







## Article

# Screening of Popcorn Genotypes for Drought Tolerance Using Canonical Correlations

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**Abstract:** Getting around the damage caused by drought is a worldwide challenge, particularly in Brazil, given that economy is based on agricultural activities, including popcorn growing. The purpose of this study was to evaluate popcorn inbred lines under water stressed (WS) and well-watered (WW) conditions regarding agronomic attributes, root morphology, and leaf “greenness” index (SPAD index), besides investigating the viability of indirect selection by canonical correlations (CC) of grain yield (GY) and popping expansion (PE). Seven agronomic, six morphological root traits were evaluated and SPAD index at five different dates during grain filling. The WS (−29% less water than WW) affected significantly the GY (−55%), PE (−28%), increased the brace and crown root density, and more vertically oriented the brace and crown angles. Higher SPAD index is associated with a higher yield, and these measures were the only ones with no significant genotype × water condition interaction, which may render concomitant selection for WS and WW easier. For associating the corrections of the different traits, CC proved to have better potential than simple correlations. Thus, the evaluation of SPAD index at 29 days after the anthesis showed the best CC, and based on the previous results of SPAD index, may be used regardless of the water condition.

**Keywords:** leaf greenness index; root morphology; water stress

## 1. Introduction

In Brazil, popcorn is a much appreciated and consumed food, especially associated with leisure time and movie theaters [1–5]. In view of the increased consumption of this type of grain, it will be necessary to expand the growing area of this crop and enhance genetic values to the cultivars to meet the rising demand for the product [2,3,6]. There is still a wide market to be developed for the growing of popcorn in Brazil, as the area planted has increased by 223% in the last five years [7].

Getting around the drought losses, which will be ever more frequent [8] due to climate change resulting from man-made actions [9], is a challenge for world agribusiness. For Brazil, whose economy

is strongly based on large-scale agricultural activities, and annual advances towards new areas with unpredictable rain regimes, the negative effects are supposed to be worse. Drought is the abiotic factor that most limits the agricultural productivity of maize [10–14]. In Brazil, agricultural production is concentrated in the second growing season (February to July), after the soybean harvest [15]. This period also happens during the dry seasons and may contain strong variations in rainfall [8]. Consequently, there is urgency in selecting high productive genotypes under drought conditions, as well as identifying efficient traits for genotypic discrimination in environments with water stress.

Induction of leaf senescence is a mechanism of response of plants to water stress conditions [12,14]. Under soil water stress conditions, it is often seen that maize genotypes with delayed leaf senescence—stay green—are the highest productive ones, and that is why breeders select them as germplasm adapted to the soil water stress condition [16–20]. As stated by some authors, stay-green cultivars are the best option for drought-prone environments [21,22]. This characteristic can be easily measured by means of a spectrometric method, using SPAD index, an important tool for diagnosing plant stress [13,23–25]. Choosing the proper phenological stage for “greenness” index measurements is a major bottleneck, which is why the effective application of phenotyping methodologies has to do, in part, with the ability to apply them in critical stages associated with grain production [19].

When there are environmental constraints intrinsic to the soil—either water or nutritional—a good conformation of the root system architecture is essential for greater agricultural yield [26]. A root system adapted to the specific conditions of abiotic soil stress represents an agronomic advantage [27]. Maize genotypes with a deep and branched axial root system can access water in deeper layers, where water availability is greater [28]. According to Gao and Lynch (2016) [29], having fewer crown roots improves the water uptake capacity in maize plants under water stress.

As for the popcorn breeding for drought adaptation, in order to identify promising variables for indirect selection of grain yield and popping expansion, Kamphorst et al. (2019) [30] adopted the path analysis, and using the supertrait expanded popcorn volume per hectare as a dependent variable, they sought to get around the inconvenience of considering only one dependent variable for path analysis [31]. On the other hand, as it is a method of linear correlation between two multidimensional variables, the analysis of canonical correlations (CCA) turns out to be a more adequate statistical tool to uncover the relationships of the independent variables, when the focus is to quantify the association to more than one dependent variable. Moreover, the CCA takes into account the maximum correlation between two variable complexes [32], being a more viable option in comparison with the simple linear correlation, besides its implementation avoiding the obtaining of Type I error results [33].

The present study aimed at investigating the impact of water stress on agronomic traits, leaf greenness index, and root architectural traits of 20 popcorn inbred lines in two water conditions, and investigating the viability of using canonical correlations for the selection of easy-to-measure variables that are efficient predictors of grain yield and popping expansion.

## 2. Materials and Methods

### 2.1. Plant Material

It was evaluated a total of 20 popcorn lines (S<sub>7</sub>), whose genealogy is derived from germplasm with tropical (L61, L63, L65, L69, L70, and L71—from the “BRS-Angela” population) and temperate/tropical climate adaptation (P1, P5, and P7—from the hybrid “Zélia”; P2 and P3—from the compound “CMS-42”; P4—from South American breeds; P6, P8, and P9—from the hybrid “IAC-112”; L54, L55, and L59—from the population “Beija Flor”; and L75 and L76—from the population “Barão de Viçosa”) [34].

### 2.2. Experimental Design, Cultural Traits, and Water Conditions Applied

The experiments were conducted at the Experimental Station of the Colégio Estadual Agrícola Antônio Sarlo, in Campos dos Goytacazes city, Rio de Janeiro State, Brazil (Latitude 21°42′48″ S, Longitude 41°20′38″ O, 14 m altitude). The soil of the Experimental Station has been classified as

