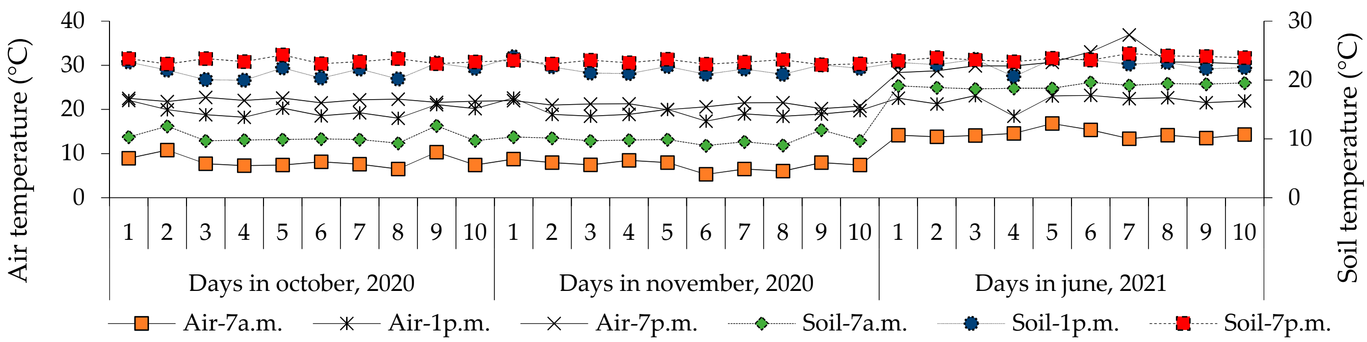

Figure 1. Mean air and soil temperature during S-F 2020 and S-S 2021 experiments with seeds of corn varieties, hybrids and crosses, established in sand at 20 cm depth.

3.2. First Stage: Variation of Characteristics of Corn Seedlings Based on Seed Size of Genotypes

Significance was found for the sources of variation in seed size and genotypes in the nine traits evaluated. In the case of interaction genotype by seed size ($p \leq 0.05$), the speed and percentage of emergence were significant, and for all the variables of dry weight with the exception of the dry weight of the mesocotyl and the length of the coleoptile and that of the mesocotyl (Table 1), there was genetic diversity observed for all the variables under study [14], both for genotypes in each seed size and between the two seed sizes. The absence of interaction genotype by environment in length and dry weight of the mesocotyl, the same as for the length of coleoptile, implies that although the small seed size reduces compared to the large seed size (Table 2), the effect was proportional and parallel for all the genotypes.

Table 1. Results from the ANOVA of square means for the length of mesocotyl and coleoptile and other variables of corn seedlings from large seed (LS) to small seed (SS) during the S-F 2020 cycle.

SV	DF	SE	PE	LOM	LOC	DWM	DWC	DWR	DWAP	DWT
SS	1	13.6 *	23,501.9 *	9.7 *	21.6 *	543.5 *	7950.2 *	28,769.3 *	15,064.5 *	164,077.9 *
R/SS	6	0.1 *	222.3 *	0.66 ^{ns}	3.4 *	57.5 *	79.6 ^{ns}	151.3 *	559.0 *	1038.1 *
G	21	1.5 *	3894.5 *	35.9 *	8.7 *	119.1 *	452.6 *	369.5 *	472.6 *	4099.3 *
G × SS	21	0.18 *	284.9 *	0.4 ^{ns}	0.5 ^{ns}	15.5 ^{ns}	84.7 *	110.3 *	113.8 *	900.1 *
Error	126	0.03	75.8	1.5	0.78	19.8	49.0	42.9	46.5	346.1
Total	175									
C.V. (%)		26.0	25.0	12.2	16.2	17.4	20.5	24.1	25.1	16.4
G and SG	21	1.2 *	2585.7 *	18.2 *	5.9 *	73.0 *	351.4 *	339.3 *	409.4 *	3612.8 *
Error	63	0.06	135.9	2.0	0.8	19.7	39.4	49.5	75.7	265.4
Total	87									
C.V. (%)		25.4	25.7	13.9	16.2	16.2	15.3	17.4	20.0	11.3
G and SP	21	0.47 *	1593.8 *	18.1 *	3.4 *	61.5 *	185.8 *	116.5 *	169.7 *	1386.5 *
Error	63	21.2	17.8	10.1	16.0	18.8	19.0	21.0	22.1	19.7
Total	87									
C.V. (%)		21.2	17.8	10.1	16.0	18.8	19.0	21.0	22.1	19.7

SV = source of variation; DF = degrees of freedom; R = repetition; G = genotype; SE = speed of emergence; PE = percentage of emergence; LOM and LOC = length of mesocotyl and coleoptile, respectively; DWM, DWC, DWR, DWAP and DWT = dry weight of mesocotyl, coleoptile, root, aerial part and total seedling, respectively; SS = seed size. * = $p \leq 0.05$; ^{ns} = non-significant.

Table 2. Weighted mean of corn seedlings from large and small seeds during the S-F 2020 cycle.

Seed Size	Groups of Genotypes	SE (sd-1)	PE (%)	LOM (cm)	LOC (cm)	DWM (mg)	DWC (mg)	DWR (mg)	DWAP (mg)	DWT (mg)
Large	Varieties	0.8 b [1]	36.3 b	9.9 b	5.6 a	26.5 a	40.7 a	38.9 a	39.7 a	145.6 a
	Hybrids	1.1 a	50.6 a	11.4 a	6.0 a	28.5 a	40.9 a	40.1 a	35.2 b	144.6 a
Small	Varieties	0.3 b	13.6 b	9.0 b	5.1 a	23.7 a	33.4 a	18.6 a	23.0 a	98.8 a
	Hybrids	0.5 a	26.7 a	10.2 a	5.2 a	24.6 a	25.9 b	14.6 b	16.9 b	82.1 b

[1] Means bearing the same letter(s) within the column are not significantly ($p > 0.05$) different according to Turkey's honest significance test; SE = speed of emergence per day (seedlings emerged per day); PE = percentage of emergence; LOM and LOC = length of mesocotyl and coleoptile, respectively; DWM, DWC, DWR, DWAP and DWT = dry weight of mesocotyl, coleoptile, root, aerial part and total seedling, respectively.

3.2.1. Speed and Percentage of Emergence in Genotypes

The seed size affected the speed of emergence (SE) and the percentage of emergence (PE) (Figure 2), which was higher ($p \leq 0.05$) when using the large seed, with a difference of 40% for SE and 51% for PE compared to the small seed.

The genotypes H-48, HS-2 and Promesa were the ones that presented the highest ($p \leq 0.05$) SE and PE for both seed sizes, compared to the commercial control AS7573. These differences for both variables were of the order of 95% with H-48 and 94% with HS-2 and Promesa with the large seed; and when using the small seed, it was 90% with H-48 and Promesa and 89% with HS-2 and H-70 for SE and 98% for PE of the three genotypes [30] compared to the control, respectively (Figure 2).

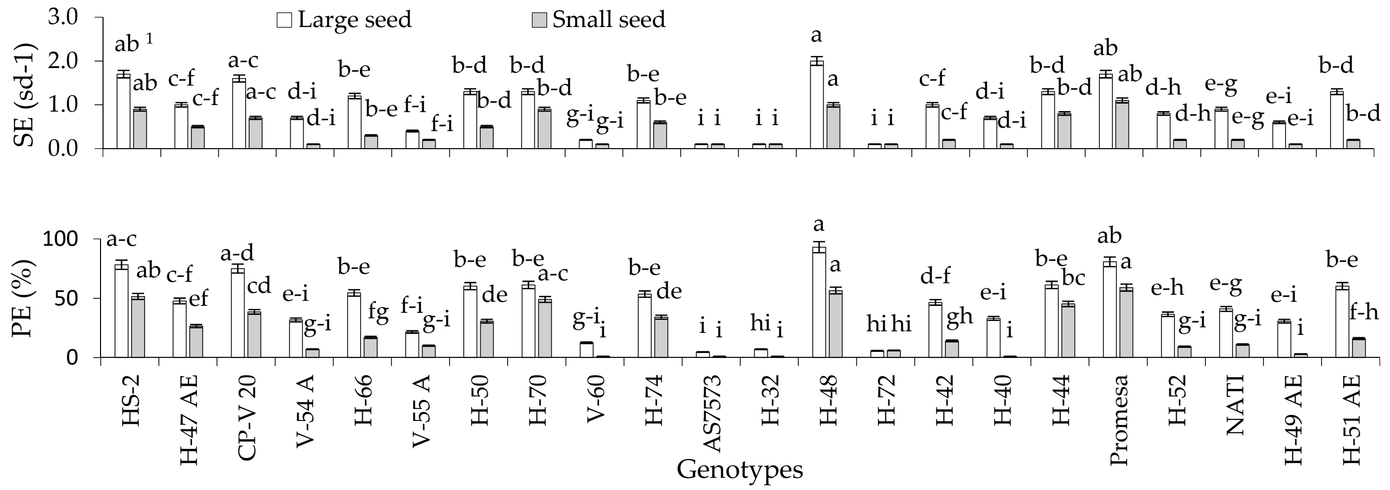

Figure 2. Speed of emergence per day (SE, seedlings emerged per day) and percentage of emergence (PE) of large and small seed corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

Some studies [30] indicate that a lack of uniformity in the emergence of seedlings happens due to the variation in the seed size contained in a commercial bag and to the variation in the depth of the seed during sowing in the entire field. In this sense, and with these results, there is evidence that the large size of the corn seed in deep sowing accelerates the SE and PE, compared to the small seed size. Similarly, studies have proven that a delay in the emergence of corn seeds reduces the grain yield by up to 50% [30].

As the depth of sowing increases, both the SE and the PE are reduced [31]. Study [31], where six levels of seed sizes/shapes were evaluated (round large, flat large, round medium, flat medium, round small and flat small) at three levels of depth of sowing (2, 4 and 6 cm), found that the increase in depth of sowing significantly reduced the SE and PE [31]. In addition, the interaction between the size/shape of the seed, the depth of sowing and the temperature showed a significant effect on the percentage of emergence of the seed and the vigor of the seedling, except in the dry weight of the seedling [31].

3.2.2. Lengths of the Mesocotyl and the Coleoptile in the Genotypes

From the 22 genotypes, the seed size affected the LOM and LOC, which were higher ($p \leq 0.05$) using the large seed with a difference of 5% for the mesocotyl and 12% for the coleoptile, compared to the small seed (Figure 3). Between genotypes, considering the commercial control AS7573 as a reference, the LOM of 12 of the 21 genotypes surpassed ($p \leq 0.05$) that of the control when the seedlings were from the large seed by 20 to 46%; and the LOC of 15 of the 21 genotypes was 14 to 39% higher. The LOM of seedlings of 20 out of the 21 genotypes from the small seed also surpassed the control by 8 to 49%, and the LOC by 2 to 32% for 15 out of the 21 genotypes. The hybrid H-48 presented the highest ($p \leq 0.05$) LOM and LOC for both seed sizes (Figure 3).

