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Heritability of Morphophysiological Traits in Popcorn for Drought Tolerance and Their Use as Breeding Indicators of Superior Genotypes

Flávia Nicácio Viana, Marcelo Moura Chaves, Samuel Henrique Kamphorst, Antônio Teixeira do Amaral Junior, Valter Jário de Lima, Jhean Torres Leite, Katia Fabiane Medeiros Schmidt, Uéliton Alves de Oliveira, Danielle Leal Lamego, Jacymara Lopes Pereira, Guilherme Ferreira Pena, Henrique Duarte Vieira, Jurandi Gonçalves de Oliveira, Rogério Figueiredo Daher, Eliemar Campostrini and Ricardo Bressan-Smith *

Laboratory of Plant Breeding, Center of Agricultural Science and Technology, Darcy Ribeiro State University of Northern Rio de Janeiro, Av. Alberto Lamego, 2000, Campos dos Goytacazes 28013-602, RJ, Brazil; flaalegre@hotmail.com (F.N.V.); marcelomoura873@gmail.com (M.M.C.); samuelkampho@hotmail.com (S.H.K.); amaraljr@uenf.br (A.T.d.A.J.); valter_jario@hotmail.com (V.J.d.L.); torresjhean@gmail.com (J.T.L.); kmedeirosschmitt@gmail.com (K.F.M.S.); uelitonalves2011@hotmail.com (U.A.d.O.); danileallamego@gmail.com (D.L.L.); lopesjacymara@gmail.com (J.L.P.); penabio2@gmail.com (G.F.P.); henrique@uenf.br (H.D.V.); jurandi.oliveira@gmail.com (J.G.d.O.); rogdaher@uenf.br (R.F.D.); campostenator@gmail.com (E.C.)

* Correspondence: bressan@uenf.br

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Abstract: This study aimed to identify the genetic control of morphological, water status, and root traits in four inbred lines and their pre-selected hybrid combinations of popcorn, and to identify the best parents and hybrids. The plants were grown under greenhouse conditions, and with two water conditions—water-stressed (WS) and well-watered (WW). We evaluated shoot biomass (SB), whole plant leaf area (WPLA), chlorophyll content, carbon isotope discrimination, net CO_2 assimilation rate, the stomatal conductance, transpiration, cumulative plant transpiration, and root weight density in three sections. The reductions in SB and WPLA are attributed to stomatal causes, given the maintenance of water condition values in water-stressed plants. In fact, the stomata were closed for long periods during plant growth under the water-stressed condition, as indicated by the more negative carbon isotope discrimination values. The root weight density, regardless of soil depth and water conditions, was higher in hybrids. There was a preponderance of non-additive effects in the control of morphological and root traits, especially in the WS condition, and heterosis was shown to be the fundamental strategy for obtaining superior hybrids. For water status traits, it is essential to choose the female parent in the crossing block, given the maternal effect expressed by net CO_2 assimilation rate, stomatal conductance, and transpiration.

Keywords: water stress; heterosis; drought tolerance

1. Introduction

Heterosis refers to the superior performance of heterozygous hybrid plants over their homozygous parental inbred lines; indeed, exploitation of heterosis has been described as beneficial and fundamental to mitigating environmental stresses, such as the stress associated with soil water limitation [1–4]. Heterosis has been a mediator in optimizing water use [1,3]. In a water-limited environment and for physiological and photosynthesis-related traits, cumulative transpiration, and root traits, Chairi et al. (2016) [4] described the effects of heterosis. In the initial growth of plants [5], in the juvenile phase [1,2], and in adult plants, heterosis has been demonstrated as an important phenomenon for confere higher biomass accumulation and grain production [6–11], which allows genotypes with higher water use efficiency and/or tolerance to water deficit to be obtained.

