agriculture

Article

Direct and Joint Effects of Genotype, Defoliation and Crop Density on the Yield of Three Inbred Maize Lines

Dejan Ranković [1,*], Goran Todorović [1], Marijenka Tabaković [1], Slaven Prodanović [2], Jan Boćanski [3] and Nenad Delić [1]

[1] Maize Research Institute, Zemun Polje, 11185 Belgrade-Zemun, Serbia; gtodorovic@mrizp.rs (G.T.); mtabakovic@mrizp.rs (M.T.); ndelic@mrizp.rs (N.D.)
[2] Faculty of Agriculture, University of Belgrade, 11080 Belgrade-Zemun, Serbia; slavenp@agrif.bg.ac.rs
[3] Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia; bocanski@polj.uns.ac.rs
* Correspondence: drankovic@mrizp.rs; Tel.: +381-11-3756-704

Abstract: The aim of this study was to observe direct and joint effects of three factors (genotypes, ecological environmental conditions and the applied crop density) on the level of defoliation intensity and yield. Three inbred lines (G) of maize (G1–L217RfC, G2–L335/99 and G3–L76B004) were used in the study. The trials were performed in two years (Y) (Y1 = 2016 and Y2 = 2017) and in two locations (L) (L1 and L2) under four ecological conditions of the year–location interaction (E1–E4) and in two densities (D1 and D2) (50,000 and 65,000 plants ha^{-1}). Prior to tasselling, the following five treatments of detasseling and defoliation (T) were applied: T1—control, no leaf removal only detasseling, T2–T5—removal of tassels and top leaves (from one to four top leaves). The defoliation treatments had the most pronounced effect on the yield reduction in G1 (T1–Tn+1 . . . T5), $p < 0.05$. The ecological conditions on yield variability were expressed under poor weather conditions (E3 and E4), while lower densities were less favorable for the application of defoliation treatments. The result of joint effects of factors was the lowest grain yield (896 kg/ha) in G3 in the variant E3D1 for T2 and the highest grain yield (11,389 kg/ha) in G3 in the variant E2D2 for T1. The smallest effect of the defoliation treatment was on the kernel row number (KRN).

Keywords: maize; detasseling; remove top leafs; location; year; properties

Citation: Ranković, D.; Todorović, G.; Tabaković, M.; Prodanović, S.; Boćanski, J.; Delić, N. Direct and Joint Effects of Genotype, Defoliation and Crop Density on the Yield of Three Inbred Maize Lines. *Agriculture* **2021**, *11*, 509. https://doi.org/10.3390/agriculture11060509

Academic Editor: Rentao Song

Received: 29 April 2021
Accepted: 28 May 2021
Published: 31 May 2021

1. Introduction

The seed production of inbred lines and F_1 hybrids requires the removal of tassels from the female parent. This removal prevents uncontrolled pollination, and then the appropriate genetic background in the seed is provided by self-pollination or by effects of anemophily [1,2] or by any other appropriate crossing. Detasseling is done prior to full maturity of anthers, when the tassel has emerged out of the leaf sheath. Detasseling is approached very carefully, it is performed either manually with a skilled labor [3,4], since the base of the tassel is not visible, or mechanically. With more intensive defoliation, there is greater certainty that the entire tassel would be removed. When knives cutters are adjusted to remove the longer top portion of the plant, the variation in the stalk height does not affect the success of detasseling. Regardless of manual or mechanical detasseling, more intensive defoliation reduces the cost of control of the presence of tassel residues on plants in the seed crop. However, more intensive defoliation can cause a decrease in the grain yield [5–8]. The loss in the grain yield results in the lower income from the seed production, which can nullify the savings achieved by the decreased defoliation intensity.

The intensity of defoliation in which there is no significant reduction in yield or the occurrence of other side effects in inbred lines and their seeds can be designated as "safe" ("harmless") treatment (level) of defoliation. The terms "optimum leaf defoliation" and "judicious defoliation" of maize plants have been used by Raza et al. [9]. These terms are related to treatments that improve the nutrient uptake and do not reduce yield. Slower seed maturation can be one of the undesirable effects of defoliation. Modarres et al. [10] stated that leafy-reduced maize inbred lines have a higher grain moisture content at harvest than leafy inbreds.

Seed producers are interested in the increase of the intensity of defoliation, but only up to a safe level. Effects of defoliation and the safe level of defoliation vary depending on different factors, such as: genotype, environmental conditions, crop density, mode, timing and intensity of defoliation. The results obtained in several studies dealing with the effects of various factors on defoliation have been published [11–15].

In one of the initial studies within this field [16], the response of six maize (*Zea mays* L.) inbreds was evaluated to 50% and 100% defoliation at the 14-leaf stage regarding grain yields and yield components. Frascaroli et al. [17] studied the response of six inbred lines of maize (B73, IABO78, Lo1016, Lo964, Mo17 and Os420) to different defoliation treatments in three environments. Raza et al. [9] investigated the effects of four-leaf excision treatments (T_1-0; T_2-2; T_3-4 and T_4-6 leaves excised from the top of maize plants) on grain yield of maize through two-year field experiments. Shekoofa et al. [18] analyzed changes in yields and yield components when all the leaves were removed from one side of the maize plants in three hybrids in three plant densities.

The aim of this study was to establish the direct and combined joint effects of three factors: genotypes (inbred lines), environment and applied cropping practices (crop density) on the safe level of defoliation, including quadruple interactions. These studies are important for the management of seed production and improvement detasseling and defoliation technology to prevent yield losses.

2. Materials and Methods

2.1. Plant Material and Field Experiment

Three maize inbred lines (G), designated as G1–L217RfC, G2–L335/99 and G3–L76B004, were used in the study. Each inbred was derived at the Maize Research Institute, Zemun Polje, Serbia. These inbreds are parental components of commercial hybrids. G1 is a female component of the hybrids ZP 341, ZP 434 and ZP 360. G2 is a female component of the hybrids ZP 555, ZP 606 and ZP 666, while G3 is a female component of the hybrids ZP 560 and ZP 600. These inbreds belong to the following heterotic groups: BSSS (G2, G3) and Iowa Dent (G1).

The three-replicate trail was set up according to the randomized complete block design (RCBD) in 2016. The distance between replicates amounted to 1 m. The elementary plot included three 5-m rows of plants with the inter-row distance of 70 cm. The size of the elementary plot was 0.35 m^2.

The studies were performed during two years (Y1 = 2016 and Y2 = 2017) in two locations in Serbia (L1 = Zemun Polje—44°52′00″ N; 20°19′00″ E and L2 = Parage—45°24′30″ N, 19°24′07″ E). The soil in L1 was degraded chernozem of the second class, while the soil in L2 was calcareous chernozem of the first class. Soil analyses were performed by standard methods for determining quality: soil pH in KCl was determined in a 1:2.5soil-1 MKCl suspension after a half-hour equilibration period; $CaCO_3$ was determined by the method Scheibler with a calcimeter; organic matter content was determined by Kotzmann's method; the Kjeldahl method was used to determine total N, while the content of available phosphorus (P_2O_5) and potassium (K_2O) was established by the standard AL-method according to Egner–Riehm (Table 1).

The trial was set up under rainfed conditions, so that the differences in ecological conditions between years and locations would be as pronounced as possible. In such a way, data were obtained for four year–location interactions: E1 (Y1L1), E2 (Y1L2), E3 (Y2L1) and E4 (Y2L2). Inbred lines were tested in two densities (D): D1 = 50,000 plants ha^{-1} and D2 = 65,000 plants ha^{-1}. Sowing was done in mid-April. The plant distance within the row was adjusted to 28.57 and 21.98 cm in D1 and D2, respectively.