The expression of the hybrid H-48 at a higher LOM and LOC compared to the other genotypes is due to a great extent because of its origin, since it has the male parent line M38, and this line is derived from the *cónico* race, which is particularly sown at greater depths (15 to 25 cm) than the normal depth in high zones of Estado de Mexico, Puebla and Tlaxcala, where corn is cultivated under the *arrope* system or the conservation of residual wetland, and where through the selection by farmers, the *cónico* race has achieved elongated mesocotyl and coleoptile that can emerge from the soil [21].

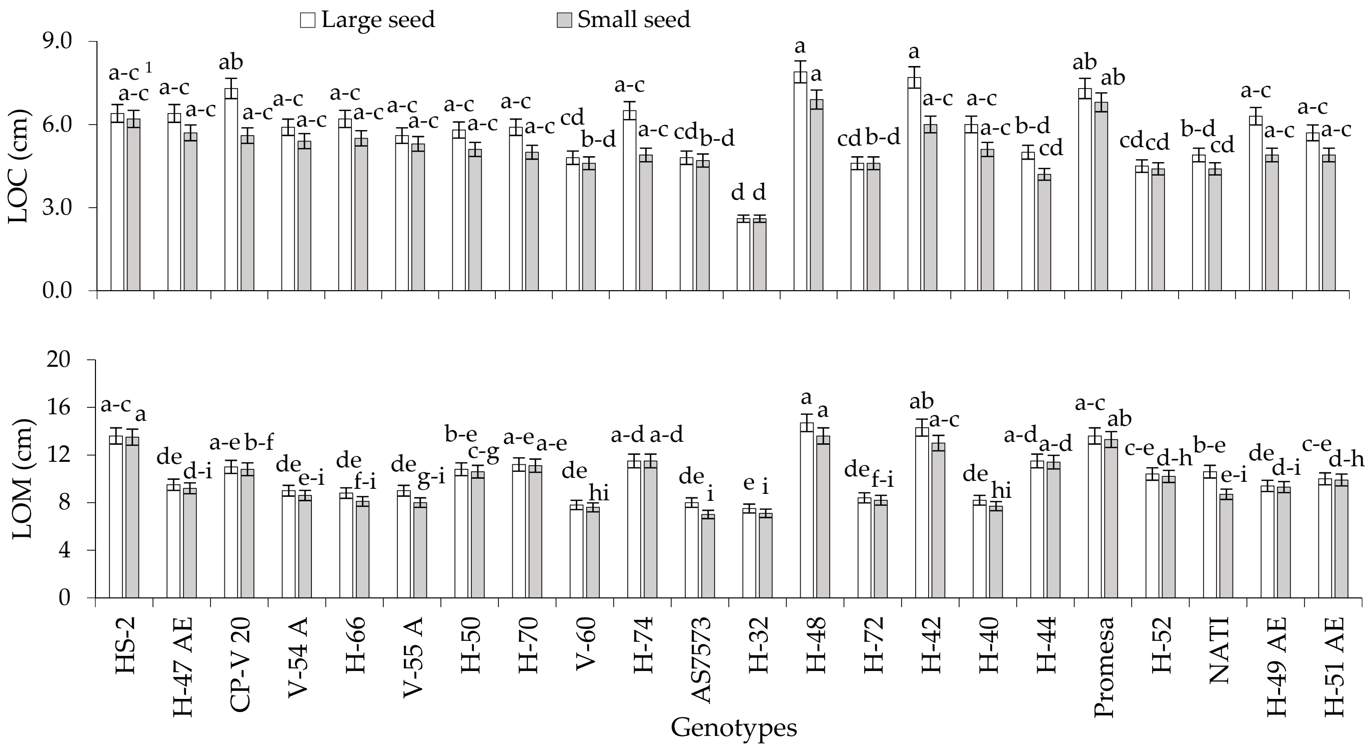

Figure 3. Length of mesocotyl (LOM) and coleoptile (LOC) of large and small seed corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly (p > 0.05) different according to Turkey's honest significance test. Vertical bars show standard error.

The elongation of the mesocotyl and the coleoptile is important because it conditions the emergence of seedlings in the establishment, and the genotypes of greater elongation, such as H-48, HS-2 and Promesa (Figure 3), have higher potential than the other genotypes evaluated to extract moisture from the soil, which could be because they also present higher DWR; these two characteristics are crucial for the growth and development of the plant, since they both take advantage of the soil's residual moisture and contribute to adapting to conditions of deep sowing or to areas prone to hydric stress in the seedling stage [5]. The LOM of H-48, with both seed sizes was 14.7 and 13.6 cm, followed by HS-2 with 13.4 cm with the small seed, exceeded the values found by [11] (4.6 to 9.3 cm) which evaluated corn seedlings IMB Syn10 and their parents (B73 and Mo17) in sand beds at a depth of 12.5 cm in a greenhouse in Iowa, USA.

Studies about the elongation of the mesocotyl of corn varieties of the Chalqueño race at a depth of 20 cm point out that the populations ST34, ST11 and ST112 presented an average elongation of 15.2 cm [22], higher than the average 10.4 cm obtained in this study. However, considering the best three parents, H-48, HS-2 and Promesa, the average was 14.0, which comes close to the value of 15.2 obtained for domesticated populations for deep sowing in the High Valleys of Chalco and Amecameca, Mexico.

Corn is a crop that is sensitive to the cold [32], where the high and low temperatures can reduce the growth and development rate of seedlings [33]. In this study, the maximum temperature present during the emergence of the 22 genotypes fluctuated between 11 and 17 °C and the minimum from 0 to 8 °C; these temperatures were lower than the optimal ones for the germination and early growth of corn seedlings, which is estimated to be between 25 and 30 °C [34]. In spring–summer sowing, which is the case of sowing in the High Valleys of Mexico, the temperatures are warmer at between 15 and 25 °C and the minimum are from 10 to 13 °C (S-S 2021). Therefore, the SE of the hybrids and varieties in both seed sizes could be underestimated and negatively affected by temperatures that are lower than normal for the spring–summer sowing. The hybrids presented a higher LOM than the varieties, and this superiority was 12% with the small seed and 13% with the large seed (Table 2).

3.2.3. Dry Weight of Mesocotyl, Coleoptile, Root and Aerial Part in the Genotypes

When the seedlings were derived from large seeds, the dry weight of the various structures of the seedlings from the varieties was the same as that of the hybrids, except in the aerial part where the varieties exceeded the hybrids by 11%. When they were derived from small seeds, the varieties were also superior ($p \leq 0.05$) to the hybrids by 22% for the dry weight of the coleoptile, 21% for the root weight, 26% for the aerial part's weight, and 17% for the total weight of the seedling (Table 2). This result is similar to that of [35], which compared varieties and hybrids, and found that the elongation of the mesocotyl and the coleoptile of the Criollo Azul variety was higher than the hybrid SB 302 Beretsen. Other studies [9] found that the double hybrid US13 presented a lower elongation of the mesocotyl compared to the Hopi and Navajo varieties, which achieved an elongation of 36 and 30 cm, dimensions higher than those of the outstanding genotypes in this study. The seed size affected the biomass production of mesocotyl, coleoptile, root, aerial part, and total weight of the seedling (Table 1), when using the large seed with a difference of 11% for DWM, 34% for DWC (Figure 4), 62% for DWR (Figure 5), 50% for DWAP (Figure 6) and 42% for DWT (Figure 7).

With the large seed, the DWM for the seedling of three out of 21 genotypes exceeded ($p \leq 0.05$) that of the control AS7573 by 30 to 32%; the DWC of 7 out of 21 genotypes was higher ($p \leq 0.05$) than that of the control by 15 to 38% (Figure 4). The DWR of the hybrid Promesa exceeded ($p \leq 0.05$) the control in 16% (Figure 5) and the DWAP in 19% (Figure 6) of genotypes. Only the variety CP-V 20 exhibited a higher DWT ($p \leq 0.05$) than the control with a difference of 18% (Figure 7).

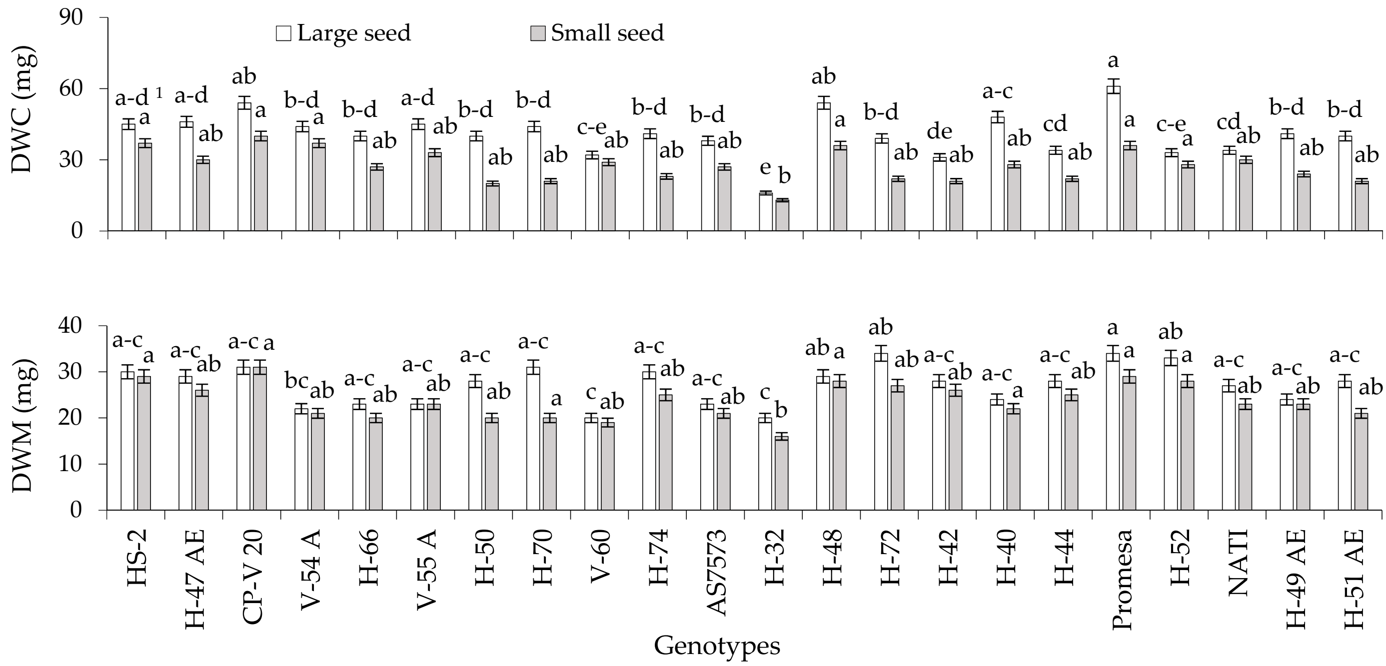

Figure 4. Dry weight of the mesocotyl (DWM) and the coleoptile (DWC) of large and small seed corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