Studies on inheritance in heterosis indicates that the trait affects the additive nature, as well as through the dominance effects. Understanding the inheritance of physiological and agronomic traits may present opportunities to increase grain yield potential and to adapt plants to water deficit conditions. In the study by Kamphorst et al. (2022) [1], it was found that shoot biomass, whole plant area, carbon isotope discrimination, net CO_2 assimilation rate, stomatal conductance, transpiration, cumulative plant transpiration, and root weight density were considered key traits for adaptation to drought tolerance in popcorn. These traits were relevant in the efficient discrimination of superior hybrids in water stress environments.

The market of the popcorn seed, belonging to the species *Zea mays* L., to the family Poaceae, subfamily Panicoideae, and tribe Maydeae [12], has offered cultivars with high yield potential, suitable for growing under the adequate availability of water in the soil [1,13,14]. However, in the current climate change situation, increasingly severe episodes of water deficit are expected, which is the main stress that compromises crop yield. Soil water limitation causes severe damage to food production, especially in tropical and subtropical regions [6,13,15–19]. Despite this situation, there is currently only one popcorn hybrid recommended for cultivation under drought stress [15,20], which can be used to mitigate the action of drought on Brazilian agricultural production. The occurrence of drought drives the search for genotypes adapted to adverse environmental conditions since, soon, it is expected that cultivation in producing areas will experience even more severe water restrictions [1,21–24].

As a result of water limitation in the soil, a series of physiological and biochemical changes are observed in popcorn crops [25]. Among the main changes includes a reduction in leaf expansion, stomatal closure, inhibition of CO_2 assimilation, reduction in cellular photochemical and metabolic activity, as well as changes in carbon partition, with negative and significant effects on plant yield [1,19,26]. Recent studies associated with plant breeding have already identified the responses of plants to water stress in the soil, and it is possible to select superior genotypes with adaptation to drought, including the possibility of using morphophysiological traits in the indirect selection for yield [1,2,14,15,20,25–29].

Therefore, concerning the study by Kamphorst et al. (2022) [1], it becomes essential to evaluate the morphological traits, as an example of water status traits, in order to elucidate how these traits are associated with drought tolerance, as well as to identify the best parents and superior hybrids in an environment with two water conditions (WC) (water stress (WS) and well-watered (WW)). Given the above, this study seeks to identify a promising genotype for water-stressed conditions, through the evaluation morphological traits, water status and root traits, selection in a popcorn breeding program.

2. Materials and Methods

2.1. Genotypes

Four S_7 lines of popcorn from the Active Germplasm Bank of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) were used. These lines were pre-selected under water deficit conditions from a cultivation of twenty lines evaluated according to the agronomic potential of each line [23]. The pre-selected lines were P6 and P7 (derived from the hybrid Zaeli, adapted to temperate/tropical climate), L61 (derived from the Beija-flor variety, adapted to temperate/tropical climate), and L75 (derived from the open-pollinated variety BRS-Angela, of tropical climate adaptation) [12]. Such lines showed contrasting performance in terms of agronomic efficiency in water use [23]; that is, the ratio between the mass of harvested matter (grain yield) and the volume of water applied to the crop during the cycle (L). Lines P6 and P7 were classified as agronomically efficient in water use (>kg L^{-1}), and L61 and L75 as inefficient (<kg L^{-1}).

The lines were crossed in a complete diallel scheme, involving crossing between all genitors, including reciprocals, resulting in 12 hybrids. Following the order of female (♀) and male (♂) parents, the hybrids obtained were P6 × P7, P6 × L61, P6 × L75, P7 × L61, P7 × L75, L75 × L61, P7 × P6, L61 × P6, L75 × P6, L61 × P7, L75 × P7, and L61 × L75.

2.2. Growing Conditions

The experiment was conducted, in a greenhouse located at the Research Support Unit (UAP) of the Center for Agricultural Sciences and Technologies (CCTA) of UENF, in 2020, in Campos dos Goytacazes, Rio de Janeiro, Brazil (21°45′16″ LS and 41°19′28″ LW). The experimental design was complete randomized blocks with three replications and two water conditions (WC): WW (well-watered) and WS (water stress), totaling 96 experimental units (16 genotypes $\times$ 2 WCs $\times$ 3 replications). The plants were cultivated in a lysimeter system, as described by Elazab et al. (2016) [30].