Standard cropping practices for maize were applied. Prior to tasseling, the following five treatments of detasseling and defoliation (T) were applied: T1—control, no leaf removal, only detasseling, T2—removal of tassel and one top leaf, T3—removal of tassel and two top leaves, T4—removal of tassel and three top leaves, T5—removal of tassel and four top leaves (Table 2).

Table 1. Soil properties.

	Soil Layer (cm)	pH		$CaCO_3$ (%)	OM (%)	total N (%)	P_2O_5 mg/100 g	K_2O mg/100 g
		KCL	H_2O					
L1Y1	0–30	7.10	8.10	1.20	2.70	0.19	39.20	20.80
L1Y2	0–30	7.30	8.40	3.30	2.60	0.19	25.90	25.70
L2Y1	0–30	7.61	8.16	5.58	2.53	0.13	18.66	24.85
L2Y2	0–30	7.64	8.22	7.42	2.77	0.13	28.17	24.85

L1Y1—Zemun Polje/2016; L1Y2—Zemun Polje/2017; L2Y1—Parage/2016; L2Y2—Parage/2017.

Table 2. Treatment of detasseling and defoliation (T).

Treatment	Removal of (Tassel + Top Leaf)
T1	T
T2	T + 1 TL
T3	T + 2 TL
T4	T + 3 TL
T5	T + 4 TL

T—Tassel, TL—Top leaf.

The removal of tassels and leaves was done by hand. All ears from the middle row were used to measure the yield. Ears were harvested manually at the stage of full grain maturity, whereby the grain yield was measured and the moisture content was determined for each sample. Then, the grain yield of all variants in the trial was calculated to 14% moisture. The moisture content was determined using a hygrometer (Perten instruments AB, AM 5200-A, Sweden).

The sample of five ears of each variant (3(G) × 2(Y) × 2(L) × 2(D) × 5(T) = 120) was drawn to determine the kernel row number (KRN), kernel number per row (KNR), the ear length (EL), and kernel weight. The kernel row number (KRN) was measured by counting the rows of kernels of each ear from the sample (five ears), KNR was established by counting three rows of kernels from each ear from the sample, EL was determined by measuring the length of five ears from each sample with a ruler (0–1000 mm), and the kernel weight (KW) was established by counting 10 × 100 kernels and then by measuring on the digital balance (Tehtnica ET 1111, max-1200.00/120.00 g, e-0.1 g, dd-0.1/0.01 g, Serbia).

2.2. Precipitation and Temperature Data

The average temperature was lower in Y1 than in Y2 in both locations (by 0.8 °C (19.40–20.2 °C) in L1 = Zemun Polje, and by 0.7 °C (18.1–18.8 °C) in L2-Parage). Y1 was characterized by a higher amount of precipitation during the growing season of maize (April–October) than Y2.

2.3. Statistical Analysis of Data

The statistical analysis encompassed 360 data on the value of grain yield (3 genotypes × 4 year–location interactions × 2 crop densities × 5 defoliation treatments × 3 replications), kernel row number, kernel number per row, 1000-kernel weight, and germination. The analysis of variance (ANOVA), type III sum of squares, was applied to estimate the significance of main factors (G, E, D and T) and their interactions. The descriptive statistics was used to describe basic features, including the mean, standard deviation, minimum, maximum and the range of variants, within each factor. Factors, for which the significance of differences between their variants in ANOVA were determined, were analyzed separately. First, the data file was split into appropriate groups, and then the univariate analysis, with the grain yield as a dependent variable, was applied. Post hoc multiple comparisons were done by the LSD test, for the significance level 0.05 and 0.01 [19]. Statistical analyses were performed using the SPSS version 20 (IBM, Armonk, NY, USA).

3. Results

3.1. Effect of Genotype, Year, Location and Density on Grain Yield and Yield Components under Different Detasseling Treatments

ANOVA showed that there were statistically significant differences among variants G, E and D, as well as among treatments of defoliation (T) (Table 3). This points out that the grain yield, morphological traits or yield components (KRN, KNR, EL, KW) significantly depended on the intensity of defoliation, as well as on effects of defoliation that were observed in this study [20]. The statistical significance obtained by the analysis of variance justifies the choice of factorial variants and provides the meaning of the factorial analysis.

Table 3. Estimation of factor contribution to grain yield, kernel rows number, kernel number per rows, ear length, 1000-kernel weight (ANOVA).

Source	[a] GY	[b] KRN	[c] KNR	[d] EL	[e] KW
G	59.563 ***	195.323 ***	140.873 ***	10.932 ***	182.668 ***
E	2495.874 ***	525.427 ***	1311.885 ***	1103.954 ***	168.828 ***
D	5.146 *	12.193 **	37.046 ***	75.143 ***	24.831 ***
T	36.308 ***	0.285ns	52.773 ***	17.891 ***	676.697 **
G × E	121.272 ***	90.942 ***	54.693 ***	56.748 ***	52.095 ***
G × D	5.574 **	4.085 *	3.506 *	4.919 **	5.215 **
G × T	1.596 ns	3.152 **	1.844 ns	0.636 ns	3.307 **
E × D	4.552 **	6.192 ***	1.189 ns	7.234 ***	2.203 ns
E × T	8.528 ***	0.883 ns	6.078 ***	5.160 ***	1.592 ns
D × T	0.789 ns	4.268 **	1.731 ns	1.918 ns	1.495 ns
G × E × D	1.671 ns	2.344 *	0.632 ns	4.874 ***	1.588 ns
G × E × T	2.681 ***	0.832 ns	2.599 ***	2.869 ***	1.271 ns
G × D × T	2.363 *	3.269 **	2.228 *	0.882 ns	0.490 ns
E × D × T	3.154 **	2.108 *	1.877 *	1.262 ns	2.061 *
G × E × D × T	1.653 *	1.940 *	1.291 ns	1.919 **	1.583 *
Error	69,510,756.000	240	289,628.150		
Total	12,261,433,404.000	360			
Corrected Total	2,617,841,433.156	359			

[a] R Squared = 0.973 (Adjusted R Squared = 0.960); [b] R Squared = 0.919 (Adjusted R Squared = 0.880); [c] R Squared = 0.953 (Adjusted R Squared = 0.930), [d] R Squared = 0.946 (Adjusted R Squared = 0.919); [e] R Squared = 0.854 (Adjusted R Squared = 0.781); KRN—kernel row number; KNR—kernel number per row; EL—ear length; KW—1000-kernel weight; * The mean difference is significant at the 0.05 level, ** The mean difference is significant at the 0.01 level, *** The mean difference is significant at the 0.001 level, ns—not significant.

High yields in the production of various crops are achieved by the selection of genotypes with the greatest possible yield potential. The statistical significance of the G × T interaction in ANOVA indicates that the most yielding inbred lines did not have the highest grain yield in all treatments of defoliation.

The favorable production conditions (location, types of soil) and the application of appropriate cropping practices are a prerequisite for achieving high grain yields [21]. However, based on the statistical significance of double interactions E × T and D × T in ANOVA, it is observed that the most favorable year–location interactions and cropping practices did not always mean the highest grain yields in all defoliation treatments.

The statistical significance of triple interactions G × D × T and E × D × T in ANOVA, as well as the statistical significance of quadruple G × E × D × T interaction in ANOVA, indicate that, in addition to the direct effects of individual factors on grain yields in various defoliation treatments, their combined effects also played a significant role.

The ANOVA results suggest that it is possible to reduce grain yield losses during defoliation by applying individual variants and by combining variants G, E and D. At the same time, the determination of an individual factor and a combination of factors that provide a significant reduction in yield losses during defoliation is very important for seed producers.