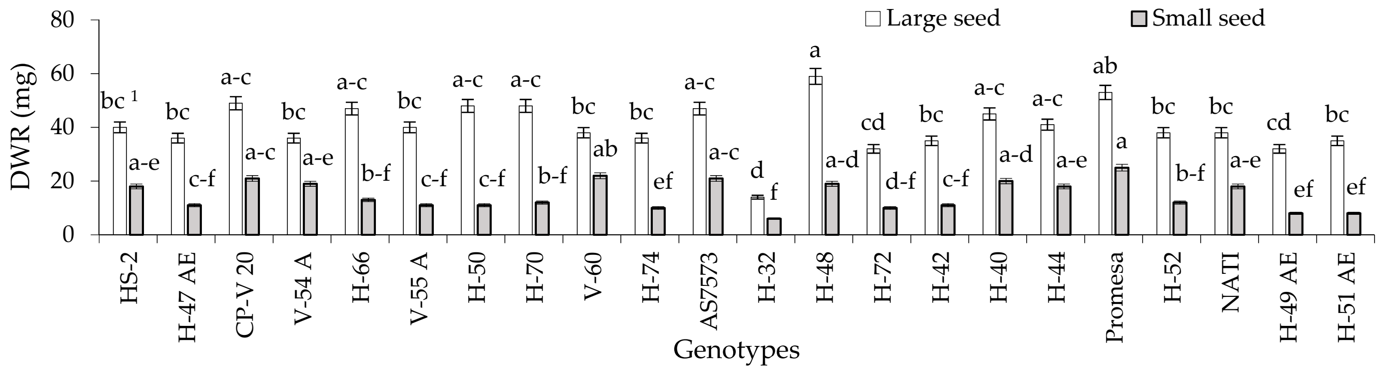

Figure 5. Dry weight of the root (DWR) of large and small seed corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

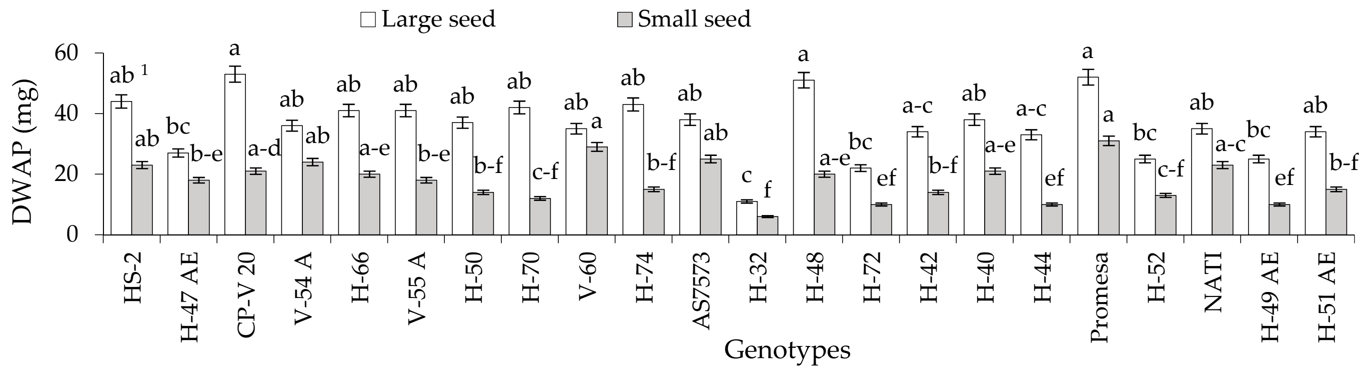

Figure 6. Dry weight of the aerial part (DWAP) of large and small seed corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

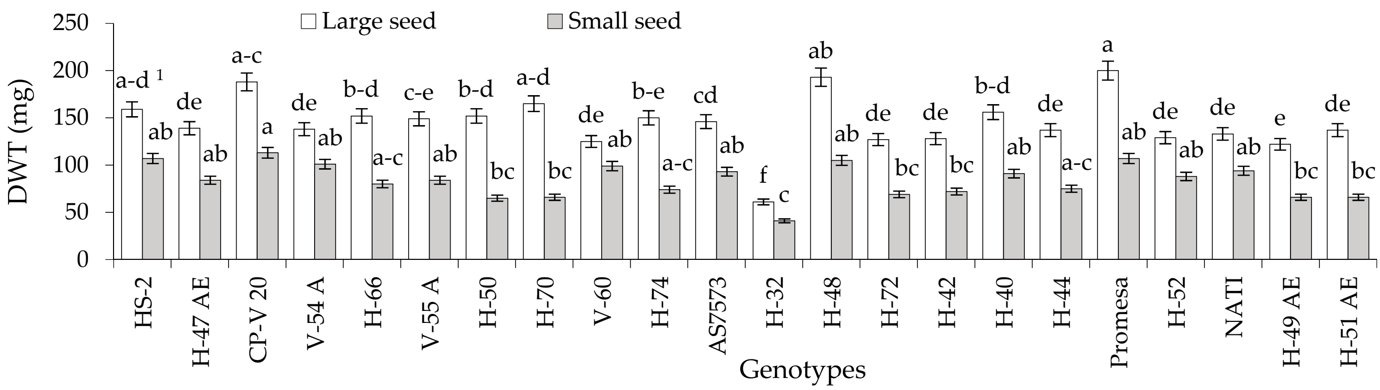

Figure 7. Total dry weight (DWT) of large and small seed corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

The soil temperature fluctuated between 8.5 and 12.9 °C during the S-F 2020 experiments and inhibited the growth of the corn seedlings [8]. In addition, these environmental conditions reduced the production of dry matter (Figures 4–7) of the organs that grow underground, such as the mesocotyl, coleoptile and roots, during the S-F 2020 cycle [8]. Another factor that affected the SE, PE, LOM and LOC is the luminosity [36], which ranged between 100 lumens (7:00 a.m.), 2000 lumens (12:30 p.m.) and 755 lumens (7:00 p.m.) during the experiments.

The hybrid Promesa presented the highest DWM (34 mg), DWC (61 mg) and DWAP (52 mg) for both seed sizes, with the exception of DWT (200 mg) that was obtained with the large seed and DWR (25 mg) with the small seed (Figures 4–7). These values are lower than the ones found by [22] (DWM between 0.72 and 1.18 g; DWC between 2.93 and 5.08 g). H-48 also stood out in terms of the DWM (28 mg) and DWC (36 mg) with the small seed, and the DWR (59 mg) and DWAP (51 mg) with the large seed. The results indicate an evident influence of the genotypes and the seed size on the DWM, DWC, DWR, DWAP (Figures 4–6) and DWT (Figure 7). There is evidence that the seed size has caused approximately 50% of the total variation in the DWAP of the seedlings [37].

3.2.4. Correlation of Characteristics of Corn Seedlings Based on Seed Size of Genotypes

All the variables correlated with each other in a positive and significant way, with the exception of the speed of emergence (SE) that was not associated with practically any of the variables analyzed and with the exception of DWT (Figure 8A). The greatest correlations were between LOM-PE (0.98 **) (Figure 8B) and DWT of the seedling and the dry weight of its respective structures: DWT-DWAP (0.74 **) (Figure 8H), DWAP-DWR (0.69 **), DWT-DWR (0.68 **) (Figure 8G), LOC-LOM (0.67 **) (Figure 8C), DWR-DWM (0.64 **) (Figure 8E), LOC-PE (0.64 **), DWT-PE (0.63 **) (Figure 8B), DWM-LOC (0.51 **) (Figure 8D) and DWR-DWC (0.46 **) (Figure 8F). Therefore, a higher production of dry matter in the seedling structures contributed positively to the greater elongation of the mesocotyl–coleoptile; for this study, this association made the H-48 and Promesa genotypes stand out for both seed sizes (Figures 2–7). In the study [11], the authors observed that, according to the correlation coefficient, the contribution of the seedling organs to emerge in deep sowing is in the following order: mesocotyl > coleoptile; and, in addition, they observed that the LOM is the main trait that is correlated to emergence in deep sowing.

Other studies [1] show that SE and PE are positively correlated with LOM and that these associations contribute to obtaining better homogeneity of seedlings in the field. Although the association between PE and LOM (0.98 **) was evident, it was not so evident between LOM and SE (Figure 8A), that is, low soil temperatures could have caused lower SE values [32], and this was not associated with the behavior of any of the variables analyzed (first stage), although it happened when the temperatures increased (second stage).

3.3. Second Stage: Variation of the Characteristics of Corn Seedlings Based on Parents and Their Crosses

Results from the first stage allow us to explain that the size of some genotypes is associated with tolerance to deep sowing during the early stage of the crop, such a PE, LOM, LOC and DWT [38]. Therefore, in this second stage, the behavior of the F_1 crosses and their parents is observed based on seed size [11], mesocotyl [39], and combination of type of mesocotyl. In this sense, as in the first stage when parents were evaluated, in this second stage, now with their crosses, significance was detected ($p \leq 0.05$) in the nine traits under study (Table 3).