Each experimental unit consisted of a plant, which was grown in a polyvinyl chloride (PVC) tube of 14 cm in diameter and 150 cm in length. The tubes were divided longitudinally into two equal parts, joined horizontally and vertically using adhesive tape. In the basal part of the tubes, adequate drainage was allowed, and there was no loss of substrates during the experimental evaluations. The substrate used consisted of 80% perlite and 20% peat, to which fertilizer was added in the form of NPK (MgO, SO_3) 20–5–8 (5–17), with micronutrients and a slow-release N source (2–3 months) (isobutylidenediurea/17 g tube^{-1}). The fertilizer corresponded to 144.7 kg N ha^{-1}, 36.2 kg P ha^{-1}, and 57.8 kg K ha^{-1}.

Before sowing, the tubes were irrigated abundantly. Subsequently, the tubes were left to rest for 72 h to percolate excess water from irrigation. After draining, the tubes were weighed in order to measure the mass at 100% water capacity. The adequate water storage capacity of each tube (equivalent to field capacity = 100%) was estimated by the difference between wet weight and dry weight. After draining, the tubes were weighed in order to measure the mass at 100% water capacity. The adequate water storage capacity of each tube (equivalent to field capacity = 100%) was estimated by the difference between wet weight and dry weight. To determine the dry weight, extra samples from the tubes that received the same treatment as the experimental samples were taken to an oven to determine the dry weight of the substrate, after maintaining a constant weight. On average, each tube had the capacity to store around 6.70 L of water (100% of field capacity). At the end of this procedure, three seeds were sown per tube. At 15 days after emergence, the excess plants were thinned. The tubes were spaced 25 cm apart with 94 cm between rows, resulting in a density of 42,553.20 plants ha^{-1}.

In the well-watered treatment (WW), the substrate was maintained at 100% of field capacity until the final evaluations (pre-anthesis period). In the water stress treatment (WS), irrigation was suspended at 15 days after emergence. The substrate was maintained at 35% of field capacity in the WS treatment until about 40 days after sowing. This value of 35% was maintained for more than 15 days, until the final evaluations. The reduction of water in the tubes occurred evenly among the different genotypes, so that those with the highest water demand received the amount of water needed to return to the same capacity as those genotypes that had the lowest water consumption. This regime was established to simulate field growth conditions with a slow decrease in water content, thus avoiding the typical situation of potted plants, which can be affected by severe water stress within a few days. To that end, the tubes were weighed and irrigated at intervals of 2–3 days.

Climatic conditions were measured over the experimental procedure. The average values of temperature, relative humidity, and solar radiation, expressed every five days, are available in Figure 1.