In plant breeding, the type of a relationship among yield components is important in order to develop genotypes with high genetic potential [22].

Grain yield can be equal with different contributions of individual yield components [23,24]. The importance of all individual factors on KNR, EL and KW was determined by the analysis of variance of morphological seed traits [25–27]. The trait KRN stood out, as defoliation treatments had no significant effects on the variation of mean values in this study.

The interaction of factors was also present in the variation of morphological traits. Double, triple and quadruple interactions with the factor T did not show statistical significance. The double interactions G × T and D × T had the least impact on the formation of KNR, El and KW. This points to the fact that attention should be paid to the selection of seed material and cropping practices in the process of crop sowing, due to the large variable possibilities of factor actions (Table 1).

3.2. Seed Yield in Trial Variants

The lowest seed grain (896 kg/ha) in the trial was detected in G3 in the variant E3D1 for T2, while the highest one (11,389 kg/ha) was recorded in the same genotype in the variant E2D2 for T1 (Table 4). This result indicates a low stability of G3 seed yields in various variants and treatments, but also the possibility to remove relatively small leaf mass, which can lead to a significant reduction in yield. According to data, G3 responded with a large reduction in grain yield in the adverse unfavorable year of 2017 compared to the genotypes G1 and G2. The reason for this may be that G3 has a longer growing season that the remaining two inbred lines, thus grain filling in this inbred under conditions of drought in 2017 ceased earlier than in G1 and G2. The first year of investigation (Y1 = 2016) was more favorable for maize production than the second one (Y2 = 2017) (Figure 1). whereby in both years, maize had more available moisture for the growth and development in L2 than in L1. Thus, in Y1, the precipitation sum in L1 and L2 amounted to 498 and 537 mm, respectively, while the corresponding sums for Y2 were 349 and 356 mm. The range (max–min) of all variants tested in the trial amounted to 10.493 kg/ha. The average yield of all trial variants was 5175.684 kg/ha, with a standard deviation of 2671.737 kg/ha and a coefficient of variation of 51.62%.

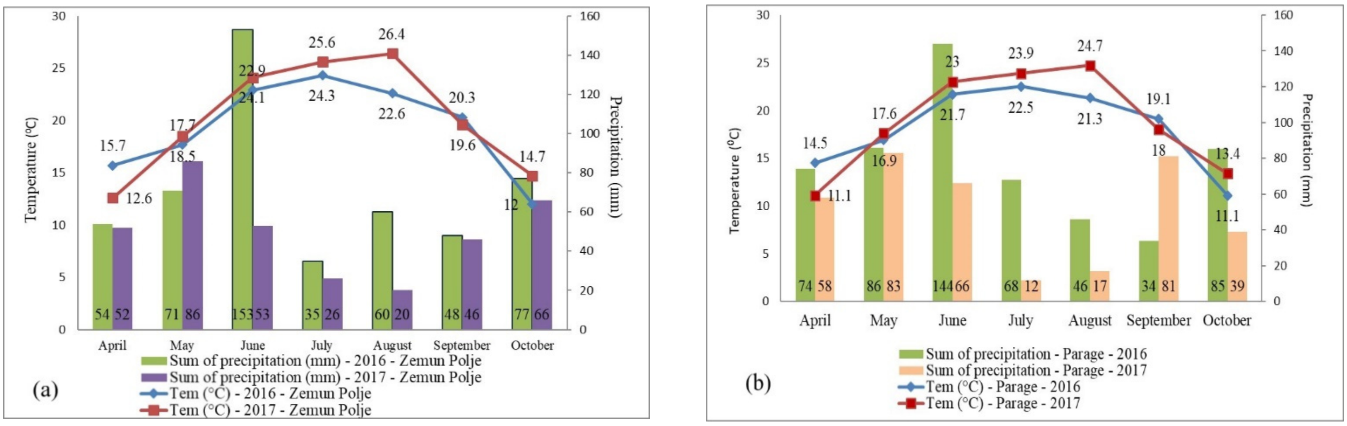


Figure 1. (**a**) Temperature and precipitation data in 2016 (Y1) and 2017 (Y2) in the location of Zemun Polje (L1); (**b**) Temperature and precipitation data in 2016 (Y1) and 2017 (Y2) in location of Parage (L2).

3.3. Descriptive Statistics and Mean Differences of Studied Factors and Defoliation Treatments

According to values obtained for G (Table 5), the highest, i.e., lowest grain yield (5549.4 and 4791.3 kg/ha, respectively) were recorded in G2 and G3, respectively. Differences among average yields of all genotypes were very significant (Table 6).

Table 4. Average yield (kg/ha) of three maize inbreed lines grown in four year–location interactions in two densities for five defoliation treatments.

G	E	D	T				
			T1	T2	T3	T4	T5
G1	E1	D1	6487	5848	5587	5717	5145
		D2	6468	6836	6158	6863	5840
	E2	D1	7149	7776	7035	6829	5777
		D2	8579	8455	8196	7216	6132
	E3	D1	3614	3876	3735	4069	3092
		D2	3559	4292	3758	3732	2967
	E4	D1	4444	3196	3686	3525	3473
		D2	4460	3445	3522	3239	3673
G2	E1	D1	7463	7312	7619	7483	6096
		D2	7089	6519	6855	7656	6158
	E2	D1	9670	8537	9064	8789	7997
		D2	9566	9839	10,000	8311	7297
	E3	D1	4022	3808	4305	2899	3233
		D2	3531	3676	3238	4027	2866
	E4	D1	2795	3452	2546	2284	2582
		D2	2050	2259	2810	3826	2444
G3	E1	D1	6464	6125	6660	6537	5561
		D2	6612	6161	6383	5203	6220
	E2	D1	10,338	9320	9464	9005	8329
		D2	11,389	9704	10,812	9215	7668
	E3	D1	1154	896	1236	1145	1074
		D2	1067	1055	1729	1147	958
	E4	D1	1547	3038	2316	2329	2125
		D2	1852	2338	2800	2808	1870

G: Genotype (G1-, G2-, G3-), E: Year–location interaction (E1—ZemunPolje 2016, E2—Parage 2016, E3—Zemun Polje 2017, E4—Parage 2017), D: Density (D1—50,000 plants/ha, D2—65,000 plants/ha), T: Treatment of defoliation (T1—control, detasseling, T2—detasseling + 1 leaf, T3—detasseling + 2 leaves, T4—detasseling + 3 leaves, T5—detasseling + 4 leaves).

Table 5. Descriptive statistics for the genotype, the year–location interaction, crop density and the defoliation treatment.

Factor	Variant	N	Yield (kg/ha)				
			Mean	S^2	Minimum	Maximum	Range
G	G1	120	5186.3	2,941,798.427	2446	8676	6230
	G2	120	5549.4	7,036,759.979	1672	10,806	9134
	G3	120	4791.3	11,730,172.849	189	11,794	11,605
E	E1	90	6437.6	697,323.256	4832	8656	3824
	E2	90	8582.0	1,946,069.211	5049	11,794	6745
	E3	90	2792.0	1,683,089.706	189	5720	5531
	E4	90	2891.2	720,879.826	1272	4791	3519
D	D1	180	5111.3	6,802,029.6465	189	11,648	11,459
	D2	180	5240.0	7,814,456.155	645	11,794	11,149
T	T1	72	5473.7	9,284,806.394	772	11,794	11,022
	T2	72	5323.6	7,478,895.490	189	10,806	10,617
	T3	72	5396.4	7,911,992.660	1041	11,090	10,049
	T4	72	5160.7	6,579,607.793	645	9883	9238
	T5	72	4524.1	5,023,261.711	811	8947	8136

G: Genotype (G1-, G2-, G3-), E: Year–location interaction (E1—Zemun Polje 2016, E2—Parage 2016, E3—Zemun Polje 2017, E4—Parage 2017), D: Density (D1—50.000 plants ha^{-1}, D2—65,000 plants ha^{-1}), T: Treatment of defoliation (T1—control, detasseling, T2—detasseling + 1 leaf, T3—detasseling + 2 leaves, T4—detasseling + 3 leaves, T5—detasseling + 4 leaves); N—samples size; S^2—variance.