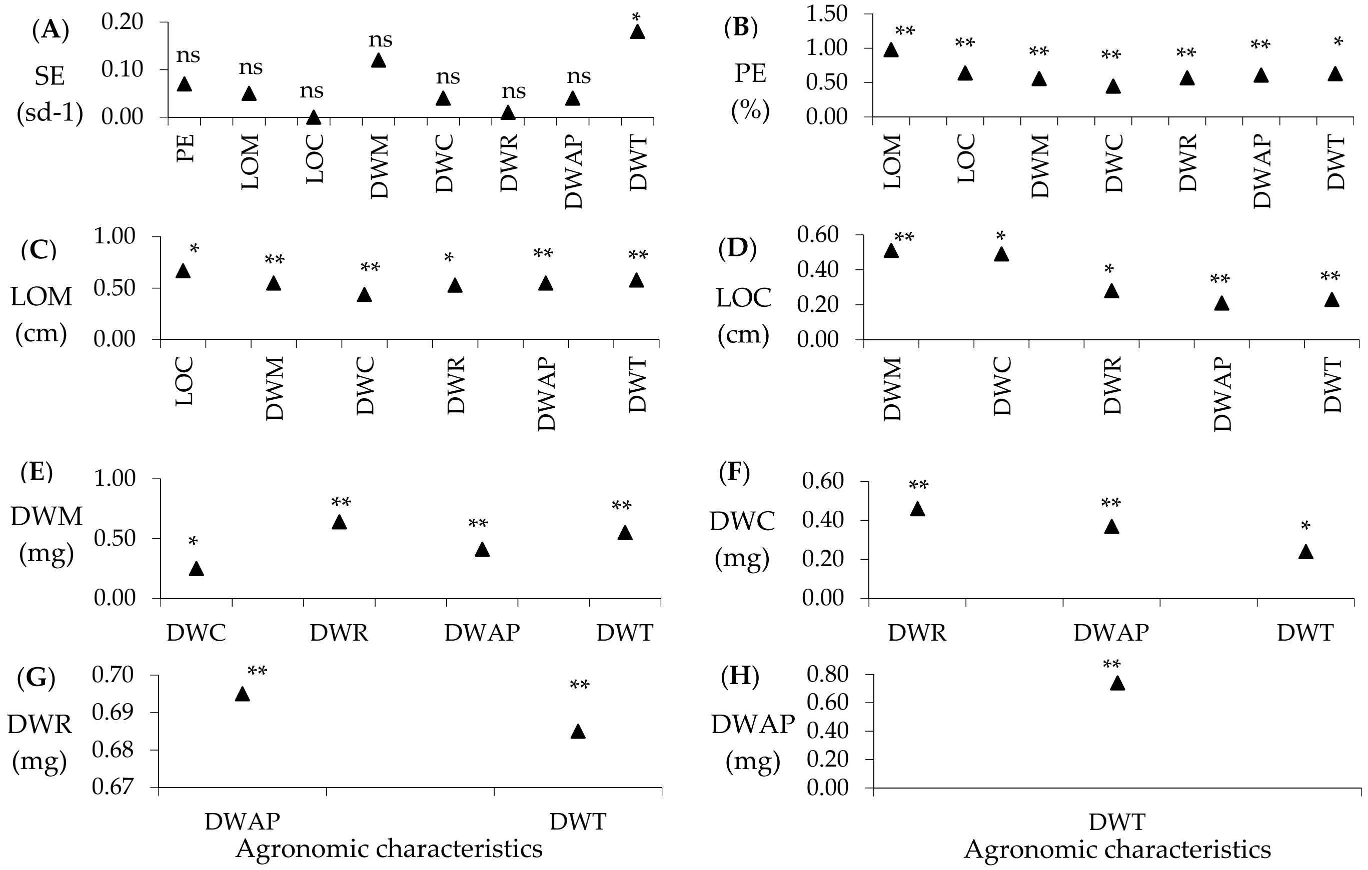

Figure 8. Pearson correlation coefficient of agronomic traits of large- and small-seeded corn seedlings established in sand at 20 cm depth during the S-F 2020 cycle. ** = significance with $p \leq 0.01$; * = significance with $p \leq 0.05$; ns = non-significant; SE = speed of emergence per day (seedlings emerged per day) (**A**); PE = percentage of emergence (**B**); LOM = length of mesocotyl, cm (**C**); LOC = length of coleoptile, cm (**D**); DWM, DWC, DWR, DWAP and DWT = dry weight of (mg) mesocotyl (**E**), coleoptile (**F**), root (**G**), aerial part and total seedling (**H**).

Table 3. Results from the ANOVA of mean squares for the length of mesocotyl and coleoptile and the dry weight of corn seedling structures based on the parent (large and small seeds) and their crosses F_1 (S-S 2021 cycle).

SV	DF	SE	PE	LOM	LOC	DWM	DWC	DWR	DWAP	DWT
Cross (F_1)	53	1.2 *	2567.5 *	26.9 *	3.6 *	204.3 *	44.2 *	38.4 *	255.9 *	1149.1 *
Error	159	0.08	20.9	2.8	0.7	31.0	8.5	6.9	39.8	103.5
Total	215									
C.V. (%)		14.3	16.3	13.6	21.6	16.7	18.5	22.2	25.5	12.4

SV = source of variation; DF = degrees of freedom; * = $p \leq 0.05$; F_1 = generation F_1; SE = speed of emergence per day; PE = percentage of emergence; LOM and LOC = length of mesocotyl and coleoptile; DWM, DWC, DWR, DWAP and DWT = dry weight of the mesocotyl, coleoptile, root, leaf and total seedling.

Figures 9–12 show the expression of the characters under study based on the combination of mesocotyls (long, medium and short) in comparison to large and small seed parents and their F_1 crosses. Based on this, if we use as criterion the seed size of parents based on SE and PE, it was seen that parents with medium and short mesocotyl for both seed sizes presented a greater difference with regard to the combination of long × long mesocotyl, with an average of 89 to 94% for SE (Figure 9A) and 85 to 94% for PE (Figure 9B), followed by long × medium and medium × medium mesocotyl crosses.

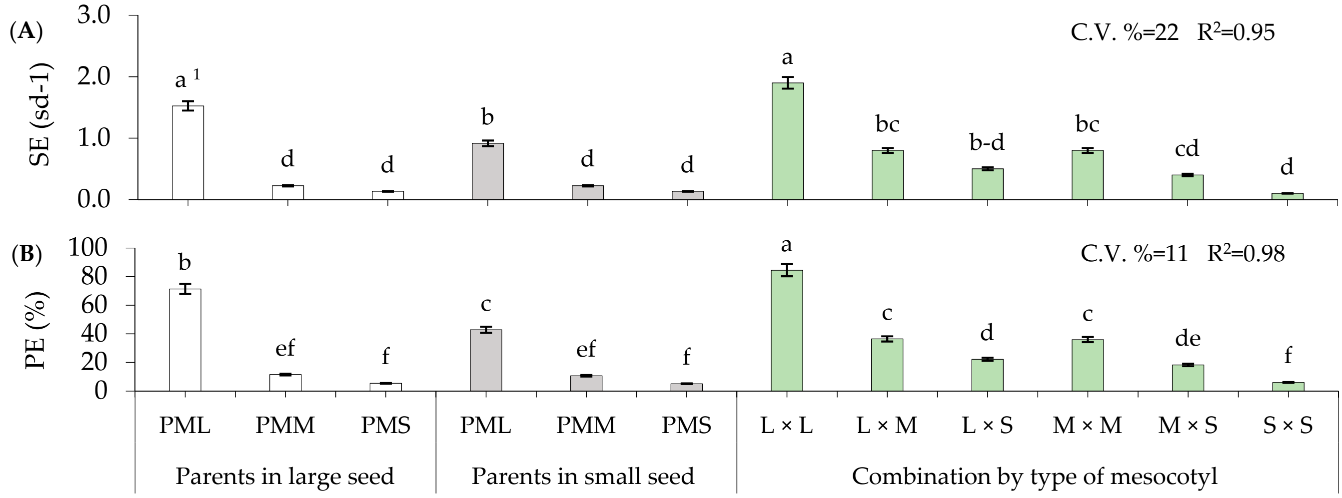

Figure 9. Weighted mean of the speed of emergence per day (SE) (seedlings emerged per day) (**A**) and percentage of emergence (PE) (**B**) of corn seedlings based on the parents (large and small seeds) and their crosses F_1 (S-S 2021 cycle) established in sand at a depth of 20 cm. Parents with long (PML) (H-48, HS-2 and Promesa); medium (PMM) (H-44, H-52 and H-70); and short (PMS) (H-49 AE, H-32 and H-40) mesocotyls. Combination by type of mesocotyl: long × long (L × L); long × medium (L × M); long × short (L × S); medium × medium (M × M); medium × short (M × S); and short × short (S × S). [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

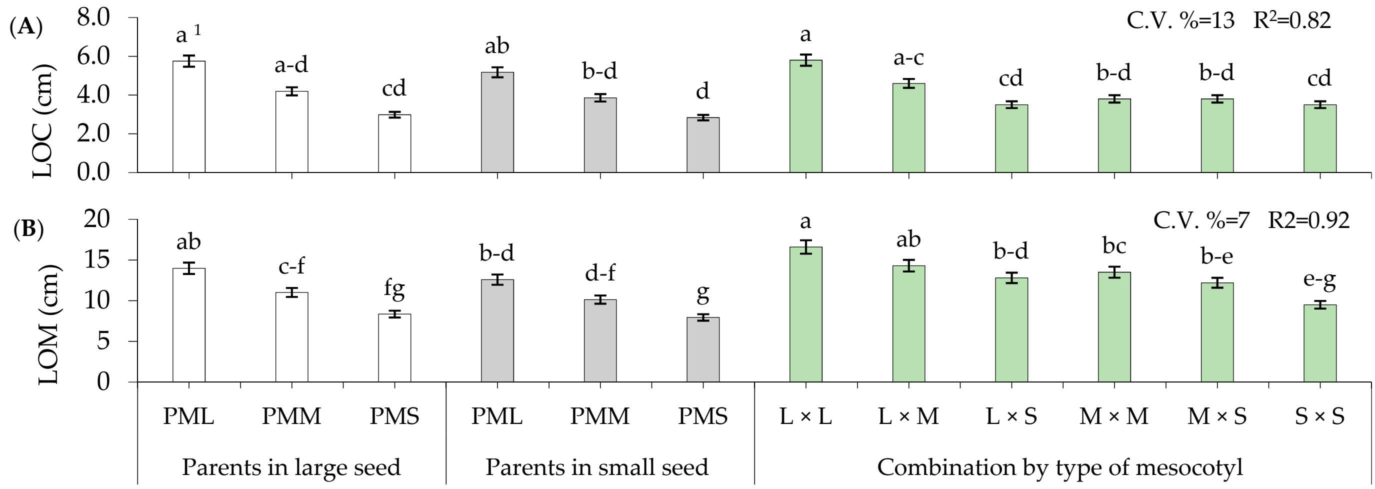

Figure 10. Weighted mean of the length of mesocotyl (LOM) (**B**) and coleoptile (LOC) (**A**) of corn seedlings based on the parents (large and small seeds) and their crosses F_1 (S-S 2021 cycle) established in sand at a depth of 20 cm. Parents with long (PML) (H-48, HS-2 and Promesa); medium (PMM) (H-44, H-52 and H-70); and short (PMS) (H-49 AE, H-32 and H-40) mesocotyls. Combination by type of mesocotyl: long × long (L × L); long × medium (L × M); long × short (L × S); medium × medium (M × M); medium × short (M × S); and short × short (S × S). [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