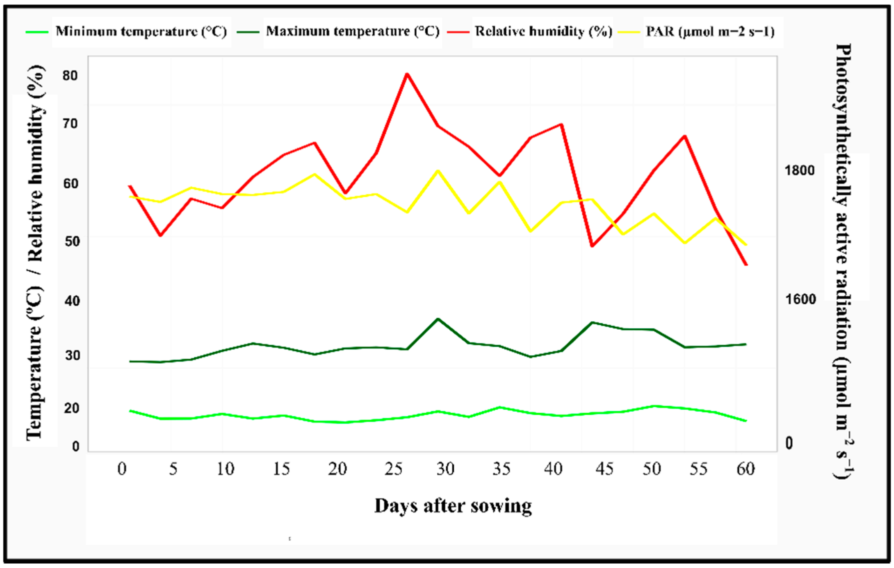

Figure 1. Average minimum (Tmin) and maximum (Tmax) air temperatures (°C), relative air humidity (RH, %), and photosynthetically active radiation (PAR, µmol m^{-2} s^{-1}) over the days after the sowing of the popcorn.

2.3. Morphological Traits

At the end of the experiment, and to determine shoot biomass (SB; g), the shoot of the plants was placed in paper envelopes and stored in an oven at 70 °C for 72 h.

The whole plant area (PA in m^2) was calculated based on pictures of each plant. The images were acquired with a Sony α6000 digital SLR (Sony Corporation, Tokyo, Japan) 24.5-megapixel resolution camera with a 23.5 × 15.6 mm sensor size, a native resolution of 6000 × 4000 pixels, and equipped with a 35 mm focal length lens. The pixel size was calculated using the Ground Sample Distance (GSD) calculator tool developed by Pix4D (https://support.pix4d.com/hc/en-us/articles/202559809-Ground-sampling-distance-GSD, accessed on 3 March 2022). The following equation was used: [(Sw.H.100)(Fr.imW)], where GSD represents the distance between two consecutive pixel centers, Sw indicates the sensor width of the camera (mm), H is the height (distance between camera and object) (m), Fr is the real focal length of the camera (mm), and imW is the image width (pixels). In our case, Sw was 23.2 mm, H = 1.95 m, Fr = 18 mm, and imW = 4608 pixels. Therefore, images were analyzed with ImageJ image processing software using a GSD of 0.054 pixels cm^{-1} [31].

The relative levels of chlorophyll concentration (CC) (Dualex index) were quantified in the middle third of the last developed leaf using a portable Dualex® meter (FORCE-A, Orsay, France). These evaluations were measured between 08:00 a.m. and 11:00 a.m., one day before harvest.

2.4. Water Status

The carbon isotope discrimination ($\delta^{13}C$) was determined in samples of the last developed leaf in all plants under both WCs. Measurements of carbon isotope composition were conducted at the Scientific Facilities of the Universidade Estadual do Norte Fluminense, in the Ecology Laboratory, using an elemental analyzer (Flash 1112 EA; Thermo Finnigan, Berman, Germany) coupled with an isotope ratio mass spectrometer (Delta C IRMS, Thermo Finnigan), operating in continuous flow. Samples of 0.7–0.8 mg of leaf dry matter from each plant, together with reference materials, were weighed and sealed into tin capsules. Isotopic results were expressed in standard δ-notation [32]: X = [(R_sample/R_standard^{-1}) × 100], where X is the $\delta^{13}C$ value, and R is the 13C. The $\delta^{13}C$ values (‰) were based on the Vienna Pee Dee Belemnite isotope standard [33].

Gas exchange was evaluated on the last two days of the experiment, first in WW conditions, and on the second day in WS conditions, between 11:00 a.m. and 2:00 p.m., using a portable infrared gas analyzer, model LI-6400 (LI-COR, Lincoln, NE, USA), with a light source (6400–40 LCF, LI-COR). During the gas exchange measurements, the photosynthetically active radiation was set to 1500 μmol m^{-2} s^{-1}, the CO_2 concentration to 400 μmol mol^{-1}, relative humidity to between 55% and 60%, and temperature to 25 °C. The net photosynthetic rate (A), stomatal conductance (gs), and transpiration rate (E) were measured in the last developed leaf of each plant.