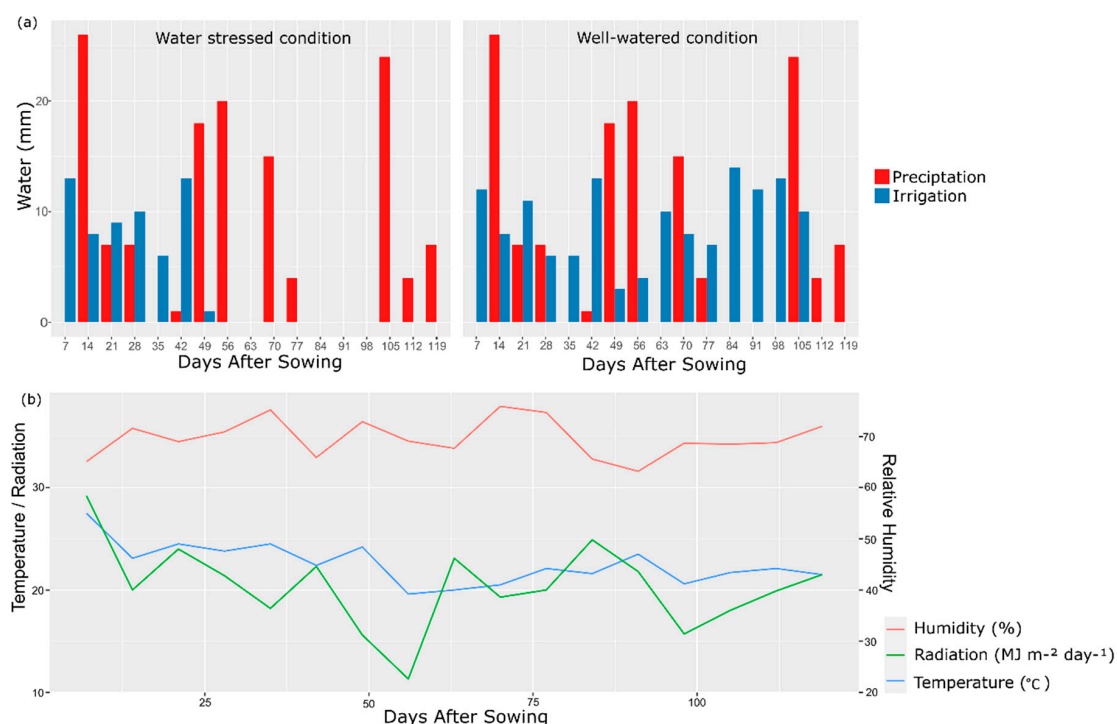
dystrophic Ultisol, presenting high levels of clay and silt. Trials were performed in the 2016 crop year, during the dry seasons, which include the period from April to August. Sowing was done on 10 April, and harvesting was carried out on 15 August.

The basal dressing application of fertilizers was made using 30 kg ha<sup>-1</sup> of N (in the form of urea), 60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (triple superphosphate), and 60 kg ha<sup>-1</sup> of K<sub>2</sub>O (potassium chloride). Top-dressing fertilization occurred 30 days after sowing (DAS), and 100 kg ha<sup>-1</sup> of N (in the form of urea) were applied.

Experimental plots comprised four 4.40 m long rows spaced 0.20 m apart between plants and 0.80 m between rows, totaling a density of 62,500 plants per hectare. The useful area of the plot was defined by 6.72 m<sup>2</sup> of the central rows. An experimental randomized complete block design with three replicates in each water condition; that is, well-watered (WW) and water stressed (WS) was conducted. The analyses of variance were performed considering genotypes effect as fixed and block affect as random.

Irrigation was used for both water regimes by using a drip system. One Katif dripper per plant was installed with a flow rate of 2.3 mm h<sup>-1</sup>. Water condition 1—well-watered (WW) was irrigated to maintain field capacity (−10 kPa), which was monitored using Decagon MPS-6 (Decagon, USA) tensiometers applied to the soil between plants at a depth of 0.20 m. Water condition 2—water stress (WS) was characterized by the suspension of irrigation at a phenological stage of male pre-anthesis. The soil reached the permanent wilting point (−1500 kPa) 12 days after the male anthesis (data not presented).

In WS condition, irrigation was interrupted 49 days after sowing (DAS), remaining this way until harvest (119 DAS). At 56, 70, 77, 105, 112, and 119 DAS, rainfall was recorded, totaling 92 mm during this period. Plants of WW condition were given an additional 78 mm of water via drip during the same period, totaling 170 mm (Figure 1a).



**Figure 1.** (a) Precipitation occurs during a trial conducted with 20 popcorn lines under well-watered condition and water-stressed condition. (b) Humidity, solar radiation, and temperature values measured in water stressed and well-watered conditions.

Throughout the crop growth and development cycle, temperature and relative humidity ranged from 12 °C to 37 °C and 23% to 97%, respectively, with a mean solar radiation of 20.35 MJ m<sup>-2</sup> day<sup>-1</sup> (maximum radiation at the experiment location was 1300 µmol m<sup>-2</sup> s<sup>-1</sup> (Figure 1b). Weather conditions were recorded in a weather station of the National Institute of Meteorology (INMET, in Portuguese), installed less than 100 m away from the experimental area.

### 2.3. Traits Evaluated

Traits were divided into three groups, or trait complex. The reference group, i.e., the so-called agronomic traits (AGRO), is the one related to grain yield and popping expansion. The group of root morphological traits (ROOT) includes the ones measured in the root system, specifically in the brace and crown roots. The third group is related to the intensity of leaf greenness (SPAD index) measured on different dates during the grain filling period.

The agronomic traits (AGRO) evaluated were grain yield (GY), popping expansion (PE), prolificacy (PR), 100-grain mass (HG), ear diameter (ED), ear length (EL), and dry matter weight (DM).

Grain yield (GY), obtained by harvesting all the plants in the useful area of the plot, was corrected to 13% humidity, and expressed in kg ha<sup>-1</sup>. Popping expansion (PE) was measured for a mass of 30 g of grains, heated by microwaves, in a special paper bag for popping, at a 1000-watt power, for a time of two minutes and fifteen seconds, and the volume of popcorn was measured in a 2000 mL graduated cylinder. Popping expansion (PE) was determined by the quotient of the volume obtained from popcorn and grain mass expressed in mL g<sup>-1</sup>. Prolificacy (PR) was established by counting the ears and expressed in mean values by the quotient of the number of ears harvested and the total number of plants in the plot. The 100-grain mass (HG) was obtained by weighing (g) three subsamples of 100 seeds each. A random sample of six plants from the plot was used to estimate the ear diameter (ED) and the ear length (EL), quantified by a caliper (cm), and to determine the aerial dry matter (DM) by weighing (g) after drying in a hot air oven at 70 °C for 72 h.