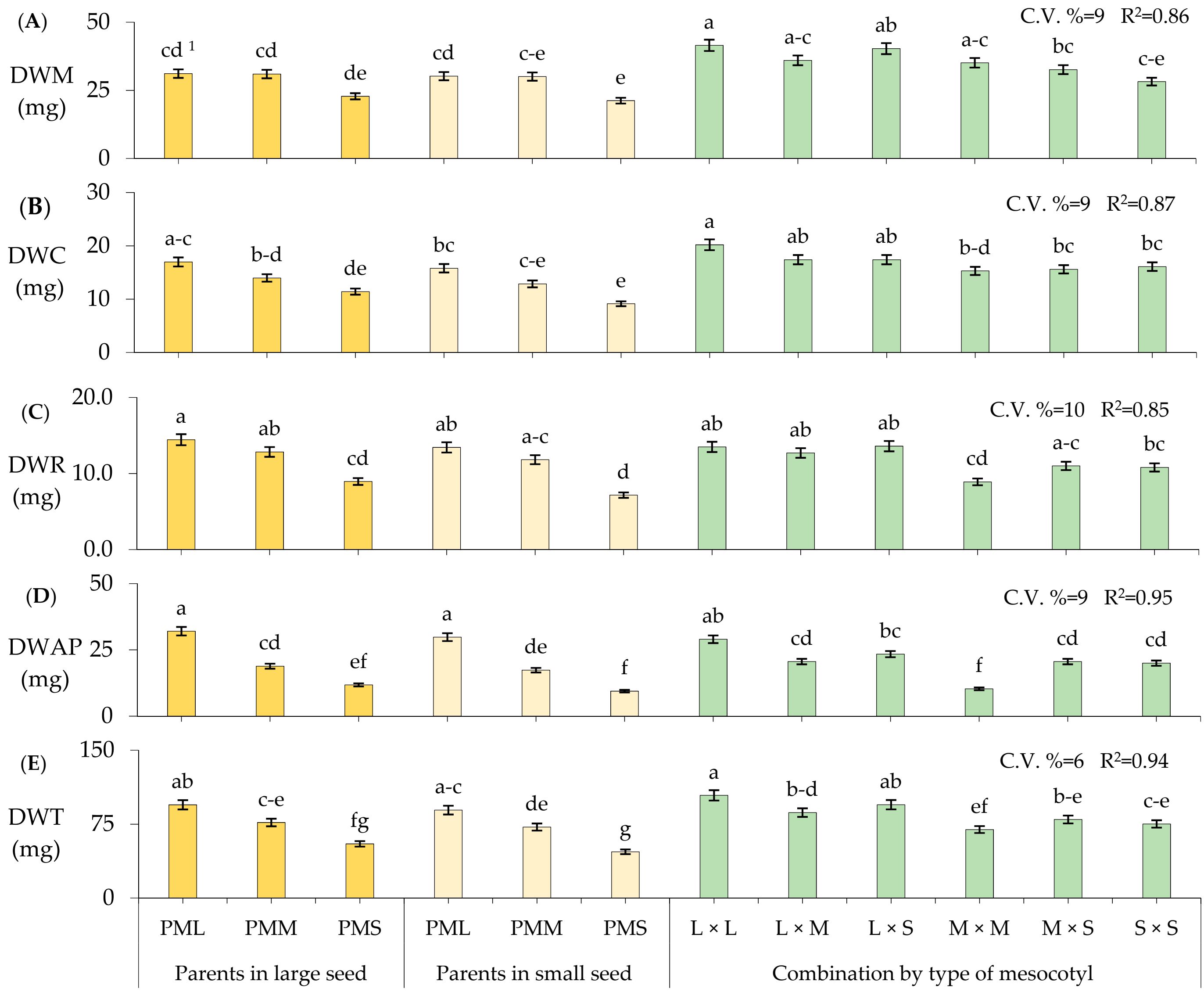

Figure 11. Weighted mean of the dry weight of mesocotyl (DWM) (**A**) and coleoptile (DWC) (**B**) and root (**C**), aerial part (**D**) and total seedling (**E**) of corn based on the parents (large and small seeds) and their crosses F_1 (S-S 2021 cycle) established in sand at a depth of 20 cm. Parents with long (PML) (H-48, HS-2 and Promesa); medium (PMM) (H-44, H-52 and H-70); and short (PMS) (H-49 AE, H-32 and H-40) mesocotyls. Combination by type of mesocotyl: long × long (L × L); long × medium (L × M); long × short (L × S); medium × medium (M × M); medium × short (M × S); and short × short (S × S). [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

Regarding the LOC and LOM, the parents with a short mesocotyl, derived from the small seed, presented a greater difference compared to the combinations of different types of mesocotyls. Therefore, the greatest variation was obtained with the combination of long × long mesocotyl with 52% for LOC (Figure 10A) and 52% for LOM (Figure 10B), followed by the combination of long × medium mesocotyl crosses. Thus, the classification by corn seed size plays a very important role for companies and for the consumers [10], and it is also seen that some parents are not suitable for deep sowing based on their seed size and mesocotyl [11].

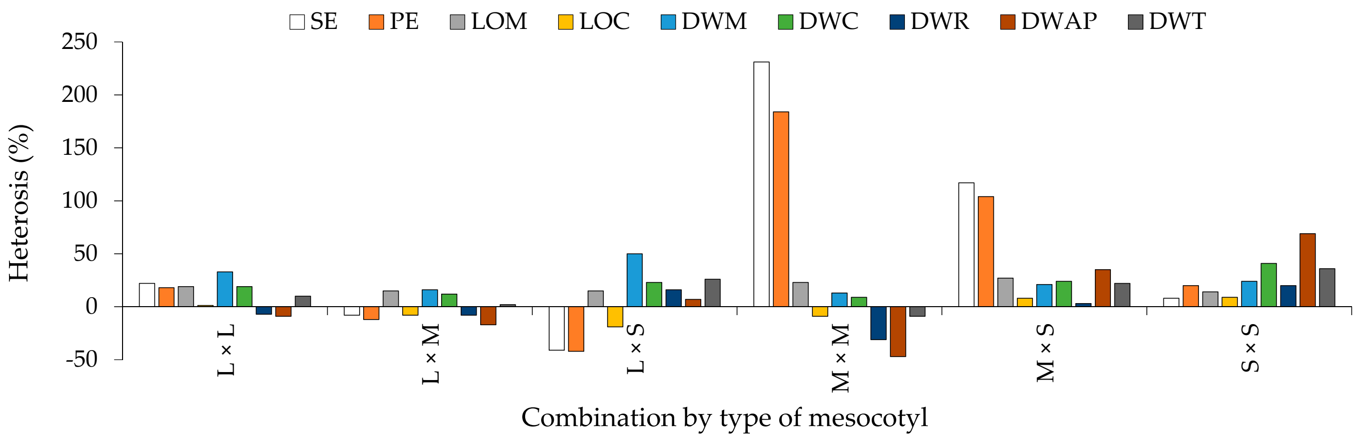

Figure 12. Percentage heterosis with respect to the average of parents for combinations of different types of mesocotyls: long × long (L × L); long × medium (L × M); long × short (L × S); medium × medium (M × M); medium × short (M × S); and short × short (S × S) (S-S 2021 cycle).

Meanwhile, based on what happened with the combinations of different types of mesocotyls, a superior effect ($p \leq 0.05$) is seen with the combination of long × long mesocotyl crosses for mesocotyl–coleoptile elongation and dry matter of all the traits of the corn seedlings, followed by the long × medium mesocotyl crosses compared to the other crosses in one direction (long × short, medium × medium, medium × short, and short × short). The study also shows that the lowest ($p \leq 0.05$) response to an increase in the elongation of the mesocotyl–coleoptile was observed when the parent with short mesocotyl participated with its combinations of crosses, with the exception of the production of dry matter in all its structures (Figure 11). Traits such as SE, PE (Figure 9A,B), LOM (Figure 10B) and DWM (Figure 11A) were superior ($p \leq 0.05$) with the combination of crosses with long mesocotyl in both seed sizes compared to parents from long, medium and short mesocotyls.

In this context, the crosses did not show ($p \leq 0.05$) superiority in DWR and DWAP, with respect to the average of their parents for both seed sizes. However, the parents with both seed sizes and based on the three types of mesocotyl showed a lower DWM, DWC and DWT. Nevertheless, the difference was greater for parents with a small seed compared to combinations of long × long mesocotyl, with a lower weight for DWM of 50% of that of the parent with a short mesocotyl and 28% of the parents with long and medium mesocotyls (Figure 11A), and similarly for DWC, 20, 30 and 55% of that of parents with long, medium and short mesocotyls (Figure 11B), respectively. Meanwhile, for DWT, a difference of 55, 31 and 14 was obtained for parents with short, medium and long mesocotyls (Figure 11E). Therefore, the combinations of long × long and long × medium mesocotyls presented a higher percentage of heterosis compared to parents with smaller seed size for SE, PE, LOM, LOC and DWT. The observed clustering pattern implied the existence of considerable genetic diversity for PE, LOM, LOC and DWT among the combinations of different types of mesocotyls. Several past studies have classified maize genotypes into clusters based on a single trait. For instance, Refs. [14,40] classified maize genotypes into heterotic groups based on the length of mesocotyl. Crosses can be planned involving genotypes from clusters of long, medium and short mesocotyls (the most divergent groups) to exploit heterosis for LOM-LOC.

In this sense, if we use the large seed as a criterion, it can be seen that the result of the crosses presented a variability from the average percentage of heterosis (H) with respect to that of the average parent for each seedling characteristic when the combinations of crosses were made; this occurred to a high degree when the crosses between long × long (22% H) (Figures 12 and 13E), medium × short (117%H) and medium × medium (231% H) (Figures 12 and 13G) mesocotyls were combined for SE, compared to the crosses between long × medium (−8% H) (Figure 12), long × short (−40% H) and short × short (8% H) (Figure 12) mesocotyls. While for PE, greater heterosis was obtained with long × long

(26% H), medium × short (104% H) and medium × medium (184% H) crosses (Figure 12), compared to crosses between long × medium (−11% H), long × short (−42% H) and short × short (19% H) (Figure 12) mesocotyls. Similarly, a higher greater heterosis was combined with the long × long (19% H), medium × medium (23% H), and medium × short (27% H) mesocotyl combinations (Figure 12) for LOM, compared to crosses of mesocotyl long × medium (15% H), long × short (15% H) and short × short (14% H) (Figure 12), since the level of heterosis is associated with the degree of genetic divergence between parent populations [41]. Studies [14] conducted on 68 corn genotypes to determine their degree of genetic variability of the LOM indicated that there is variability ($p \leq 0.01$) between the genotypes; in addition, they observed that inheritance in a broad sense was high and implied that the length of the mesocotyl is a hereditary trait. Therefore, crosses can be made to improve genotypes with less elongation of the mesocotyl [14].

Figure 13. Parents with long mesocotyl, H-48 (**A**), HS-2 (**B**) and Promesa (**C**), and with short mesocotyl (H-40) (**D**). Combination by type of mesocotyl: long × long (1 × 3) (**E**); long × medium (3 × 5) (**F**); medium × medium (5 × 6) (**G**); and short × short (7 × 9) (**H**).

Regarding the DWM, heterosis was higher when long × short (50% H) and long × long (33% H) mesocotyls (Figure 12) were combined compared to the combinations of medium × medium (13% H), long × medium (16% H), medium × short (21% H) and short × short mesocotyls (823% H), respectively (Figure 12); while the combination of medium × medium (9% H) and long × medium (12% H) mesocotyls showed a lower heterosis for DWC compared to the crosses between long × long (19% H), long × short (22% H), and short × short (41% H) mesocotyls. With the combinations of long × long, long × medium, and medium × medium mesocotyls, a negative heterosis was obtained on average for DWR, which ranged from −7 to −31% H and from −9 to −47% H for DWAP compared to the crosses of short × short, and long × short, which obtained a higher heterosis of 15 to

20% H for DWR and 6 to 69% H for DWAP. The heterosis of the combinations short × short (36% H), long × short (26%), and medium × short (22% H) for DWT was higher compared to the crosses with medium × medium mesocotyl, which was negative (−9% H). This fact could indicate that heterosis will be higher as parents who participate in the cross are more complementarily homozygous. Therefore, the F_1 are genetically heterozygous [42], uniform in all traits, and they reach great vigor and present a higher degree of uniformity than their parents.

The effects that combinations of different types of mesocotyls showed for each trait of the seedling evidenced the classification of parents to be superior [43], for example, those that are derived from long (H-48, HS-2 and Promesa) and medium (H-44 and H-52) mesocotyls, which show greater expression of traits associated with deep sowing tolerance like elongation of mesocotyl [39] and coleoptile and dry matter (Figures 9–11). In fact, they can be of use to direct breeders in the selection of cultivars adapted to environments of stress such as drought [44]. In particular, in the micro-regions of the High Valleys of Mexico, corn is sown under conditions of "irrigation tips", residual and seasonal moisture, with low temperature events (frosts) [45] and where the seed must frequently be placed at around 20 to 30 cm to reach the required moisture level; this leads to low PE and population density, with "re-sowing" becoming necessary, which increases the production costs and affects negatively the yield [46].