The total amount of water transpired from each plant during all the growth periods (the cumulative plant transpiration) (ET; dm^3 $plant^{-1}$) was recorded throughout the growth cycle. To this end, before irrigation, each tube was weighed, and the substrate surface of each was covered with plastic to avoid direct evaporation from the substrate surface.

2.5. Root Traits

At the end of the experiment, the tubes were opened to separate the substrate from the roots. First, the entire volume of the substrate was cut into equal sections. Each section had a length of 0.30 m, measured from the upper surface of the tubes to the lower end. The following tube sections were studied: 0–30 cm (a), 60–90 cm (c), and 120–150 cm (e). These layers were selected to represent the different extremes of the soil profile. Subsequently, the samples were gently shaken and washed with water, using a screen to remove the soil. Finally, the root samples were washed with distilled water and lightly dried with a paper towel. The dry root weight density (RWD) was determined separately for each layer. Each root section was placed in a paper envelope and oven-dried at 60 °C for 72 h. The root weight density of each soil section (RWDsec, g m^{-3}) was calculated as proposed by Elazab et al. (2012) [34] using the following expression: RWD_sec = RB_sec/($\pi \times R^2 \times L$); where RB_sec is the dry root biomass of the soil section (g), R is the tube radius (0.07 m), and L is the length of the tube section (0.30 m).

2.6. Statistical Analysis

Combined variance analysis was performed according to the following statistical model: Yijk = μ + Gi + B/WCjk + WCj + G × WCij + εijk, where Yijk = observation of the ith genotype in the jth environment in the kth block; μ = general constant; Gi = fixed effect of the ith genotype; B/WCjk = effect of the kth block in WC j; WCj = fixed effect of the jth WC with NID; G × WCij = fixed effect of the interaction between the ith genotype and the jth WC; and εijk = mean experimental random error associated with observation Yijk, with NID (0, σ2). The table with the comparison and significant effects of the combined ANOVA is provided in the Supplementary Material (Table S1). Individual analysis of variance was performed for each WC according to the following statistical model: Yij = μ + Gi + Bj + εij, where Yij is the observation of the i-th genotype in the j-th block; μ is the general constant; Gi is the fixed treatment effect; Bj is the random block effect; and εij is the experimental error. Given the comparison between the WC, the proportional reduction (%) of each variable was calculated as: $Y = 100 - [(Y_{WS}/Y_{WW}) \times 100]$, where Y is the overall mean of the variable WS and WW conditions. To estimate general combining ability (GCA) and specific combining ability (SCA) and reciprocal effects (RE) in relation to morphological traits, water status and rood traits, the combining abilities were analyzed by the method I diallel analysis, as proposed by Griffing (1956) [35], in which p^2 combinations are included, and the genotype effect is considered fixed. This model analyzed the four parents, hybrids, and reciprocal combinations. The statistical model considered for the analysis was: Yij = μ + gi + gj + sij + rij + εij, where Yij = mean value of the hybrid combination (i ‡ j) or the parent (i = j); μ = general mean; gi, gj = effects of the general combining ability of the ith or jth parent (i, j = 1, 2, 3, and 4); sij = effect of specific combining ability for crosses between parents of order i and j; rij = reciprocal effect that measures the differences resulting from parent i or j when used as the male or female parent in cross ij; and εij = mean experimental error associated with observation of order ij [NID (0, σ^2). Effects for the source of genotype variation were considered to be fixed. The analyses were performed using GENES software [36].