It is interesting that the most yielding genotype was characterized by higher yield stability than the least yielding genotype, based on values of their standard deviations. The lowest standard deviation and the smallest range of variation were detected in G2.

Table 6. Differences in mean yield (kg/ha) of genotypes.

Gn	Gm	Gn–Gm	Sig.	Standard Error
G1	G2	−363 **	0.000	69.488
	G3	394 **	0.000	
G2	G3	758 **	0.000	

G: Genotype (G1-, G2-, G3-), Gn—first column of factor G; Gm—second column of factor G; Gn–Gm—differences between the mean values of the factors; ** LSD significant at the 0.01 level.

Based on the E values (Table 5), the highest (8582 kg/ha), i.e., lowest (2792 kg/ha) grain yield was recorded in E2, i.e., E3, respectively. There was a significant difference among all year–location interactions, except between E3 and E4 (Table 7).

Table 7. Differences in mean yield (kg/ha) over the year–location interactions.

En	Em	En–Em	Sig.	Standard Error
E1	E2	−2144 **	0.000	80.226
	E3	3645 **	0.000	
	E4	3546 **	0.000	
E2	E3	5789 **	0.000	
	E4	5690 **	0.000	
E3	E4	−99	0.218	

E: Year–location interaction (E1—Zemun Polje 2016, E2—Parage 2016, E3—Zemun Polje 2017, E4—Parage 2017); En—first column of factor E; Em—second column of factor E; En–Em—differences between the mean values of the factors; ** LSD significant at the 0.01 level.

This result points out that producers of hybrid maize seeds can expect a significant variation in yields under rainfed conditions [28–31]. Significantly lower yields obtained in E3 and E4 than in E1 and E2 are a consequence of unfavorable weather conditions for maize production in 2017 than in 2016. The second production year lacked sufficient amounts of precipitation for obtaining higher yields. The sum of precipitation from April to October amounted to 349 and 356 mm in E3 and E4, respectively. The significant differences between E1 and E2 suggest that very different yield can be achieved in different locations during the same year. One of the interesting results is that the smallest yield deviation (835.06 kg/ha) was recorded under conditions of E1, in which the second highest yield (6437.6 kg/ha) was obtained. This leads to a conclusion that the certain year–location interactions contribute to the establishment of yield stability over all observed factors, i.e., they reduce their effects, in conjunction with management systems and plant populations [32–35].

Based on D values (Table 3), the higher yield (5240.0 kg/ha) was obtained with the higher density (D2) and vice versa the lower density (D1) with the lower yield (5111.3 kg/ha). The crop density affects the habitat and the assimilation surface of individual plants. A high sowing density shades the crop canopy, reduces the light transmittance within a population and accelerates leaf senescence, all of which affect photosynthesis of maize and both the accumulation and the distribution of substances and limit the grain development [36–38]. Considering the above stated, it should not be forgotten that the D × E interaction was significant. The range of variation in both sowing densities was equal (11,459 kg/ha). The obtained results on D (Table 3) indicate that in order to obtain higher yields in the seed production of maize inbred lines, the denser sowing should be preformed.

Concerning the T variants, the highest average yield (5473.7 kg/ha) was achieved in the variant T1, and the lowest (4524.1 kg/ha) in the variant T5 (Table 3). Pereira [39] considered that the reduction in the assimilation leaf area during defoliation was the main cause of the yield reduction. Compared to the control (T1), significantly lower yields were obtained in T4 and T5 (Table 8).

Table 8. Differences in mean yield (kg/ha) for defoliation treatments.

Tn	Tm	Tn–Tm	Sig.	Standard Error
T1	T2	150	0.095	89.695
	T3	77	0.389	
	T4	313 **	0.001	
	T5	950 **	0.000	
T2	T3	−72	0.418	
	T4	162	0.071	
	T5	799 **	0.000	
T3	T4	235 **	0.009	
	T5	872 **	0.000	
T4	T5	636 **	0.000	

T: Treatment of defoliation (T1—control, detasseling, T2—detasseling + 1 leaf, T3—detasseling + 2 leaves, T4—detasseling + 3 leaves, T5—detasseling + 4 leaves); Tn—first column of factor T; Tm—second column of factor T; Tn–Tm—differences between the mean values of the factors; ** LSD Significant at the 0.01 level.

The largest reduction in the assimilation leaf area was in the variant T5, in which four leaves were removed, hence it is logical that the lowest grain yield was obtained in the defoliation treatment. Allometry is clearly observed with the frequency as a response of plants to the compensation attributed to variations in plant populations [40]. Vasilas and Seif [16] estimated that 50% defoliation at the 14-leaf stage reduced grain yields of maize inbreds by 2.5–17.0%. In this study, mean values of grain yields in T2 and T3 were similar and did not differ significantly from the control treatment, so it can be concluded that the removal of one or two leaves represented the safe intensity of defoliation. This statement is in agreement with the conclusion drawn by Raza et al. [9] that excision of two leaves from the top of maize plants significantly improved the light interception to lower strata leaves and accelerated the biomass partitioning to maize kernels. The standard deviation of the grain yield in this study decreased with the increased defoliation intensity.

3.4. Joint Effects of Studied Factors on the Yield Loss in Different Defoliation Treatments

In regard to the seed production of maize inbred lines, it is important to know the effects of individual factors on grain yields and yield losses in different defoliation treatments (Table S1). Nevertheless, it should be borne in mind that factors are not expressed each for itself, but they are inter-related, which is in accordance with the conclusions made by other authors [41,42].

According to all individual genotype × year–location interaction (Table 9), G3 in E1, as well as G2 and G3 in E3, did not have a significant loss of grain yield in any defoliation treatment.

On the contrary, G1 in E4 had a significant yield loss in all defoliation treatments. It is noticeable that the levels of safe defoliation in the same year–location interaction varied over observed genotypes, but also these levels for the same genotype differed in dependence on E. This suggests that seed producers have the ability to reduce yield losses by producing inbred lines in a particular year–location interaction in the defoliation variant. At the same time, it is important that these conditions provide achieving high yields of the inbred line (Tables S2 and S3).

The last analysis in this study refers to the dissection of the quadruple G × E × D × T interaction. It was observed that there was a wide range of joint effects of G, E and D on grain yield losses in different defoliation treatments (Table 10).

Effects observed for individual factors can be greatly altered in quadruple interactions. For example, although a higher crop density D2 was found to reduce losses due to defoliation, by observing actual values of yield losses due to defoliation over locations, quite the opposite effect or the neutral effect of crop density, can be observed (Table S4). For instance, yield losses of G1 in E2 are greater in D2 than in D1, in absolute values and significance.

Table 9. Joint effect of genotype × year–location interaction on yield losses (kg/ha) in different defoliation treatments.