The means analysis of each of the 36 crosses is presented next and is grouped based on the selection criterion [14] (Figures 14–18). In this sense, it can be seen that the crosses 1 × 2, 1 × 3 and 2 × 3, from crosses of long × long mesocotyl, are viable combinations to be considered in a genetic improvement program, since they presented useful traits for stress tolerance (abiotic and biotic) during the early stage of the crop [5] and of deep sowing, such as elongation of mesocotyl–coleoptile (Figure 15) and dry matter production of the corn seedling structures (Figures 16–18) [5]. However, although a lower DWT was exhibited by all seedling structures, another viable combination was the cross of long × medium mesocotyl (Figure 15), because it showed a favorable response to the elongation of the mesocotyl compared to its parents for both seed sizes, in addition to a better SE and PE compared to the small seed (Figure 9).

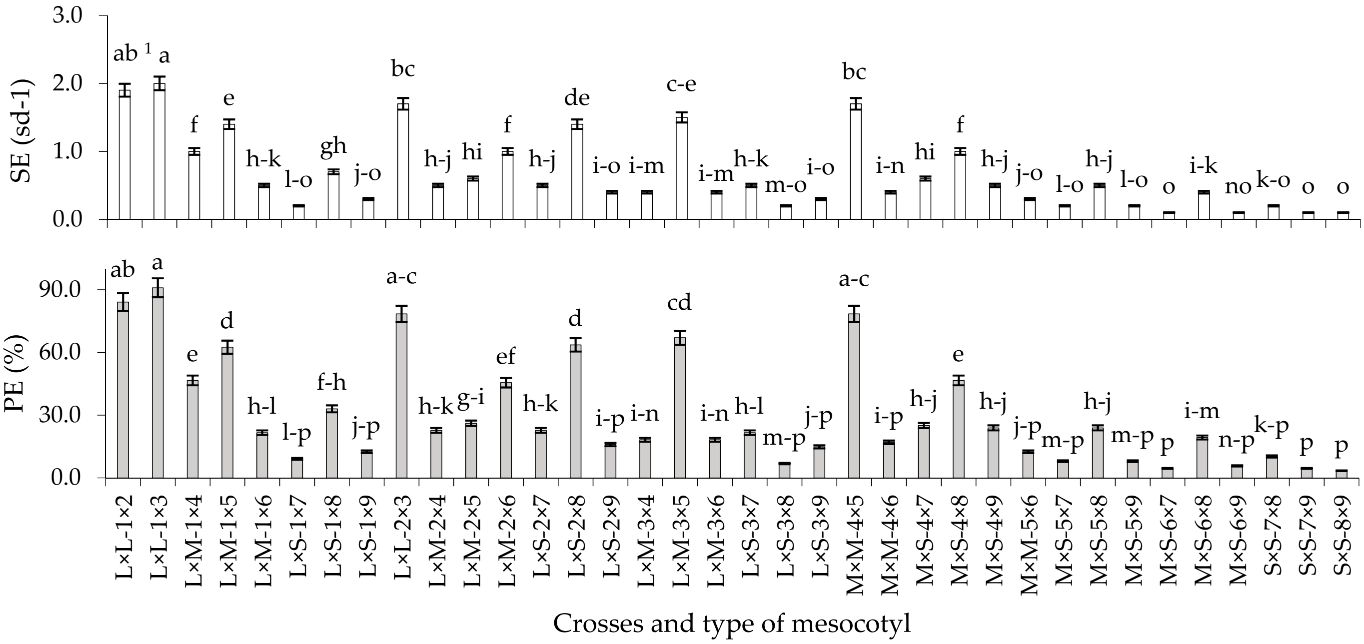

Figure 14. Speed of emergence per day (SE, seedlings emerged per day) and percentage of emergence (PE) of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly (p > 0.05) different according to Turkey's honest significance test. Vertical bars show standard error.

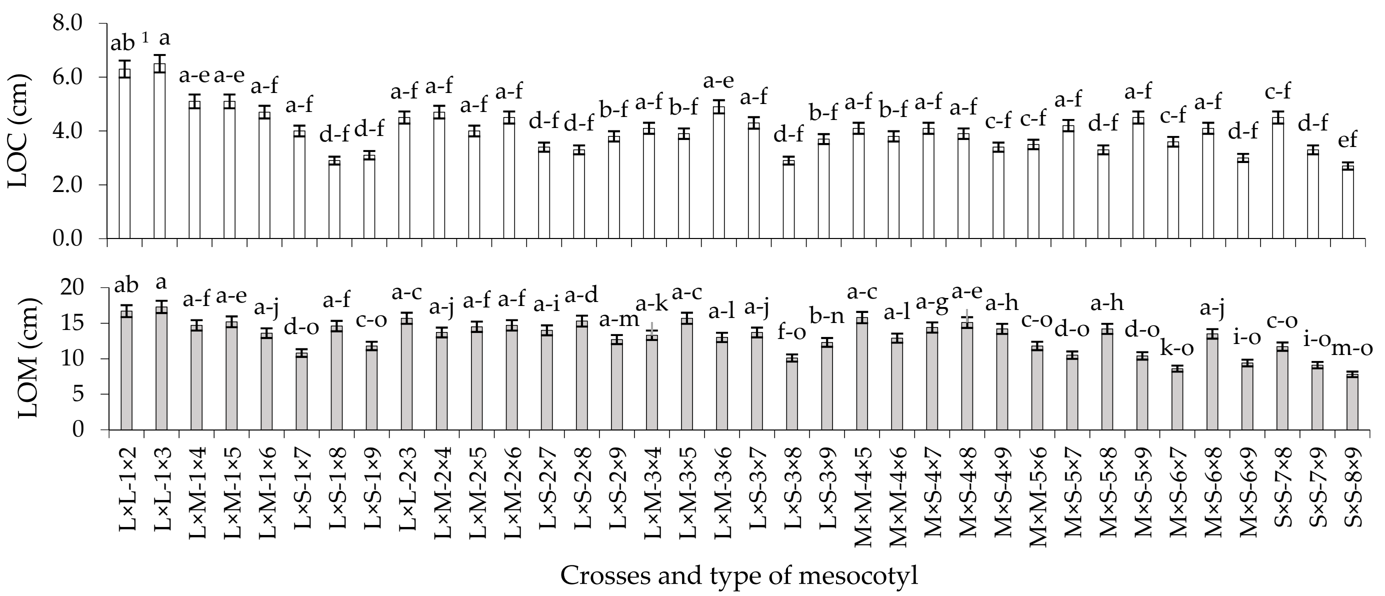

Figure 15. Length of mesocotyl (LOM) and coleoptile (LOC) of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

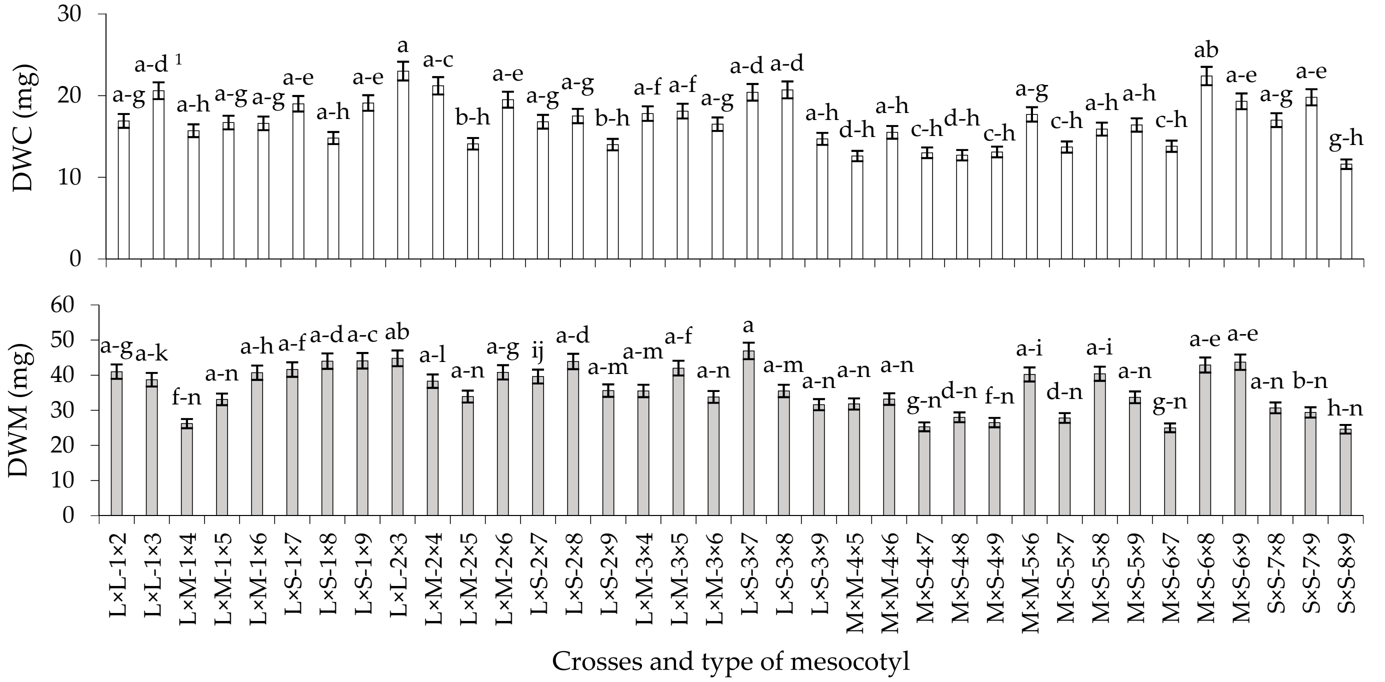

Figure 16. Dry weight of mesocotyl (DWM) and coleoptile (DWC) of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

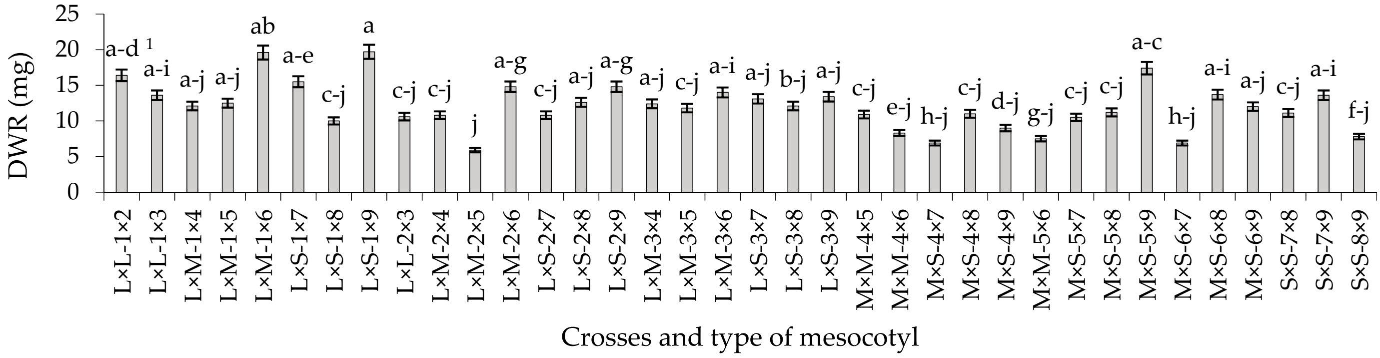

Figure 17. Dry weight of root (DWR) of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