3. Results

3.1. Genetic Variability in the Different WCs and the Impact of Applied Water Stress

In general, independent of WC, among the morphological traits (shoot biomass (SB), whole plant area (PA), and chlorophyll concentration (CC) (Dualex index)), the sources of variation, genotype (G), general combining ability (GCA), specific combining ability (SCA), and reciprocal effect (REC) expressed significant differences (Table 1). Two exceptions were observed: REC in WS for SB and CC; and WW for PA and CC. The averages of SB, PA, and CC in the WW condition were 102.28 g, 0.62 m^2, and 29.11, respectively; in WS, the averages were 74.73 g, 0.46 m^2, and 25.96, in that order. The imposed water restriction caused a reduction of 26.9%, 25.8%, and 10.8% in the averages of SB, PA, and CC, respectively (Figure 2).

Table 1. Summary of diallel analysis of variance according to Method I of Griffing (1956), containing the estimates of means for morphological traits, water status, and root traits of four parents and their hybrid combinations of popcorn grown under contrasting water conditions (WW and WS).

	SV/Traits	WW					WS				
		G	GCA QM	SCA QM	REC QM	$\bar{x}$	G	GCA QM	SCA QM	REC QM	$\bar{x}$
Morphological traits	Shoot Biomass (g)	1795.93 **	5009.76 **	1553.88 **	431.05 ^{ns}	102.28	891.35 **	1423.28 **	1309.28 *	207.46 ^{ns}	74.73
	Whole Plant area (m^2)	0.007 **	0.009 **	0.011 **	0.001 *	0.62	0.014 **	0.013 **	0.019 **	0.009 ^{ns}	0.46
	Chlorophyll concentration (Dualex index)	81.79 *	51.16 **	161.24 *	17.65 ^{ns}	29.11	15.85 **	32.04 **	19.62 **	3.97 *	25.96
Water status	Carbon isotope discrimination ($\delta^{13}C$)	0.168 **	0.504 **	0.075 *	0.093 ^{ns}	−12.16	0.270 *	0.656 **	0.223 ^{ns}	0.125 ^{ns}	−13.1
	Net CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1})	38.61 **	29.18 **	54.79 ^{ns}	27.15 **	29.08	24.74 **	41.90 *	7.63 **	33.27 **	24.12
	Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.0022 **	0.0024 **	0.0019**	0.0025 **	0.14	0.0011 **	0.0016 *	0.0006 *	0.001 **	0.1
	Transpiration (mmol H_2O m^{-2} s^{-1})	1.083 **	0.678 **	1.368 ^{ns}	1.001 **	4.26	0.559 **	0.916 ^{ns}	0.233 **	0.705 **	3.03
	Cumulative plant transpiration (dm^3 $plant^{-1}$)	48.89 **	138.89 **	45.82**	6.97 ^{ns}	18.03	20.08 **	19.56 **	36.14 *	4.29 ^{ns}	12.73
Root traits	RWD_a (cm)	17,114.45 **	24,279.33 ns	21,879.75 **	8766.70 **	220.91	2602.32 **	555.36 **	5339.91 **	888.19 **	132.07
	RWD_c (cm)	3739.59 **	5285.68 **	3541.11 **	3165.02 **	72.34	539.77 **	162.64 **	1050.24 **	217.85 **	40.39
	RWD_e (cm)	1052.96 **	1825.53 ns	971.83 **	747.81 ^{ns}	54.33	319.71 **	118.40 **	635.11 **	104.97 **	31.43

SV: sources of variation; WW: well-watered; WS: water stress; G: source of variation for genotype; GCA: source of variation of the general combining ability; SCA: source of variation of specific combining ability; REC: source of variation of the reciprocal effect; MS: mean square effect, followed by ^{ns}, *, and **, when not significant or significant at 5% and 1%, respectively, by the F test; and estimates of genotype mean obtained from four parents and twelve hybrids evaluated in two different water conditions.