G	Tm	T1-Tm			
		E1	**E2**	**E3**	**E4**
G1	T2	135	−252	−497 **	1132 **
	T3	605 **	248	−160	848 **
	T4	187	841 **	−314	1070 **
	T5	985 **	1909 **	557 **	879 **
G2	T2	361	430	35	−433
	T3	40	86	5	−256
	T4	−294	1068 **	314	−633 *
	T5	1149 **	1971 **	727	−90
G3	T2	394	1352 **	135	−988 **
	T3	16	726	−372	−859 *
	T4	668	1754 **	−36	−869 *
	T5	647	2865 **	95	−298

G: Genotype (G1-, G2-, G3-), E: Year–location interaction (E1—Zemun Polje 2016, E2—Parage 2016, E3—Zemun Polje 2017, E4—Parage 2017); T: Treatment of defoliation (T1—control, detasseling, T2—detasseling + 1 leaf, T3—detasseling + 2 leaves, T4—detasseling + 3 leaves, T5—detasseling + 4 leaves); Tm—column of factor T2, T3, T4, T5; T1–Tm—differences between the mean values of the factors.* The mean difference is significant at the 0.05 level, ** The mean difference is significant at the 0.01 level.

Table 10. Joint effect of genotype, year–location interaction and crop density on yield losses (kg/ha) in different defoliation treatments.

E	Tm	T1-Tm					
		G1		**G2**		**G3**	
		D1	**D2**	**D1**	**D2**	**D1**	**D2**
E1	T2	639 *	−368	151	570	338	450
	T3	900 **	310	−155	235	−196	228
	T4	770 **	−395	−20	−567	−73	1409
	T5	1342 **	627 *	1367 **	931	902	391
E2	T2	−627	123	1133 **	−273	1018	1685 **
	T3	114	383	606	−434	875	577
	T4	320	1363 **	880 *	1256	1333 *	2174 **
	T5	1372 **	2446 **	1673 **	2269 **	2010 **	3721 **
E3	T2	−262	−733 **	214	−145	258	12
	T3	−121	−199	−282	293	−82	−662
	T4	−455	−173	1123	−496	9	−79
	T5	522	592 **	789	665	80	109
E4	T2	1248 **	1016 *	−657 *	−209	−1491 **	−485
	T3	757 *	938 *	249	−760	−769 *	−948
	T4	918 **	1221 **	511	−1776 **	−782 *	−956
	T5	971 **	787	213	−394	−578	−17

G: Genotype (G1-, G2-, G3-), E: Year–location interaction (E1—ZemunPolje 2016, E2—Parage 2016, E3—ZemunPolje 2017, E4—Parage 2017), D: Density (D1—50,000 plants/ha, D2—65,000 plants/ha), Tm: Treatment of defoliation (T1—control, detasseling, T2—detasseling + 1 leaf, T3—detasseling + 2 leaves, T4—detasseling + 3 leaves, T5—detasseling + 4 leaves); T1–Tm—the differences between the mean values of the factors; * The mean difference is significant at the 0.05 level, ** The mean difference is significant at the 0.01 level.

G1 in E4 for D1 had very significant losses in all defoliation treatments including the largest ones (1248 kg) at the weakest defoliation intensity (T2). G3 under the same ecological conditions (E4) and the same density (D1) had no losses in any treatment, and in T2 there was a significant increase in the yield by 1491 kg/ha.

Interestingly, T4 and T5, very significantly reduced the yield in relation to T1, but did not express significance in the yield reduction in 14 and 13 out of 24 interactions, respectively.

Numerous patterns of these most complex interactions represent a chance for seed producers to reduce yield losses due to defoliation. At that, one should be very careful, because the selection of the appropriate combinations of factors for the most intensive safe level of defoliation has to be such as to provide high crop yields. G3E1D2 in T5 and G2E2D2 in T4 are the examples for such interactions for safe defoliation.

3.5. Effects of Defoliation on Morphological and Physiological Traits of Ears and Kernels

The maize yield depends on the morphological properties of kernels and ears, as well as on their stability and the relationship with environmental conditions [43,44]. The kernel row number, kernel number per row and the ear length are some of properties of yield components, the variability of which leads to a decrease or increase in total and kernel weight [45,46]. The results show that when these traits were expressed, different interactions occurred to different extents due to actions of factors (Table S5). The actions of genotypes, ecological factors, treatments and their interactions significantly affected variations in KNR and EL. With regard to the increase of quantitative values of KNR and EL, the variation in all GT combinations under the effects of E1 and E2 was significant. KNR and EL gradually increased from G1 to G3. This linearity repeated up to the T4 treatment. The application of the T5 treatment changed effects of factors, first of all, ecological impacts (E), and therewith the direction of changes of means (Figure 2). The climate changes are most noticeable in temperature oscillations, which lead to the change in the duration of the growing season, which greatly affects the environment, genetic and physiological processes and the limitation of effects of yield components [47].

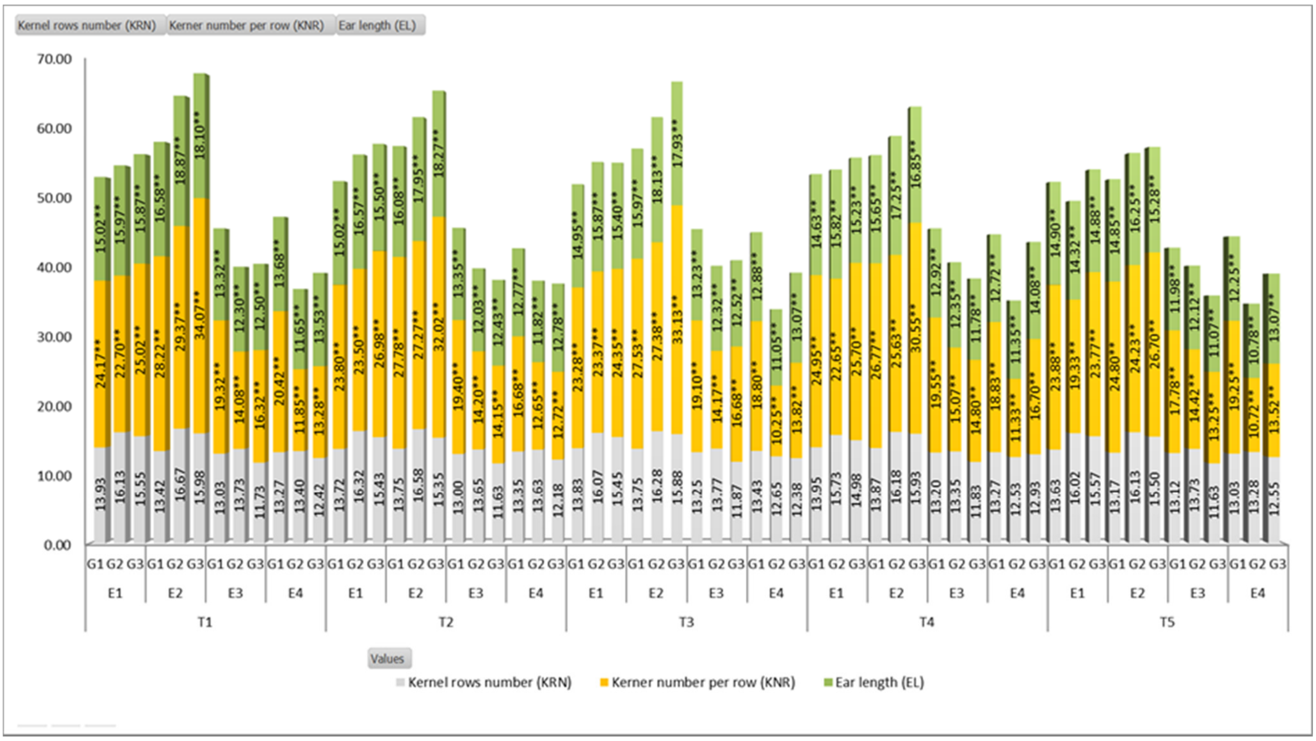


Figure 2. Effects of genotypes (G), Year–location interaction (E) and defoliation (T) on the kernel row number (KRN), number of kernels per row (KNR), the ear length and variability of means by the application of ANOVA. ** Significant at the 0.01 level; * significant at the 0.05 level, without asterisks—not significant.