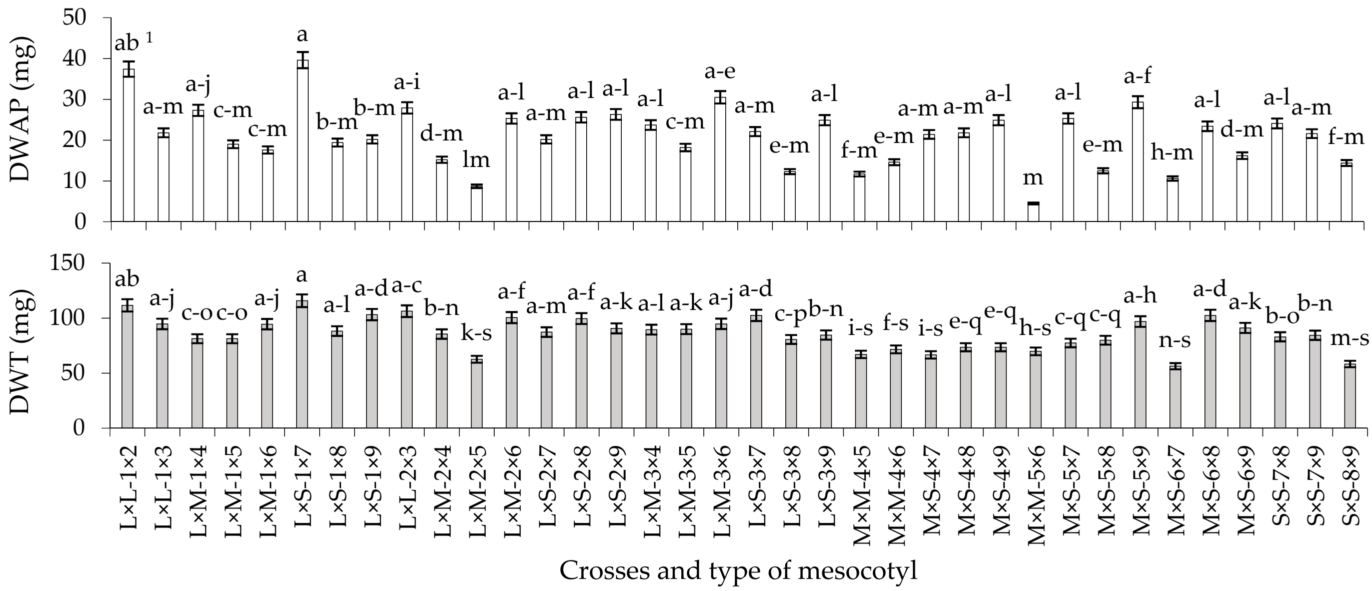

Figure 18. Dry weight of the aerial part (DWAP) and total weight (DWT) of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. [1] Means carrying the same letter(s) in the vertical axis data set are not significantly ($p > 0.05$) different according to Turkey's honest significance test. Vertical bars show standard error.

The crosses of long × long mesocotyl were superior to the rest of the crosses in terms of the average expression of each of the traits under study (Figures 14–18); these differences ($p \leq 0.05$) vary with respect to the combinations of long × medium mesocotyls for SE in 58%, PE in 57% (Figure 14), LOM in 14% and LOC in 21% genotypes (Figure 15), respectively. Seed germination is a fundamental process, which directly influences the development of maize plants and further affects grain yields [47]. It is suggested that F_1 hybrid seeds show superior performance compared to their inbred parental lines in terms of seed germination [48]. It has also been proved that the embryo expansion rate at the early stages of maize seed germination is one of the heterosis traits [49]. Seed germination is a critical stage in plant life cycle that directly determines the establishment of the seedlings. Previous studies have proved that F_1 hybrid seeds have a better performance over both parents during the germination stage [49] and DWM [40], such the crosses 1 × 2, 1 × 3 and 2 × 3, from crosses of long × long mesocotyl. Therefore, to improve the efficiency of early-stage maize breeding in maize, it is necessary to pay attention to the selection of the main basic materials, that is, tall parent, medium parent and female parent, as well as consider the influence of heterosis [40].

The long × long mesocotyl crosses also showed greater ($p \leq 0.05$) dry weight for each structure of the corn seedling compared to the long × medium mesocotyl combinations by 13% for DWM, 14% for DWC 14% (Figure 16), 6% for DWR (Figure 17), 29% for DWAP and 17% for DWT (Figure 18). For crosses of long × short mesocotyl, the difference ($p \leq 0.05$) for SE and PE was 74% (Figure 14), LOM was 23%, LOC was 40%, DWM was 3%, DWC was 14%, DWR was 0% (Figure 16), DWAP was 19% and DWT was 9%. Meanwhile, for the crosses of medium × medium mesocotyl, these differences ($p \leq 0.05$) were for SE in 58%, PE in 57%, LOM in 19%, LOC in 34% (Figure 15), DWM in 15%, DWC in 24% (Figure 16), DWR in 34%, DWAP in 64% and DWT in 33% genotypes (Figure 17). In sowing at 1, 5 and 10 cm of depth [50], these authors observed that the endogamous line of sweet corn Ltx05 presented a higher LOM at emergence, promoted by a greater depth of sowing and which contributed to an unequal PE; however, the physiological mechanisms of the LOM and their relationship with SE and PE in response to deep sowing continue to be unknown. Previous studies have shown that the mesocotyl is the key promoter of SE and PE and, therefore, the establishment of seedlings in the field [40] and that of corn varieties with long mesocotyls have increased the rates of SE and PE of the top soil layer, as well as produced a greater emergence of seedlings [22]. Other studies indicate that in order to optimize the early vigor of corn seedlings in shallow and deep sowing in the field, it is fundamental to establish genotypes with large seed sizes, since this type of seed presents superiority in the elongation of the mesocotyl, SE and PE, than seeds with a small size [1]. Other studies [5] point out that although elongation processes of the mesocotyl are still an object of study, they are influenced by genetic and environmental signals to guide their growth and optimize the early vigor of the seedlings [2]. In these stages, the elongation of roots and mesocotyl perform a critical role under the surface of the soil, which concludes with the plant's emergence.

In relation to the contrast between the crosses of long × long mesocotyl and the crosses of medium × short and short × short mesocotyls, the differences were higher ($p \leq 0.01$); for example, for the crosses with medium × short mesocotyl, SE was 79%, PE 78% (Figure 14), LOM 26%, LOC 34% (Figure 15), DWM 21%, DWC 20% (Figure 16), DWR 18% (Figure 17), DWAP 29% and DWT was 23% (Figure 18). For crosses with short × short mesocotyl, SE was 95%, PE 93%, LOM 43%, LOC 40%, DWM 32%, DWC and DWR were 20%, DWAP was 31% and DWT was 28%, respectively. Other studies [1] indicate that some lines have developed long mesocotyls because of the genetic diversity of corn, which is why they can be sown deeper, while others cannot. In this context, the lengthening of the mesocotyl in response to water scarcity [51] in times of global warming could be used to develop more resistant corn plants. In study [22], the contribution of elongation and dry weight of mesocotyl to the capacity of emergence at deep sowing was evident; in addition, the study showed that with a greater LOM in corn seedlings of native varieties, the production of DWM is higher compared to those with less elongation.

The approach for improving crops is to select genotypes based on the identification of superior phenotypes in the production of dry matter [12] (Figure 18), as well as different attributes of the aerial system (shape, size or pigmentation of the seed) [11]. Jointly, the traits of the organs that grow under the soil surface are used as possible selection markers [40]. Deep sowing is an agricultural practice commonly used in high altitudes or in drier habitats. Its implementation is based primarily on the seed size and the capacity of the seedling in germination to take advantage of a larger area of exploration and the residual moisture of the soil to generate more dry matter in the upper part (leaves) and inferior part (root, mesocotyl and coleoptile) of the plant [52].

Correlation of the Characteristics of Corn Seedlings Based on Parents and Their Crosses

The random crosses for different sizes (long, medium and short) of mesocotyl had a high ($p \leq 0.01$) positive relationship among the traits, SE, PE, LOM, LOC and DWM, of seedlings in this stage (Figures 19–21), which was different from the first stage when a favorable correlation was not obtained ($p \leq 0.05$) between the SE for PE, LOM, LOC and

DWM during the early stage of the crop and deep sowing (Figure 8A), with the exception of PE (Figure 8B) and all other traits under study. Meanwhile, for this second stage, the SE and PE did not present a favorable correlation ($p \leq 0.05$) for DWC, DWR, DWAP and DWT. Therefore, Pearson correlation indicates that the synergies or antagonisms [40] of SE (Figure 19A), PE (Figure 19B), LOM (Figure 20A) and DWM (Figure 21A) in F_1 crosses developed the mechanism of tolerance for deep planting (20 cm) of corn [40].

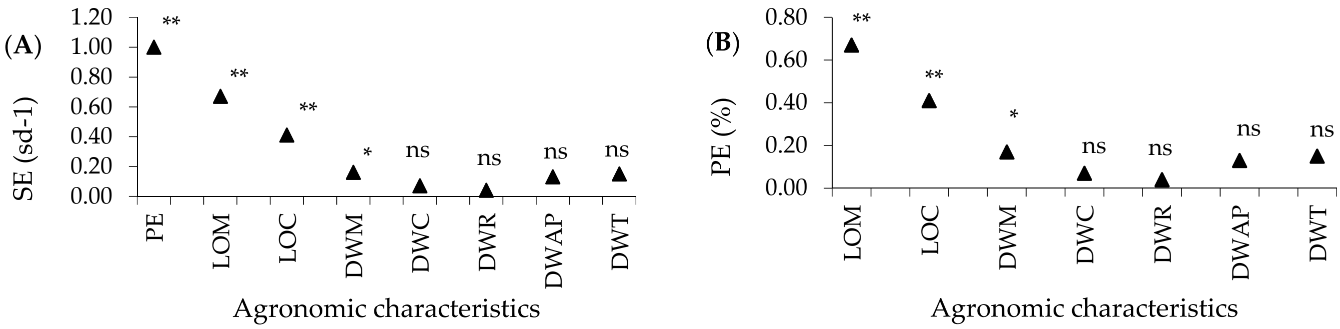

Figure 19. Pearson correlation coefficient of agronomic traits of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. ** = significance with $p \leq 0.01$; * = significance with $p \leq 0.05$; ns = non-significant; SE = speed of emergence per day (seedlings emerged per day) (**A**); PE = percentage of emergence (**B**).