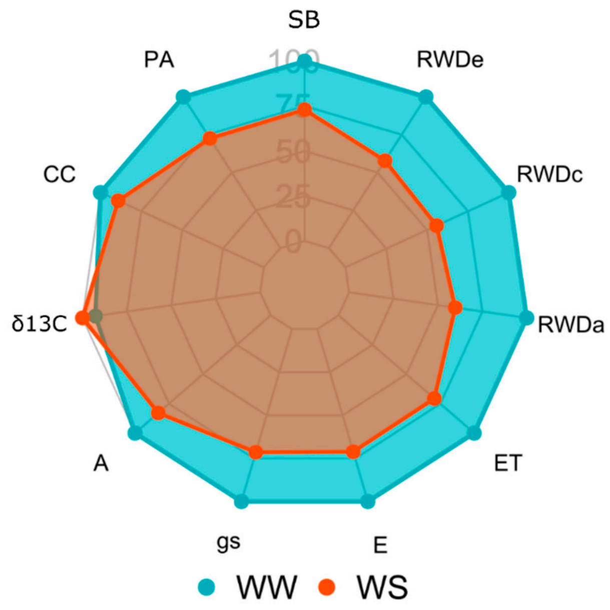

Figure 2. Comparison of average estimates obtained under water stress (WS) and well-watered (WW) conditions for morphological traits, water status, and root traits in popcorn parents and hybrids. WS: water stress; WW: well-watered; SB: shoot biomass; PA: whole plant area; CC: chlorophyll concentration (Dualex index); $\delta^{13}C$: carbon isotope discrimination; A: net CO_2 assimilation rate; gs: stomatal conductance; E: transpiration; ET: cumulative plant transpiration; RWDa: root weight density of the section a (0–30 cm); RWDc: root weight density of the section c (60–90 cm); RWDe: root weight density of the section e (120–150 cm).

For the source of variation G, all traits related to water status—carbon isotope discrimination ($\delta^{13}C$), net CO_2 assimilation rate (A), stomatal conductance (gs), transpiration (E), and cumulative plant transpiration (ET)—showed statistical differences (Table 1). For the SCA variation sources, the traits $\delta^{13}C$ and gs showed significant differences in WW and $\delta^{13}C$ in WS (Table 1).

The $\delta^{13}C$ trait expressed an average of −12.16 in WW and −13.10 in WS. The imposed water limitation caused a reduction in the averages of the characteristics of A, g_s, E, and ET, considering the averages obtained in WW of 29.08 µmol CO_2 m^{-2} s^{-1}, 0.14 mol H_2O m^{-2} s^{-1}, 4.26 mmol H_2O m^{-2} s^{-1}, and 18.03 dm^3 $plant^{-1}$; and in WS of 24.12 µmol CO_2 m^{-2} s^{-1}, 0.10 mol H_2O m^{-2} s^{-1}, 3.03 mmol H_2O m^{-2} s^{-1}, and 12.72 dm^3 $plant^{-1}$ (Table 1). These traits also followed the trend of reducing magnitudes of the values, with the greatest differences between the WW treatment and the WS treatment occurring for E and ET, which had a decrease of about 30%, followed by g_s, which showed a reduction of about 28%, and by A, which expressed a decrease of 17% in the WS condition (Figure 2).

Regarding root growth traits, and regardless of WC, most root traits showed significant differences for the sources of variation G, GCA, SCA, and REC (Table 1). Concerning GCA, exceptions were observed in WW for sections RWD_a and RWD_e. Given the comparison between the WS and WW condition, the averages of RWD_a, RWD_c, and RWD_e were reduced by 84.8%, 85.5%, and 69.6% (Figure 2). Following this same order of traits, the mean estimates were 220.91 cm, 72.34 cm, and 54.33 cm in WW, while in WS, the respective magnitudes were 132.07 cm, 40.39 cm, and 31.43 cm (Table 1).

3.2. Importance of Quadratic Components

In the morphological traits, quadratic components (Φ) with negative values of the general combining ability were observed in WW for shoot biomass (SB) in REC (−4.81) and whole plant area (PA), also for REC (−0.00002). In the WS condition, a comparable situation occurred for chlorophyll concentration (Dualex index) in REC (−0.52). These negative values should be interpreted as estimates of an actual value equal to zero (Table 2).