The 1000-kernel weight under the effects of genotypes, ecological conditions and defoliation treatments did not vary significantly. The largest kernel weight was obtained in the combination of G2E3 factors in the defoliation variants of T1, T3 and T4 (Figure 3). Studies indicate that 1000-kernel weight is more stable across environment and different levels of plant competition than other yield components [48].

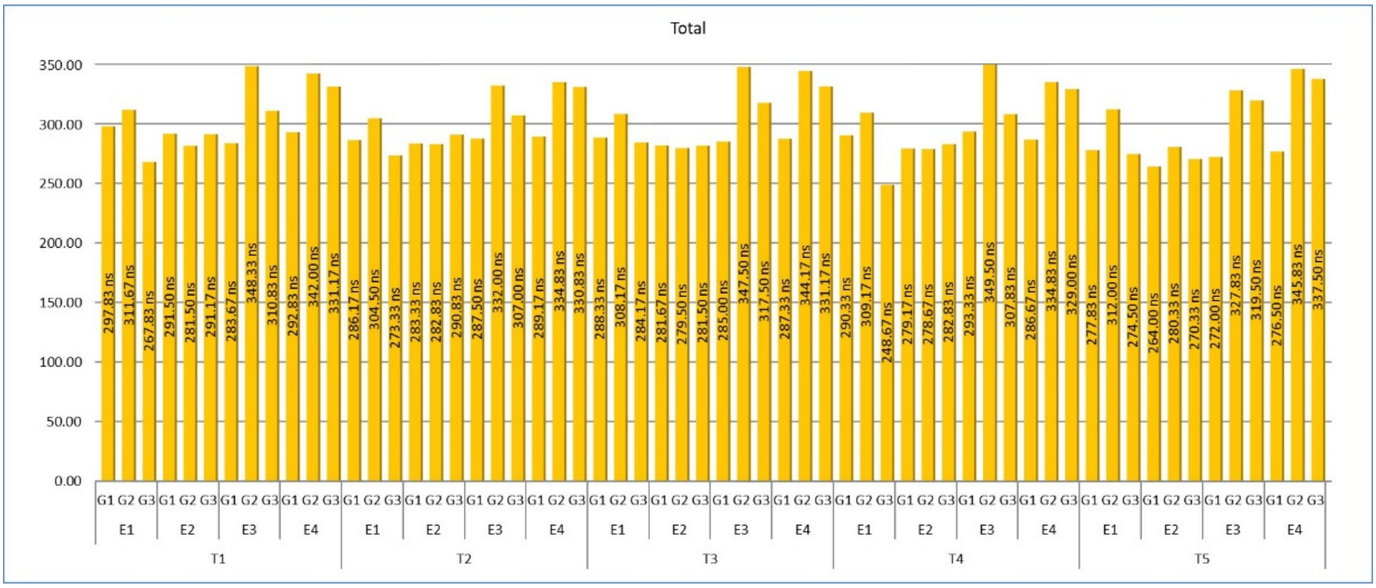
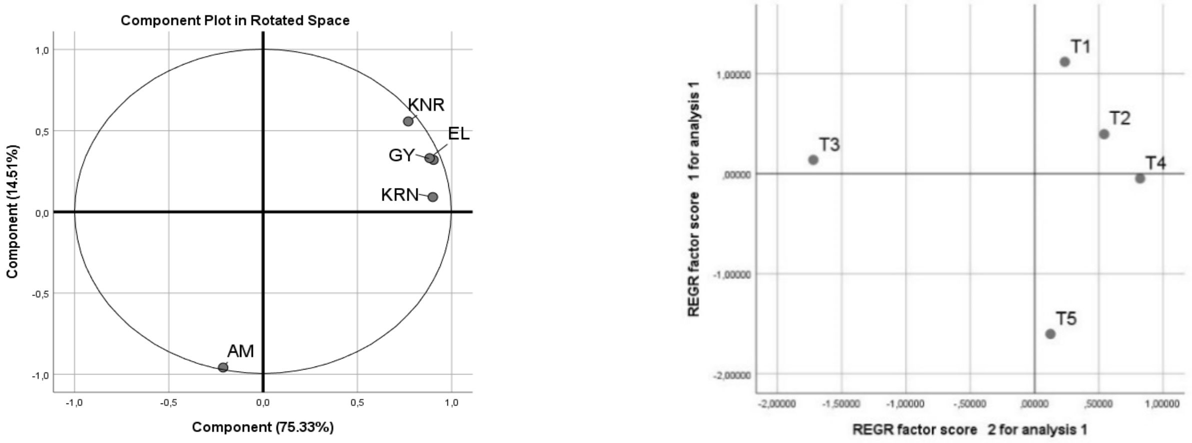


Figure 3. Effects of genotypes (G), Year–location interaction (E) and defoliation (T) on 1000-kernel weight (KW), seed germination (GR) and variations in means by the application of ANOVA, ns—non significant.

Two most important factors affecting the variability of morphological traits and grain yield were singled out by the factorial analysis. The PCA analysis shows that two components represented 88.84% of the variation obtained (Figure 4a). According to the distribution of means, two groups of properties were differentiated: KNR, KRN EL and GY on one hand and KW on the other. As much as 75.33% of variability of morphological traits was influenced by the applied T1, T2, T3 and T4 defoliation treatments. By observing the distribution of regression factor score values (Figure 4b) of the research variant of defoliation, it was determined that most of the coefficients were located on the right side of the diagram as well as the component matrix of morphological properties (Figure 4b). It can be concluded that the first component was a set of cropping practices that determined the formation of morphological properties and yield. The second factor affected KW with 14.51% and high negative regression score values. The significant loss of KW occurred when four leaves (T5) were removed in the process of defoliation.



(**a**) (**b**)

Figure 4. (**a**) Plot of principal component analysis of the variables in the study concerning the effects of the detasseling and kernel properties (KRN, KNR, El, KW) and grain yield (GY). KRN-kernel row number, KNR—kernel number per row, EL—ear length, KW—1000-kernel weight; (**b**) regression factor score of principal comment analysis of the detasseling and kernel properties (KRN, KNR, El, KW).

4. Conclusions

The genotype, the year–location interaction and the crop density expressed characteristic direct effects and a wide spectrum of joint effects on the grain yield loss of maize inbred lines in different defoliation treatments. The genotype productivity was not related to yield losses during defoliation. It is more likely that the level of losses was predominantly affected by the architecture and morphological traits of genotypes, physiological processes, etc. Environmental conditions, especially those related to rainfall, were more unfavorable for the maize production in 2017 than in 2016, which resulted in significantly lower yields obtained in E3 and E4 than in E1 and E2. The rank of genotypes by yields (G2—5549.4 kg/ha, G1—5186.3 kg/ha and G3—4791.3 kg) deviated, to some extent, from the rank of genotypes by yield stability, based on values of their coefficients of variation (G1—33.07%, G2—47.80% and G3—71.48%). The increase of the defoliation intensity was less reflected upon the reduction of grain yields in absolute values (kg/ha) under unfavorable than under more favorable environmental conditions, which is logical, considering that grain yields under these conditions were lower. The higher the crop density was, the lower the yield losses during defoliation were, whereby it is important that higher seed yields can be obtained at higher densities. The joint effects of observed factors pointed out to numerous possibilities to reduce yield losses during defoliation. From the practical point of view, only those joint effects of the genotype, the year–location interaction and the crop densities that simultaneously provide high crop yields are important for the reduction of losses due to defoliation. Based on obtained results, the highest yields at very intensive but safe levels of defoliation (T5 and T4) were achieved under favorable conditions in terms of sufficient water supply of plants (2016) and in higher sowing density (65,000 pants ha^{-1}) for the genotype G3 on degraded chernozem (location of Zemun Polje), and for the genotype G2 on calcareous chernozem (location of Parage). The loss of the leaf area to a certain critical point favored the formation of the kernel row number, number of kernels per row, as well as 1000-kernel weight, after which all the traits were decreased. In the physiological process of germ maturation, the leaf area did not significantly affect quality up to the point of overcoming the "safe" removal of leaves, which was more than four leaves in these trails.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11060509/s1, Table S1: Effect of genotype on differences grain yield between treatments of defoliation, Table S2: Effect of year on differences grain yield between treatments of defoliation, Table S3: Effect of location on differences grain yield between treatments of defoliation, Table S4: Effect of density on differences grain yield between treatments of defoliation, Table S5: Effect of treatments of defoliation on differences physical propertis of maize hybrid seed.