The root system traits evaluated were brace root number (BN), brace root angle (BA), brace root density (BD), crown root number (CN), crown root angle (CA), and crown root density (CD).

The root architectural traits were quantified following the methodology suggested by Traschel et al. (2011) [27], with modifications. After harvesting, the soil of the different water conditions (WW and WS) received 50-mm irrigation, to make it easier to remove the plants mechanically with the use of shovels. The root system of two plants per plot was removed in a 40-cm diameter and 25-cm deep soil cylinder. The roots in the soil cylinders were washed until the soil was completely removed. From the root categories—brace (B) and crown (C), the traits measured were root angle (A) (BA and CA), obtained using a degree protractor and expressed in relation to the soil (°); root number (N) (BN and CN), obtained by counting the structures; and mean density (D) of lateral roots (BD and CD), obtained using a diagrammatic scale proposed by Traschel et al. (2011) [27]. Root density values vary from 1 to 9, in which higher values indicate higher density.

The trait associated with the intensity of leaf greenness (SPAD index) was estimated by three readings in the middle third of the third leaf counted from the apex and below the flag leaf by means of the portable chlorophyll meter, model SPAD-502 “Soil Plant Analyzer Development” (Minolta, Japan). The SPAD index were measured on five different days after male anthesis (DAA), as follows: 17 DAA (S<sub>1</sub>), 22 DAA (S<sub>2</sub>), 29 DAA (S<sub>3</sub>), 36 DAA (S<sub>4</sub>), and 42 DAA (S<sub>5</sub>). Measurements were taken when the soil water potential was below the permanent wilting point (>−1500 kPa) (data not provided). SPAD index was measured in a random sample of six useful plants of the experimental plot.

### 2.4. Analysis of Variance and Statistical—Genetic Parameters

The analysis of variance was individually performed for each water condition (WS and WW) taking into account the linear model:  $y_{ij} = \mu + g_i + b_j + e_{ij}$ , in which the mean ( $\mu$ ) and genotype effect ( $g_i$ ) were considered to be fixed; and block ( $b_j$ ) and error ( $e_{ij}$ ), random.

The statistical-genetic parameters coefficient of variation ( $CV$ ) and heritability ( $H^2$ ) were estimated from the individual analyses by the estimators:  $CV = \sqrt{MSe}/\mu \times 100$ , in which  $MSe$  is the mean square of error; and  $H^2 = MSg - MSe/MSg$ , in which  $MSg$  is the mean square of the genotype effect.

To verify the occurrence of genotype interaction with water conditions, a joint analysis was carried out by considering the following linear model:  $y_{ijk} = \mu + g_i + w_j + gw_{ij} + b/w_{jk} + e_{ijk}$ , in which the mean ( $\mu$ ), the genotype effect ( $g_i$ ), and the water condition effect ( $w_j$ ) were considered to be fixed, and the block within condition effect ( $b/w_{jk}$ ) and the error ( $e_{ijk}$ ), as random.

## 2.5. Correlation Analysis

For each water condition, a simple linear correlation analysis was applied using the Pearson method, involving all variables between themselves using the following model:  $r = \sigma_{xy}^2 / \sigma_x^2 \times \sigma_y^2$ , in which  $r$  is the phenotypic correlation coefficient between variables  $x$  and  $y$ ,  $\sigma_{xy}^2$  is the phenotypic covariance between variables  $x$  and  $y$ ,  $\sigma_x^2$  is the phenotypic variance of  $x$ , and  $\sigma_y^2$  is the phenotypic variance of  $y$ .

Existing correlations between trait complex were estimated by means of the canonical correlation analysis, taking into account the following groups: AGRO  $\times$  ROOT traits and AGRO  $\times$  SPAD traits.

The canonical correlations were estimated by combining the covariances within and between the traits of the variable complexes of the two groups ( $S_{11}$ ,  $S_{22}$ , and  $S_{12}$ ) and the weight of the traits of each group:  $a' = [a_1 \ a_2 \ \dots \ a_p]$  is the vector  $1 \times p$  of weights of the traits of group I; and  $b' = [b_1 \ b_2 \ \dots \ b_q]$  is the vector  $1 \times q$  of weights of the traits of group II.

The first canonical correlation is defined by that which maximizes the relationship between  $X_1$  and  $Y_1$  by means of the expression:

$$r_1 = C\hat{o}v(X_1, Y_1) / \sqrt{\hat{V}(X_1) \cdot \hat{V}(Y_1)}, \text{ in which } C\hat{o}v(X_1, Y_1) = a'S_{12}b; \hat{V} = a'S_{11}a; \hat{V} = b'S_{22}b.$$

The simple linear and canonical correlations were separately estimated for the means obtained in water condition (WS and WW), so they could be compared. The multicollinearity diagnostic among the traits in each pair of variable complexes was carried out based on the ratio between the highest and lowest eigenvalue of the inverse matrix and interpreted by the classification of Montgomery and Peck (1981) [35]. All statistical analyses were conducted in the GENES software [36].

## 3. Results

### 3.1. Genetic Variability and Effects of Different Water Conditions on SPAD Index and Agronomic (AGRO) and Root (ROOT) Traits

There is genetic variability among genotypes evaluated in both water conditions (WS and WW) for all agronomic traits, for the different times of greenness index measurements (SPAD index) and for traits related to the root system, except BN in WW condition. The experimental coefficient of variation (CV) was below 30.0% for the traits under evaluation. Generally, it was noted that the CV values were lower for the agronomic and root system traits under WS condition and for the different moments of SPAD index under WW condition (Table 1).

**Table 1.** Summary of analysis of variance, means, coefficients of variation (CV), and coefficient of genotypic determination of agronomic traits ( $H^2$ ), different dates of measurement of greenness (SPAD index), and traits related to the root system evaluated in popcorn lines under water-stressed (WS) and well-watered (WW) conditions.