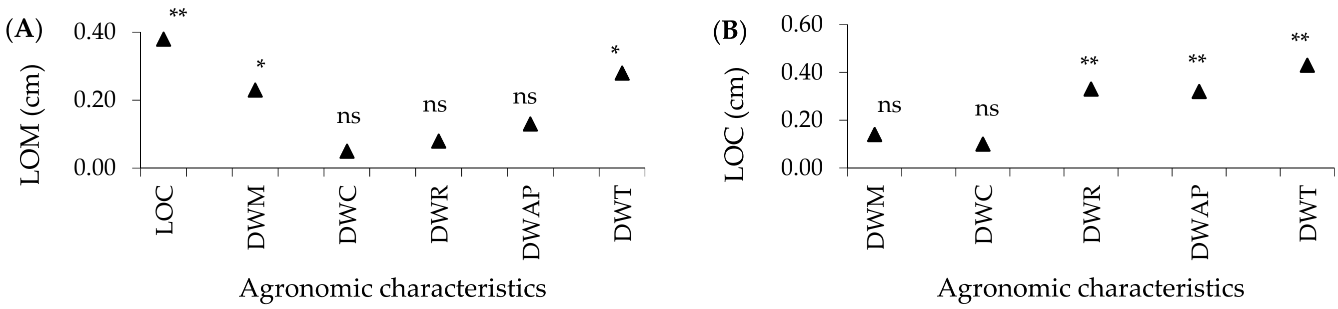

Figure 20. Pearson correlation coefficient of agronomic traits of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. ** = significance with $p \leq 0.01$; * = significance with $p \leq 0.05$; ns = non-significant; LOM = length of mesocotyl, cm (**A**); LOC = length of coleoptile, cm (**B**).

In the first stage (S-F 2020 cycle) of this study, traits such as SE–PE–LOM–LOC were not correlated, which can be due to genetic and environmental factors [1] like the broad fluctuations in soil temperatures throughout the day during the emergence process, which contribute to the development of the mesocotyl "in spiral". The exact minimum temperatures [53] of the soil that can cause such spiral development are not duly documented, although it is clearly not an unlikely occurrence in sowing during April and the beginning of May in the United States, when daily temperatures of the soil can drop to 4.5 °C, and affect [54] the relationship between SE–PE–LOM–LOC. Another factor that could significantly inhibit the expression and correlation of some characteristics of the corn seedling during the first and second stages is the spectral quality of the light emitted [36] in both experiments, which ranged between 100 lumens (7:00 a.m.), 2000 lumens (12:30 p.m.) and 755 lumens (7:00 p.m.). Other studies revealed that light-mediated inhibition of maize mesocotyl and coleoptile elongation was closely related to the dynamics of phytohormones accumulation and lignin deposition in these tissues [6]. In this sense, in the present study, Figure 19 shows that LOM showed a strong significant and direct relationship with SE (0.67 **) (Figure 19A), PE (0.67 **) (Figure 19B), LOC (0.38 **) (Figure 20A), DWM (0.23 *) (Figure 20A) and DWT (0.28 *) (Figure 20A). Meanwhile, LOC presented a higher correlation with SE (0.41 **) (Figure 19A), PE (0.41 **) (Figure 19B), DWT (0.43 **) (Figure 20B), DWR (0.33 **) (Figure 20B) and DWAP (0.32 **) (Figure 20B).

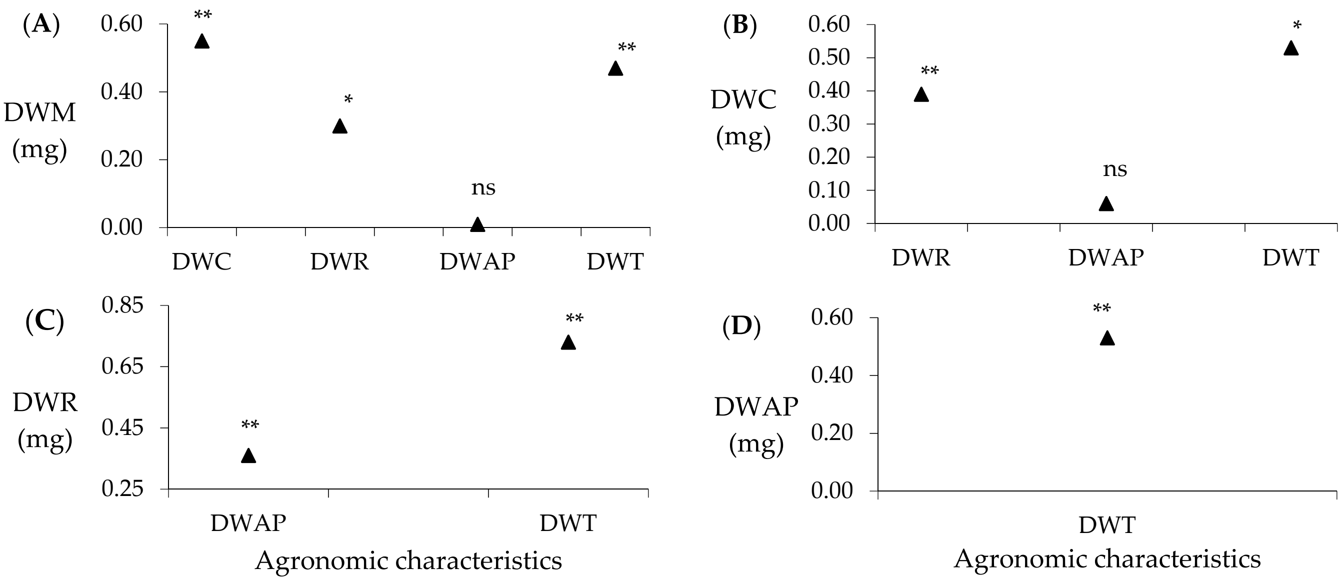

Figure 21. Pearson correlation coefficient of agronomic traits of corn seeds from crosses F_1 (S-S 2021 cycle) established in sand at 20 cm depth. ** = significance with $p \leq 0.01$; * = significance with $p \leq 0.05$; ns = non-significant; DWM, DWC, DWR and DWAP = dry weight of (mg) mesocotyl (**A**), coleoptile (**B**), root (**C**), aerial part and total seedling (**D**).

Figure 21 shows a high correlation of DWT of the seedlings with their corresponding structures: DWT-DWR (0.73 **) (Figure 21C), DWT-DWAP (0.53 **) (Figure 21D), DWT-DWC (0.53 **) (Figure 21B) and DWT-DWM (0.47 **) (Figure 21A). Studies in corn seedlings [9] point out that the emergence of some varieties in deep sowing was correlated with PE and with the capacity of elongation of the mesocotyl and dry matter in the roots, with the exception of DWAP. Studies [55] have shown that tolerance to deep sowing in corn is related to the LOM, and this is because a greater elongation of the mesocotyl promotes the emergence of corn seedlings [3]. Other studies [39] indicate that in deep sowing, SE and PE are highly correlated with the elongation capacity of the mesocotyl, since failures in emergence directly reduce the population of productive plants [55], one of the main determinants of corn yield [56], which is why the potential for grain yield will decrease if the population of productive plants is substantially lower than the optimal population [3].

4. Conclusions

In the germplasm of liberated corn from the High Valleys of Mexico, significant variation was found for speed and percentage of emergence, elongation of mesocotyl–coleoptile and the production of dry matter of mesocotyl, coleoptile, root and aerial part for both seed sizes, obtaining a better expression on average with the large seed compared to the small seed. Similarly, the expression of the traits presented variations based on combinations of different types of mesocotyls (long, intermediate and short) and between crosses within the type of combination. This indicates the strong influence of the genetic variation of corn genotypes based on seed size, parents and their crosses, on mesocotyl–coleoptile elongation and other characteristics of the corn seedlings.

The hybrid with the greatest elongation of mesocotyl and coleoptile was H-48 in both seed sizes, followed by HS-2 with the small seed. The highest dry weight of mesocotyl, coleoptile, root and aerial part was found in the hybrids Promesa and H-48. It was also found that all the combinations of different types of mesocotyls, with the exception of long × long for dry weight of the aerial part, medium × medium for root dry weight, and short × short for speed and percentage of emergence, presented superiority compared to small-seeded parents. However, when the parent had a large seed, the crosses between parents contrasting in the length of mesocotyl, the elongation of mesocotyl and coleoptile and the dry weight of all the seedling traits was superior in the long × long crosses, compared to the long × medium, long × short, medium × short, and short × short crosses. Superiority compared to their parents was only exhibited by the long × long crosses; in the rest of the crosses, no superiority was observed in relation to the elongation of the coleoptile and their dry weights, dry weight of root, aerial part and total, compared to the average of the parents.

All the characteristics of the seedlings, derived from small seed genotypes, showed a greater variation of heterosis compared to the combinations of different types of mesocotyls. Meanwhile, between different types of mesocotyls, the long × long, medium × medium, and medium × short combinations presented a higher percentage of heterosis for speed of emergence and length of mesocotyl. Similarly, this was observed for the medium × medium combination for percentage of emergence; long × long and long × short for length of coleoptile and dry weight of mesocotyl; and the long × short, medium × short, and short × short combinations for dry weight of the coleoptile, root, aerial part and total seedling.

A high positive correlation was found between the elongation of mesocotyl–coleoptile and the percentage of emergence and the production of total dry matter in parents and crosses. Based on the positive expression on the elongation of mesocotyl–coleoptile and other characteristics and heterosis, the crosses that stood out were 1 × 2, 1 × 3 and 2 × 3 that were derived from combinations between long mesocotyls, followed by the 4 × 5, 4 × 6 and 5 × 6 crosses that were derived from crosses of intermediate mesocotyls.

The elongation of the mesocotyl–coleoptile of some corn genotypes could be a useful criterion to classify their parents, to the extent that they can be useful in guiding breeders in the selection of cultivars adapted to stress environments from deep sowing during the early stage of establishment.

Author Contributions: Conceptualization, A.V.G., I.B.R., F.C.G., M.d.C.M.C. and A.E.C.; methodology, A.V.G. and I.B.R.; validation, F.C.G., M.d.C.M.C. and A.E.C.; formal analysis, A.V.G. and I.B.R.; investigation, A.V.G., I.B.R., F.C.G., M.d.C.M.C. and A.E.C.; resources, A.V.G. and I.B.R.; writing—original draft preparation, A.V.G., I.B.R., F.C.G., M.d.C.M.C. and A.E.C.; writing—review and editing, A.V.G., I.B.R., F.C.G., M.d.C.M.C. and A.E.C.; visualization, F.C.G., M.d.C.M.C. and A.E.C.; supervision, I.B.R., F.C.G., M.d.C.M.C. and A.E.C.; project administration, A.V.G. and I.B.R.; funding acquisition, A.V.G. and I.B.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed by the College of Postgraduates (COLPOS), with institutional resources to support students of the postgraduate course in Genetic Resources and Productivity-Genetics (No. 12011027) and by the National Council of Humanities, Science and Technology (CONAHCYT) with resources postgraduate scholarship (No. 765690) to Antonio Villalobos González in the call for National CONAHCYT 2020 scholarships.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors wish to thank Juan Virgen Vargas and Alejandro Espinosa Calderón from the National Institute for Forest, Agriculture and Livestock Research (INIFAP) and Aquiles Carballo Carballo from Colegio de Postgraduados (COLPOS) for supplying the seed for this study. We also thank José Apolinar Mejía Contreras and Martha Hernandez Rodríguez for his contributions to this study.

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

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