Author Contributions: Conceptualization, S.P., J.B. and G.T.; methodology, J.B.; software, M.T.; validation, D.R., N.D. and G.T.; formal analysis, S.P., M.T.; investigation, D.R.; resources, G.T., N.D.; data curation, D.R., G.T.; writing—original draft preparation, M.T.; writing—review and editing, S.P., J.B.; visualization, M.T., D.R.; supervision, S.P., J.B., N.D.; project administration, G.T., N.D.; funding acquisition, D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Education, Science and Technological Development, Republic of Serbia, Grant no. 451-03-68/2020-14/200040 (Maize Research Institute, Zemun Polje, Belgrade-Zemun).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Acknowledgments: The authors thank the Ministry of Education, Science and Technological Development, Republic of Serbia, for the financial support.

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

References

1. Costa, L.; Carvalho, I.; Ferreira, L.; Szareski, V.; Pimentel, J.; Troyjack, C.; Barbosa, M.; Da Silva, J.; Conte, G.; Villela, F. The effects of different mechanical detasseling methods on hybrid maize seed production. *Genet. Mol. Res.* **2019**, *18*, 18207. [CrossRef]
2. De Pelegrin, J.; Szareski, J.; Demari, H. Yield components of hybrid based on the plant population and artificial defoliation. *Aust. J. Basic Appl. Sci.* **2016**, *10*, 136–142.
3. Baretta, D.; Nardino, M.; Carvalho, I.; de Pelegrin, A.; Ferrari, M.; Szareski, V.; Barros, W.; de Souza, V.; de Oliveira, A.; da Maia, L. Estimates of genetic parameters and genotypic values prediction in maize landrace populations by REML/BLUP procedure. *Gen. Mol. Res.* **2017**, *16*, 16029715. [CrossRef] [PubMed]
4. Nardino, M.; Baretta, D.; Carvalho, I.R.; Olivoto, T.; Follmann, D.N.; Pelegrin, A.; Szareski, V.J.; Lautenchleger, F.; Rosa, T.; Barbosa, M.H. Environment Stratification in the Evaluation of Corn Hybrids in Southern Brazil. *J. Agric. Sci.* **2018**, *10*, 333–342. [CrossRef]
5. Ghețe, A.B.; Haș, V.; Vidican, R.; Copândean, A.; Ranta, O.; Moldovan, C.M.; Crișan, I.; Duda, M.M. Influence of detasseling methods on seed yield of some parent inbred lines of turda maize hybrids. *Agronomy* **2020**, *10*, 729. [CrossRef]
6. Heidari, H. Effect of defoliation and $\frac{1}{2}$ ear removal treatments on maize seed yield and seed germination. *Biharean Biol.* **2015**, *11*, 102–105.
7. de Toledo Alvim, K.R.; de Brito, C.H.; Brandão, A.M.; Gomes, L.S.; Lopes, M.T.G. Quantificação da área foliar e efeito da desfolha em componentes de produção de milho. *Cienc. Rural* **2010**, *40*, 1017–1022. [CrossRef]
8. Sarca, T.R. *Chapter, X. Maize*; Romanian Academy: Bucharest, Romania, 2004; pp. 345–450.
9. Raza, M.A.; Feng, L.Y.; van der Werf, W.; Iqbal, N.; Khan, I.; Hassan, M.J.; Ansar, M.; Chen, Y.K.; Xi, Z.J.; Shi, J.Y. Optimum leaf defoliation: A new agronomic approach for increasing nutrient uptake and land equivalent ratio of maize soybean relay intercropping system. *Field Crops Res.* **2019**, *244*, 107647. [CrossRef]
10. Modarres, A.; Hamilton, R.; Dwyer, L.; Stewart, D.; Dijak, M.; Smith, D. Leafy reduced-stature maize for short-season environments: Yield and yield components of inbred lines. *Euphytica* **1997**, *97*, 129–138. [CrossRef]
11. Magalhães, P.C.; Durães, F.O.M.; Oliveira, A.C.d.; Gama, E.E.G. Efeitos de diferentes técnicas de despendoamento na produção de milho. *Sci. Agric.* **1999**, *56*, 77–82. [CrossRef]
12. Komatuda, A.S.; Dos Santos, C.M.; De Santana, D.G.; De Souza, M.A.; De Brito, C.H. Influência de métodos de despendoamento na produtividade e na qualidade das sementes de milho. *Rev. Bras. Milho Sorgo* **2006**, *5*, 359–368. [CrossRef]
13. Moreira, J.N.; Silva, P.S.L.; Silva, K.; Dombroski, J.L.; Castro, R.S. Effect of detasseling on baby corn, green ear and grain yield of two maize hybrids. *Hortic. Bras.* **2010**, *28*, 406–411. [CrossRef]
14. Carvalho, I.; Nardino, M.; Pelegrin, A.; Ferrari, M.; Demari, G.; Szareski, V.; Souza, V. Path analysis and Annicchiarico method applied in relation to protein in corn grains. *Aust. J. Basic Appl. Sci.* **2016**, *10*, 300–306.
15. Safari, A.R.; Roshan, N.M.; Barimavandi, A.R.; Amiri, I. Effect of defoliation and late-season stress on yield, yield components and dry matter partitioning of grain corn in Kermanshah region, Iran. *Adv. Environ. Biol.* **2013**, *7*, 47–55.
16. Vasilas, B.; Seif, R. Pre-Anthesis Defoliation Effects on Six Corn Inbreds 1. *Agron. J.* **1985**, *77*, 831–835. [CrossRef]
17. Frascaroli, E.; Casarini, E.; Conti, S. Response of maize inbred lines to a defoliation treatment inducing tolerance to cold at germination. *Euphytica* **2005**, *145*, 295–303. [CrossRef]
18. Cumagun, C.J.R.; Manalo, J.O.; Salcedo-Bacalangco, N.A.; Ilag, L.L. Cellulose decomposing ability of Trichoderma in relation to their saprophytic survival. *Arch. Phytopathol. Plant Protect.* **2009**, *42*, 698–704. [CrossRef]
19. Sheridan, J.C. *SPSS: Analysis without Anguish: Version 12.0 for Windows*; John Wiley & Sons: Hoboken, NJ, USA, 2005.
20. Fox, T.; DeBruin, J.; Haug Collet, K.; Trimnell, M.; Clapp, J.; Leonard, A.; Li, B.; Scolaro, E.; Collinson, S.; Glassman, K. A single point mutation in Ms44 results in dominant male sterility and improves nitrogen use efficiency in maize. *Plant Biotechnol. J.* **2017**, *15*, 942–952. [CrossRef] [PubMed]
21. García-Martínez, H.; Flores-Magdaleno, H.; Ascencio-Hernández, R.; Khalil-Gardezi, A.; Tijerina-Chávez, L.; Mancilla-Villa, O.R.; Vázquez-Peña, M.A. Corn Grain Yield Estimation from Vegetation Indices, Canopy Cover, Plant Density, and a Neural Network Using Multispectral and RGB Images Acquired with Unmanned Aerial Vehicles. *Agriculture* **2020**, *10*, 277. [CrossRef]
22. Golba, J.; Studnicki, M.; Gozdowski, D.; Mądry, W.; Rozbicki, J. Influence of genotype, crop management, and environment on winter wheat grain yield determination based on components of yield. *Crop Sci.* **2018**, *58*, 660–669. [CrossRef]
23. Mądry, W.; Rozbicki, J.; Gozdowski, G.; Golba, J.; Piechociński, M.; Studnicki, M.; Wery, J.; Shili-Touzi, I.; Perrin, A. Typology of grain yield formation patterns by yield components in winter wheat cultivars grown across Polish environments. In Proceedings of the AGRO 2010, the XI ESA Congress, Montpeilier, France, 29 August–3 September 2010; European Society for Agronomy (ESA): Conthey, Switzerland, 2010; pp. 601–602.
24. Slafer, G.A.; Savin, R.; Sadras, V.O. Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crop. Res.* **2014**, *157*, 71–83. [CrossRef]
25. Irfanullah, H.A.; Hussain, A.A.I.; Khan, M.W.; Ahmadzai, M.D. Yield and yield attributes of maize (Zea mays L.) as affected by detasseling and potassium fertilization. *Pure Appl. Biol. (PAB)* **2017**, *6*, 958–964.
26. Gao, Z.; Sun, L.; Ren, J.-H.; Liang, X.-G.; Shen, S.; Lin, S.; Zhao, X.; Chen, X.-M.; Wu, G.; Zhou, S.-L. Detasseling increases kernel number in maize under shade stress. *Agric. For. Meteorol.* **2020**, *280*, 107811. [CrossRef]
27. Uribelarrea, M.; Cárcova, J.; Borrás, L.; Otegui, M.E. Enhanced kernel set promoted by synchronous pollination determines a tradeoff between kernel number and kernel weight in temperate maize hybrids. *Field Crop. Res.* **2008**, *105*, 172–181. [CrossRef]