Traits	Water Condition (WC)												Mean Squares (MS) of Joint Analysis	
	Water Stressed (MS)						Well Watered (MS)						WC	Genotype $\times$ WC
	Block	Genotype	Error	Mean	CV%	$H^2$	Block	Genotype	Error	Mean	CV%	$H^2$		
	(DF = 2)	(DF = 19)	(DF = 38)				(DF = 2)	(DF = 19)	(DF = 38)				(DF = 1)	(DF = 19)
Agronomic traits (AGRO)														
GY	137418	55,9525 **	66,624	1139.11	22.65	88.09	60,1287	1,839,610 **	336,794	2548.07	22.77	81.69	$5.9 \times 10^7$ **	$70.2 \times 10^5$ *
PE	2.75	36.4 **	3.35	20.91	8.75	90.79	2.52	52.27 **	16.39	29.35	13.79	68.62	2136.4 **	28.35 **
PR	0.00	0.031 *	0.01	0.86	14.08	53.22	0.10	0.060 **	0.02	1.02	12.32	73.64	0.785 *	0.073 **
HG	0.89	5.533 **	0.53	9.69	7.49	90.44	1.33	5.52 **	1.72	12.67	10.36	68.75	265.9 **	1.71 <sup>ns</sup>
ED	9.04	20.06 **	2.75	27.94	5.94	86.27	10.59	17.70 **	2.39	29.12	5.31	86.46	41.8 **	3.45 **
EL	3.38	6.18 **	1.14	12.62	8.46	81.49	2.97	5.57 **	0.81	12.72	7.10	85.34	0.25 <sup>ns</sup>	0.79 <sup>ns</sup>
DM	521.8	6812.3 **	1807.8	313.94	13.54	73.46	21,931.2	10,358.4 **	2557.9	368.31	13.73	75.31	$8.8 \times 10^4$ *	2614 <sup>ns</sup>
SPAD index														
S1(17DAA)	33.39	31.77 **	9.35	43.54	7.02	70.55	15.79	53.35 **	4.66	48.83	4.42	91.25	838 **	6.40 <sup>ns</sup>
S2(22DAA)	17.23	63.53 **	8.45	40.32	7.21	86.69	9.03	38.32 **	7.10	48.96	5.44	81.47	2239 **	9.81 <sup>ns</sup>
S3(29DAA)	1.52	64.42 **	8.88	39.70	7.50	86.20	24.45	37.26 **	4.59	48.15	4.45	87.67	2145 **	7.68 <sup>ns</sup>
S4(36DAA)	30.62	100.18 **	22.51	32.50	14.59	77.52	34.45	59.94 **	9.69	45.98	6.77	83.82	5452 **	24.11 <sup>ns</sup>
S5(42DAA)	39.71	82.73 **	24.20	18.81	26.15	70.74	49.96	108.62 **	13.30	39.35	9.26	87.75	12663 **	45.84 **
Root traits (ROOT)														
BN	0.53	6.472 **	0.50	9.12	7.75	92.27	2.77	3.94 <sup>ns</sup>	2.79	8.67	19.25	29.27	6.03 <sup>ns</sup>	5.15 **
BA	14.82	71.01 **	8.12	50.23	5.67	88.55	7.89	243.40 **	31.45	37.20	15.07	87.07	5094 **	48.73 **
BD	0.00	4.002 **	0.16	5.98	6.75	95.91	0.14	4.276 **	0.31	5.26	10.66	92.63	15.95 **	1.43 **
CN	2.60	14.82 **	1.61	16.29	7.78	89.13	1.02	7.16 **	1.52	15.31	8.05	78.74	28.8 <sup>ns</sup>	6.01 **
CA	4.33	83.81 **	9.08	53.08	5.67	89.16	9.30	166.32 **	11.10	42.63	7.81	93.32	3276 **	38.84 **
CD	0.07	4.737 **	0.14	5.62	6.64	97.05	0.40	2.836 **	0.20	4.12	10.86	92.91	67.45 **	1.76 **

MS: mean squares; WC: water condition; DF: degrees of freedom; GY: grain yield ( $\text{Kg ha}^{-1}$ ); PE: popping expansion ( $\text{mL g}^{-1}$ ); PR: prolificacy; HG: 100-grain mass (g); ED: ear diameter (mm); EL: ear length (cm); DM: dry matter (g); S1, S2, S3, S4 e S5 = SPAD index at 17, 22, 29, 36 and 42 days after male anthesis, respectively; root traits: BN: brace number; BA: brace angle ( $^\circ$ ); BD: brace density; CN: crown number; CA: crown angle ( $^\circ$ ) and CD: crown density. Symbols <sup>ns</sup>, \*, and \*\* represent not significant, significant at 5.0%, and 1.0% probability, by the F test, respectively.

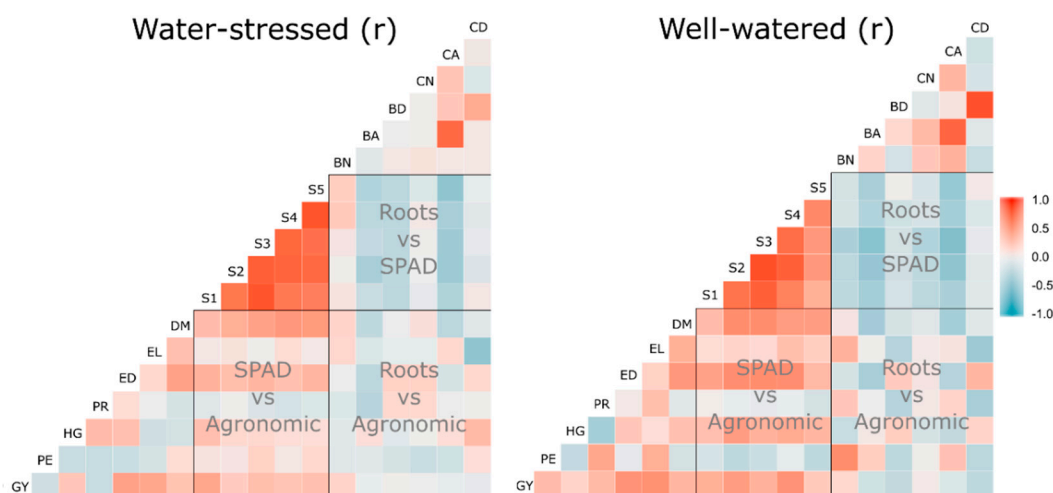
Among the agronomic traits, grain yield (GY), popping expansion (PE), and 100-grain mass (HG) presented higher heritability ( $H^2$ ) under WS condition, while the other ones—namely, prolificacy (PR), ear diameter (ED), ear length (EL), and dry matter weight (DM)—presented higher  $H^2$  under WW condition. Under the WS condition, the means of GY and PE were reduced by 55.3% and 28.7% compared with WW condition, respectively. Only the EL did not present a significant difference in mean between the different water conditions (WC). The means of PR, ED, and DM were significantly lower under WS condition, in the order of 15.7%, 4.1%, and 14.8%, respectively (Table 1). Significant genotype  $\times$  water condition (Gen  $\times$  WC) interactions were observed in GY, PE, PR, and ED (Table 1).

The SPAD index, under WW condition, presented higher values of  $H^2$  in the different measurement dates regarding WS condition. Given the comparison of WS and WW condition, the proportional mean reductions were 10.8% ( $S_1$ ), 17.6% ( $S_2$ ), 17.5% ( $S_3$ ), 29.3% ( $S_4$ ), and 52.2% ( $S_5$ ) for the measurements taken 17, 22, 29, 36, and 42 days after the anthesis (DAA). In the joint analysis, a difference was seen between the means of SPAD index between water conditions, with significance in the Gen  $\times$  WC interaction only in the measurement carried out at 42 days ( $S_5$ ) (Table 1).

With respect to root traits (ROOT), apart from the crown root angle (CA),  $H^2$  were superior under WS condition. The mean of brace root (BN) and crown (CN) number did not differ in both water conditions; all other root traits, however, expressed means with higher values under WS condition. In the joint analysis, a significant Gen  $\times$  WC interaction was noted for all root traits (Table 1).

### 3.2. Phenotypic Correlations within and among AGRO, SPAD Index, and ROOT Trait Groups under WS and WW Conditions

Correlations of greater expression, all in positive direction, among the traits of the AGRO group were seen between GY  $\times$  ED (0.43), GY  $\times$  EL (0.40), and ED  $\times$  DM (0.42), under WS condition, and between GY  $\times$  ED (0.50), GY  $\times$  EL (0.52), PE  $\times$  PR (0.40), ED  $\times$  DM (0.49), and ED  $\times$  DM (0.49), under WW condition (Figure 2).



**Figure 2.** Phenotypic correlation among 18 traits evaluated in 20 popcorn inbred lines under two different water conditions. Agronomic traits—GY: grain yield; PE: popping expansion; PR: prolificacy; HG: 100-grain mass; ED: ear diameter; EL: ear length and DM: dry matter). SPAD index— $S_1$ ,  $S_2$ ,  $S_3$ ,  $S_4$ , and  $S_5$  = SPAD index at 17, 22, 29, 36, and 42 days after male anthesis, respectively; Roots traits—BN: brace number; BA: brace angle; BD: brace density; CN: crown number; CA: crown angle and CD: crown density.

Correlations among the traits of the AGRO and SPAD index groups were more expressive under WW condition. All associations observed between GY  $\times$   $S_4$  (0.51), ED  $\times$   $S_2$  (0.53), ED  $\times$   $S_3$  (0.54), and ED  $\times$   $S_4$  (0.53) were positive. Under WS condition, the most intense association estimate occurred between GY  $\times$   $S_1$  (0.40).

The correlation of greater expression between the traits of the AGRO and ROOT group, under WW condition, in positive direction, occurred between PE  $\times$  BN (0.54) and, under WS condition, in negative direction, between EL  $\times$  CD (−0.46) (Figure 2).

Correlations between the traits of the SPAD index and ROOT group mainly denote negative associations under WW condition. The leaf greenness index, measured 22 DAA ( $S_2$ ), was moderately correlated with CA (−0.46), and the one measured 29 DAA ( $S_3$ ) was more intensely associated with BA (−0.48) and CA (−0.53). Under WS condition, the CA trait was correlated with  $S_2$  (22 DAA),  $S_3$  (29 DAA), and  $S_5$  (42 DAA), with values of −0.40, −0.39, and −0.45, respectively (Figure 2).

The traits of the SPAD group showed high correlations between themselves, irrespective of the water condition. Regarding the ROOT group, it was observed a high correlation between BA  $\times$  CA, under both water conditions, ( $\approx 0.72$ ), while the correlation between BD  $\times$  CD (0.82) was expressive only under WW condition.

### 3.3. Canonical Correlations among AGRO, SPAD Index, and ROOT Trait Groups in WS and WW Conditions

The multicollinearity classification applied to the phenotypic correlation matrix between the two pairs of variable complexes presented a weak multicollinearity for the pair AGRO and ROOT, and moderate for the pair AGRO and SPAD. For that reason, the canonical correlation analysis (CCA) could be carried out with the traits evaluated [37].

There were significant correlations between AGRO and SPAD index groups of traits under both water conditions (WS and WW) (Table 2). The correlation was significant in the first three canonical pairs under WS condition. In the first canonical pair ( $r = 0.92$ ), the variable DM was associated in the opposite direction with the SPAD index in  $S_1$  (17 DAA) and in the same direction with  $S_2$  (22 DAA). The second canonical pair ( $r = 0.74$ ) associated higher GY values and lower EL values with higher  $S_1$  values and lower  $S_3$  values. The third canonical pair ( $r = 0.68$ ) associated PE with all measurement dates of the SPAD index, pointing out that higher values in  $S_1$ ,  $S_3$ , and  $S_5$  and lower  $S_2$  and  $S_4$  were related to the lower PE (Table 2).

**Table 2.** Correlation and coefficients of canonical pairs estimated between SPAD index and agronomic traits in 20 popcorn inbred lines under two different water conditions.