28. Zhou, B.; Yue, Y.; Sun, X.; Ding, Z.; Ma, W.; Zhao, M. Maize kernel weight responses to sowing date-associated variation in weather conditions. *Crop J.* **2017**, *5*, 43–51. [CrossRef]
29. Mi, N.; Cai, F.; Zhang, Y.; Ji, R.; Zhang, S.; Wang, Y. Differential responses of maize yield to drought at vegetative and reproductive stages. *Plant Soil Environ.* **2018**, *64*, 260–267.
30. Mafouasson, H.N.A.; Gracen, V.; Yeboah, M.A.; Ntsomboh-Ntsefong, G.; Tandzi, L.N.; Mutengwa, C.S. Genotype-by-environment interaction and yield stability of maize single cross hybrids developed from tropical inbred lines. *Agronomy* **2018**, *8*, 62. [CrossRef]
31. Khaki, S.; Wang, L.; Archontoulis, S.V. A cnn-rnn framework for crop yield prediction. *Front. Plant Sci.* **2020**, *10*, 1750. [CrossRef] [PubMed]
32. Reeves, G.W.; Cox, W.J. Inconsistent responses of corn to seeding rates in field-scale studies. *Agron. J.* **2013**, *105*, 693–704. [CrossRef]
33. Edwards, J.W. Genotype× environment interaction for plant density response in maize (*Zea mays* L.). *Crop Sci.* **2016**, *56*, 1493–1505. [CrossRef]
34. Xu, W.; Liu, C.; Wang, K.; Xie, R.; Ming, B.; Wang, Y.; Zhang, G.; Liu, G.; Zhao, R.; Fan, P. Adjusting maize plant density to different climatic conditions across a large longitudinal distance in China. *Field Crop. Res.* **2017**, *212*, 126–134. [CrossRef]
35. Hmielowski, T. Maize yield potential. *CSA News* **2018**, *63*, 8–9. [CrossRef]
36. Falihzade, F.; Mojadam, M.; Lack, S. The effect of source-sink restriction and plant density changes on the role of assimilate remobilization in corn grain yield. *Int. J. Agric. Res.* **2013**, *5*, 2459–2465.
37. Li, R.; Liu, P.; Dong, S.; Zhang, J.; Zhao, B. Increased maize plant population induced leaf senescence, suppressed root growth, nitrogen uptake, and grain yield. *Agron. J.* **2019**, *111*, 1581–1591. [CrossRef]
38. Cao, Y.J.; Wang, L.C.; Gu, W.R.; Wang, Y.J.; Zhang, J.H. Increasing photosynthetic performance and post-silking N uptake by moderate decreasing leaf source of maize under high planting density. *J. Integr. Agric.* **2021**, *20*, 494–510. [CrossRef]
39. Da Silva, S.; Pereira, L.; Sbrissia, A.; Hernandez-Garay, A. Carbon and nitrogen reserves in marandu palisade grass subjected to intensities of continuous stocking management. *J. Agric. Sci.* **2015**, *153*, 1449. [CrossRef]
40. Bonser, S.P.; Aarssen, L.W. Interpreting reproductive allometry: Individual strategies of allocation explain size-dependent reproduction in plant populations. *Perspect. Plant Ecol. Evol. Syst.* **2009**, *11*, 31–40. [CrossRef]
41. Ona, A.D.; Muntean, L.; Haș, V.V.; Varga, A. Combining ability for yield of single-cross hybrids derived from maize composites (*Zea mays* L.). *Not. Bot. Horti Agrobot. Cluj Napoca* **2019**, *47*, 465–469. [CrossRef]
42. Tokatlidis, I.S. Adapting Maize Crop to Diverse Agro-Ecosystems. In *Sustainable Agriculture Reviews*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 97–117.
43. Novacek, M.; Mason, S.; Galusha, T.; Yaseen, M. Twin rows minimally impact irrigated maize yield, morphology, and lodging. *Agron. J.* **2013**, *105*, 268–276. [CrossRef]
44. Testa, G.; Reyneri, A.; Blandino, M. Maize grain yield enhancement through high plant density cultivation with different inter-row and intra-row spacings. *Eur. J. Agron.* **2016**, *72*, 28–37. [CrossRef]
45. Assefa, Y.; Vara Prasad, P.; Carter, P.; Hinds, M.; Bhalla, G.; Schon, R.; Jeschke, M.; Paszkiewicz, S.; Ciampitti, I.A. Yield responses to planting density for US modern corn hybrids: A synthesis-analysis. *Crop Sci.* **2016**, *56*, 2802–2817. [CrossRef]
46. Milander, J.J.; Jukic, Z.; Mason, S.C.; Glausha, T.; Kmail, Z. Plant population influence on maize yield components in Croatia and Nebraska. *Crop Sci.* **2016**, *56*, 2742–2750. [CrossRef]
47. Sadras, V.O.; Slafer, G.A. Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. *Field Crop. Res.* **2012**, *127*, 215–224. [CrossRef]
48. Eichenberger, S.; Miguez, F.; Edwards, J.; Knapp, A. Changes in kernel filling with selection for grain yield in a maize population. *Crop Sci.* **2015**, *55*, 521–526. [CrossRef]