Traits		Canonical Pairs									
		Water-Stressed					Well-Watered				
		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
SPAD index	S1(17DAA)	−1.16	1.74	1.19	−0.13	0.12	1.14	0.82	−0.34	1.43	−0.49
	S2(22DAA)	2.14	−0.07	−0.92	−1.42	2.18	0.01	−1.22	1.41	0.61	−1.63
	S3(29DAA)	−0.33	−1.64	1.05	2.04	−1.19	0.03	0.09	0.79	−1.86	2.41
	S4(36DAA)	0.04	0.45	−1.84	0.50	−0.97	−2.55	−0.34	−0.31	2.46	0.51
	S5(42DAA)	−0.10	0.27	1.20	−0.80	−0.63	1.20	1.48	−0.90	−2.51	−1.00
Agronomic (AGRO)	GY	0.32	1.78	−0.30	−0.76	0.06	−0.14	−0.68	2.10	1.03	1.33
	PE	0.13	−0.45	−0.81	0.25	0.17	0.27	0.02	0.62	−0.12	0.09
	PR	0.33	−0.15	−0.16	0.33	0.94	0.42	1.15	−1.06	0.36	−0.76
	HG	−0.10	0.05	0.39	0.73	0.78	−0.10	−1.16	1.76	0.36	0.07
	ED	−0.30	−0.64	0.04	1.20	−0.70	0.67	0.52	−0.54	−1.13	−0.47
	EL	−0.11	−1.04	−0.34	−0.09	−0.74	0.18	0.47	−1.32	0.04	−1.04
	DM	0.90	−0.23	0.29	−0.04	0.28	−0.94	0.65	−0.08	0.15	−0.40
CC		0.92 **	0.74 **	0.68 **	0.51 <sup>ns</sup>	0.20 <sup>ns</sup>	0.90 **	0.77 **	0.53 <sup>ns</sup>	0.49 <sup>ns</sup>	0.34 <sup>ns</sup>
DF		35	24	15	8	3	35	24	15	8	3
$\chi^2$		194.6	93.7	51.5	18.2	2.1	175.9	88.2	39.2	21.2	6.7

CC: canonical correlations; DF: degrees of freedom. AGRO—GY: grain yield; PE: popping expansion; PR: prolificacy; HG: 100-grain mass; ED: ear diameter; EL: ear length and DM: dry matter. SPAD index— $S_1$ ,  $S_2$ ,  $S_3$ ,  $S_4$ , and  $S_5$  = SPAD readings at 17, 22, 29, 36, and 42 days after male anthesis, respectively. Symbols <sup>ns</sup>, \*\* represent not significant, significant at 1.0% probability.

Under WW condition, and considering the AGRO and SPAD index groups of traits, the first canonical pair ( $r = 0.90$ ) associated the measurements  $S_1$  (17 DAA),  $S_5$  (42 DAA), and  $S_4$  (36 DAA) with the variables ED and DM. The  $S_1$  and  $S_5$  relationships occurred in the same direction of ED and in the opposite direction of DM, while  $S_4$  related itself in the opposite direction of ED and in the same direction of DM. The second canonical pair ( $r = 0.77$ ) indicates the association of measurements  $S_1$ ,  $S_5$ , and  $S_2$  (22 DAA) with the variables HG and PR. The measurements  $S_1$  and  $S_5$  related themselves in the same direction with PR, but in the opposite direction with HG, while  $S_2$  presented an inverse relationship with the traits PR and HG (Table 2).

In the canonical correlation estimated between the groups of traits, AGRO and ROOT, under WS condition, the first three canonical pairs were significant, with values of  $r = 0.89$ ,  $r = 0.84$ , and  $r = 0.65$ , from the first to the third pair, respectively. In this condition, the first canonical pair indicates that the lower the BN, the lower the PE and DM and the higher the ED. The second canonical pair points to lower PR and higher PE concomitant with lower DB and higher CD. The third canonical pair showed inverse correlation between BA and DM (Table 3).

**Table 3.** Correlation and coefficients of canonical pairs estimated between roots morphology and agronomic traits in 20 popcorn inbred lines under two different water conditions.

Traits		Canonical Pairs											
		Water-Stressed						Well-Watered					
		1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th
Roots	BN	−0.71	0.40	−1.59	0.44	0.01	0.20	−0.26	−0.01	0.86	−0.30	−0.01	0.34
	BA	−0.34	0.48	0.93	0.72	0.92	0.58	−0.49	1.42	0.26	0.38	0.39	−0.88
	BD	0.32	−0.94	0.05	1.27	−1.47	0.20	0.32	0.33	0.38	0.95	0.49	0.03
	CN	0.37	−0.05	−0.25	−0.34	−0.21	0.96	0.71	0.06	0.23	−0.42	0.24	−0.61
	CA	0.07	0.28	0.01	−1.10	−0.96	−1.00	−0.21	−1.47	−0.48	−0.61	0.39	0.90
	CD	0.25	1.59	−0.32	−0.80	1.20	−0.29	0.41	0.39	−0.38	−0.76	−0.04	0.52
Agronomic (AGRO)	GY	−0.42	−0.16	0.10	−1.27	1.04	1.11	0.02	1.21	0.47	1.97	0.18	1.17
	PE	−0.89	0.97	−0.50	0.56	0.37	−0.07	−0.12	0.13	−0.73	0.59	−0.68	0.34
	PR	0.18	−0.37	0.59	0.84	0.40	−0.02	−0.64	−0.28	−0.52	−1.60	−0.40	0.10
	HG	0.22	−1.12	0.34	0.05	0.14	−0.90	0.70	0.27	−0.17	1.68	0.10	0.45
	ED	0.94	0.38	−0.43	0.53	−0.88	−0.43	0.61	−0.70	−0.28	−0.80	0.09	0.07
	EL	0.17	−0.16	0.19	0.55	−1.58	0.13	−0.66	−1.48	−0.27	−1.10	−0.36	−0.22
	DM	−0.63	−0.43	−0.84	0.33	0.64	−0.15	0.09	0.11	0.59	−0.58	−0.75	−0.30
CC		0.89 **	0.84 **	0.65 **	0.52 <sup>ns</sup>	0.44 <sup>ns</sup>	0.13 <sup>ns</sup>	0.88 **	0.82 **	0.68 **	0.55 <sup>ns</sup>	0.36 <sup>ns</sup>	0.22 <sup>ns</sup>
DF		42	30	20	12	6	2	42	30	20	12	6	2
$\chi^2$		203.2	121.8	58.0	28.9	12.5	0.9	201.9	121.4	62.2	29.4	10.3	2.6

CC: canonical correlations; DF: degrees of freedom. AGRO—GY: grain yield; PE: popping expansion; PR: prolificacy; HG: 100-grain mass; ED: ear diameter; EL: ear length and DM: dry matter. ROOTS—BN: brace number; BA: brace angle; BD: brace density; CN: crown number; CA: crown angle and CD: crown density. Symbols ns, \*\* represent not significant, significant at 1.0% probability.

Under WW condition, the canonical correlations estimated between the AGRO and ROOT groups showed significance in the first ( $r = 0.8$ ), second ( $r = 0.82$ ), and third ( $r = 0.68$ ) canonical pair. As per the first canonical pair, the higher the CN, the lower the HG and EL, and the higher the ED and PR. The second canonical pair showed that the higher the BA, the higher will be GY and the lower will be ED and EL, while the third canonical pair suggested a decrease in PE in plants with lower BN (Table 3).

#### 4. Discussion

The soil water limitation strongly affected the traits GY (−55.3%), PE (−28.8%), and HG (−23.5%). Scientific studies assign GY reduction, under drought conditions, to a smaller number of grains yielded per area [19]. The water stress in the soil, throughout anthesis phase, critical period for the crop [38,39], affects the viability of the pollen grain, stigma receptivity, and fertilization [40], resulting in a lower number of grains yielded. In this study, the reduction in GY is explained by the decrease in HG. In WS condition, the insufficiency of photoassimilates for grain filling was pointed out as the cause for the smaller size of these structures [18]. In the evaluation of popcorn hybrids under soil water stress, it was noted, on average, 30% reduction in HG [41]. Prolificacy (−15.7%), ear diameter (−4.1%), ear length

(−0.8%), and dry matter (−14.8%) traits experienced the impact of soil water limitation to a lesser extent. Irrigation was interrupted 15 days before the male anthesis, in which the plants are at the end of their plant development, starting the emission of tassels. Hence, traits like PR, ED, EL, and DM, developed before the anthesis, tend to be less impacted, as they are already partially developed [39]. Durães et al., 2004 [39] described that, in the pre-flowering period, the plants have already reached or are close to the end of the plant development. It should be stated that, in maize, PR presents a high genetic correlation with GY, both under adequate water conditions of growing [42] and under stress conditions [43], being used as a selection trait in breeding programs for WS conditions.

SPAD index (leaf greenness), generated by the evaluation of a portable chlorophyll meter (SPAD), turned out to be a trait of high potential for genotypic discrimination in materials evaluated under different water conditions. At 17 DAA, when the first measurement was taken, differences between genotypes and environments were observed. Moreover, SPAD index decreases faster under WS conditions [13,23,24], because the leaf senescence occurs more sharply under WS in comparison with the WW condition. The foliar senescence is coordinated by reproductive plant stadium, but also strongly influenced by the environment [44]. Thus, SPAD index is an essential tool for diagnosing plant stress [13,23–25]. Actually, spectrometric methods are efficient to evaluate the different water conditions and the performance of cereals [45].

SPAD index measurements suggest a photosynthetic capacity of the leaf tissues, since that this index is correlated with chlorophyll concentration, and enable to obtain better inferences of photosynthesis [25]. Kamphorst et al. (2020) [46] evaluated SPAD values popcorn inbred lines contrasted to grain yield in drought conditions. The authors showed that maximum SPAD values were obtained more later (11 days after anthesis) in WW, when compared with WS (two days after anthesis). Maximum SPAD index values are related to late leaf senescence, due to high chlorophyll concentration and photosynthetic carbon assimilation [46]. In addition, Gregersen (2008) [47], showed that nutrients mobilization from senescent leaves to reproductive organs are important to plant growth in drought conditions. Actually, Killi et al. (2017) [48] evaluated maize varieties with contrasting drought tolerance in water-stressed condition and showed no physiological or morphological traits that may be associated with enhanced yield in the drought tolerant maize variety. As such, the respective yield characteristics of the drought and sensitive maize varieties may be the result of differences in the partitioning of photosynthate between reproduction and vegetative structures [48].

Plants increased the brace root and crown density, displaying the phenotypic root plasticity of adaptive response to WS condition. Maize plants with reduced lateral root branching make greater axial root elongation possible, and the greater depth of rooting promotes water acquisition in deeper layers of dry soils [49]. Because of these characteristics, the metabolic root demand of these genotypes is lower, which may contribute to higher growth, with consequent greater grain yield [50]. The drought tolerant maize varieties exhibited higher investment in root-systems, allowing greater uptake of the available soil water [48]. In this way, lower root density is important for drought tolerance and should be considered as a selection goal in maize breeding programs [49,51].

The brace and crown root angles are directed more vertically towards the soil for the acquisition of water. Trachsel et al. (2011) [27] report that, in water-limited environments, genotypes with larger angles, close to 90°, of the roots in relation to the soil present adaptive advantages to find water. A root ideotype with three characteristics, Steep, Cheap, and Deep, may represent an advantage for water acquisition in deeper soil layers [29]. Steep suggests greater adaptive value to drought for plants with root angles close to 90° in relation to the soil [29].

Agronomic and root traits suffered genotype × water condition interaction, which may make concomitant selection for WS and WW conditions difficult. A number of papers describe the presence of G × WC interaction between common maize traits evaluated with and without drought stress [15,52]. Plant breeders hardly ever select root traits because of the high plasticity in response to soil conditions and measurement difficulty, incurring in low heritability values [50].

Higher SPAD index was associated with higher yield values; in addition, these measurements can be reliable for plant selection considering the absence of genotype  $\times$  water condition interaction in the present work. Researches in this area have focused on secondary morphological and physiological traits easy to measure under field conditions, which correlate with grain yield [19,20,23,39,53,54]. The use of secondary traits of simple measurement and high adaptive value can increase the efficiency of the selection under stressful condition [55]. A suitable secondary trait is that of high heritability, low cost, easy-to-measure, stable during the measurement period, and, above all, genetically associated with grain yield under stress conditions [56]. Additionally, in recurrent selection programs with maize, it has been said that the mean yield of tropical germplasm grains has increased after eight cycles, when genotypes with delayed leaf senescence were prioritized under intermediate and severe water stress conditions (rain-free winter season in Mexico) [16].

The choice of the appropriate phenological stage for SPAD index measurements is essential for consistent phenotyping of plants under drought conditions. In the view of Cairns et al. (2012) [19], the effective application of phenotyping methodologies relies, partly, on the applicability of these methodologies in the critical stages associated with grain yield. Accordingly, given the results presented herein, the evaluation in  $S_2$  and  $S_4$ , under WS, and in  $S_1$ , under WW conditions, is recommended. The proper stage of evaluation to identify differences among genotypes by means of spectral indices is the phenological stage of grain filling [10,19,20,23].

On the basis of an agronomic and root characterization and using different dates of measurement of the SPAD index, it was sought, in this research, to identify traits with variability and that do not present genotype  $\times$  water condition ( $G \times WC$ ) interaction, but that present desirable associations for the traits GY and PE, to favor the indirect selection process of superior genotypes. Firstly, the ideal scenario would be the absence of  $G \times WC$  interaction in relation to the traits GY and PE. A second promising scenario would be to find a variable associated with GY and PE in both environments. The third promising scenario would be to find a variable associated with GY and PE only in the environment under WS condition, but not presenting  $G \times WC$ , and which would consequently have the role of ensuring a reliable selection of drought tolerant genotypes in the environment under WW condition. These scenarios were not observed.

Considering the results of the agronomic traits, the  $G \times WC$  interactions proved to be an obstacle for the selection of high productive genotypes under drought by direct via. In addition to the traits GY and PE have significant  $G \times WC$  interaction, the traits that did not present  $G \times WC$  interaction (HG, EL, and DM) expressed either weak or negative correlation with PE (Figure 2). Similar to the agronomic traits, the root traits expressed significant  $G \times WC$  interaction for all their variables, making their use in indirect selection in an irrigation environment unviable (Table 2), and although, under WS condition, the root traits BN, in the first canonical pair, and BD and CD, in the second canonical pair, are associated with PE, their use is unviable because it is a destructive, indirect and costly measure [27].

The SPAD index did not present significant  $G \times WC$  interaction in the evaluations from  $S_1$  (17 DAA) to  $S_4$  (36 DAA); hence, they have potential to be used in an environment under WW condition (Table 1). Regarding the root traits, the variables correlated similarly to what was verified by Trachsel et al. [27], which emphasize the association between the two pairs of root traits BA and CA, and DB and CD. When observed the Pearson linear correlation (Figure 2), however, the correlation between the measures of SPAD index and GY under WW condition were positive, while regarding PE, they were negative or weak. This emphasizes that selection for these two traits is a challenge already addressed by many authors, who have reported observing negative correlations between GY and PE [3,6,31,57].

Conversely, the  $S_3$  SPAD index values correlated negatively in the second and third canonical pairs with GY and PE, respectively (Table 2). Taking these two canonical pairs into account, the smaller the  $S_3$  (29 DAA), the greater the GY and PE. Even though the association in the second and third canonical pairs is less relevant in relation to the first one, the results of  $S_3$  association should be considered.

Results of Pearson correlations and canonical correlations (CCA) were in contrast in various situations. However, Pearson linear correlation is a multiple univariate method, whereas CCA considers

the interaction between groups of traits. In accordance with Thompson (2005) [33], the CCA better illustrates the reality of traits interacting with each other. For this reason, correlation patterns in CCA are sensitive to changes with the addition or exclusion of a simple variable.

Given that the root and SPAD index traits were well represented, it is assumed that the CCA should be regarded as a selection index for GY and PE increase. In findings from previous analysis, Kamphorst et al. (2019) [30] support this statement using the same genotypes because, as well as in this work, the research cited above concluded that SPAD index measures are associated with simultaneous increase of GY and PE under WS condition, but having as dependent variable the product of GY and PE, that is, volume of popcorn per hectare. Kamphorst et al. (2019) [30] performed a single SPAD index measurement at 35 DAF, equivalent to the S4 measurement, which, in this study, was negatively and with high intensity associated with PE in the third canonical pair and under WS condition. For this reason, CCA meets the need to better understand the relationship among traits and the choice of the best variable for efficient use in indirect selection.

The canonical correlation analysis in popcorn crops has great potential for use in identifying non-destructive and easily quantified traits, which are associated with GY and PE, especially as a result of the negative association between GY and PE proposed by some authors [3,6,31,57]. Even in relation to the path analysis, canonical correlations are of interest, as they allow analyzing the association between different groups of variables simultaneously and not only fixing a variable as dependent. According to the findings of this study and the characteristics of the variable that was highlighted as being optimal for the selection of the high productive genotypes under drought by indirect via, it proved to be possible to identify variables with potential for use in indirect selection or even to compose a selection index for the discrimination of the high productive genotypes under water stress condition based on well-watered condition. The SPAD index, measured at 29 DAA, was significant because it associated grain yield and popping expansion in an environment with limited soil water conditions, as well as the measurements at 22 and 36 DAA, which combined more closely with grain yield.

## 5. Conclusions

Major guidance has been given regarding the most adequate traits for the development of research correlated to water shortage in popcorn in regard to the determination of the proper phenological stages for measurement. The leaf senescence, estimated by SPAD index, can be applied to identify more productive genotypes under drought conditions, resulting from a non-destructive procedure using a portable chlorophyll meter (SPAD) [14,20,58].

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