Table 2. Analysis of variance and quadratic components for three groups of traits evaluated in popcorn, with exposure to different water conditions (WS: water stress, and WW: well-watered), according to the Method I proposed by Griffing (1956) for a diallel involving four inbred lines, their F_1′s, and reciprocal hybrids.

	SV/Traits	WW						WS					
		GCA		SCA		REC		GCA		SCA		REC	
		Φ_g	%	Φ_s	%	Φ_{rc}	%	Φ_g	%	Φ_s	%	Φ_{rc}	%
Morphological traits	Shoot biomass	189.57	13	364.64	78	−4.81	0	54.33	4	396.64	94	14.68	3
	Whole plant area	0.0003	43	0.0033	40	−0.00002	0	0.0005	6	0.0053	59	0.001	36
	Chlorophyll concentration (Dualex index)	1.86	3	51.6	93	1.86	3	1.04	20	4.18	80	−0.52	0
Water status	Carbon isotope discrimination ($\delta^{13}C$)	0.013	58	0.003	30	0.004	12	0.024	36	0.047	53	0.008	11
	Net CO_2 assimilation rate	0.92	5	15.91	79	3.35	17	1.49	25	0.53	0	4.54	75
	Stomatal conductance	0.00007	8	0.00043	49	0.00031	43	0.00006	15	0.00015	34	0.00021	51
	Transpiration	0.018	4	0.37	72	0.13	25	0.03	23	0.03	8	0.09	69
	Cumulative plant transpiration	5.15	34	10.2	66	−1.37	0	0.72	6	11.25	92	0.32	3
Root traits	RWD_a	958.3	11	6866.58	76	1247.78	14	13.12	1	1699.72	93	107.94	6
	RWD_c	212.97	12	1122.25	55	498.44	34	5.64	1	341.05	90	31.79	8
	RWD_e	68.67	16	264.84	62	95.08	22	2.5	1	192.23	95	7.76	4

SV: sources of variation; WW: well-watered; WS: water stress; G: source of variation for genotype; Importance (expressed in %) of the quadratic components related to the general (Φ_g) and specific (Φ_s) combining abilities and the reciprocal effects (Φ_{rc}).

In general, although the mean squares related to the general combining ability and the reciprocal effect were significant (Table 1), the quadratic component associated with the specific combining ability was the most important to determine the expression of the traits, as well as to explain most of the observed genetic variability (Table 2). The relative importance of the quadratic components related to CEC in WW was 78% for SB, 40% for PA, and 93% for CC; in WS, 94% for SB, 59% for PA, and 80% for CC (Table 2).

The effect of Φs related to specific combining ability was the most important in explaining the genetic variability in most water status traits in WW, with percentage estimates of 79% for A, 49% for gs, 72% for E, and 66% for ET (Table 2). Nevertheless, in WS, there was a prevalence of the Φrc effect related to the reciprocal effect in the expression of genetic variability for A (75%), gs (51%), and E (69%) (Table 2).

For root traits, independent of WC, the quadratic component Φs related to SCA was the most prevalent (Table 2). For these traits, in WW, the values were 76% for RWDa, 55% RWDc, and 62% for RWDe; whereas, in WS, the percentage estimates were 93% for RWDa, 90% for RWDc, and 95% for RWDe (Table 2).

3.3. Effects of General Combining Ability (GCA) in Different Water Conditions

For the morphological traits, in both WC, it was observed that for SB, the parents P6 and P7 presented positive estimates of the effects of GCA, while L61 presented a negative estimate. For PA, only parents P6 and P7 showed positive estimates of the effects of GCA in both WC. The parents P7 and L75 were the ones that presented the highest positive estimates of the effect of GCA concerning the CC trait, regardless of WC (Figure 3).

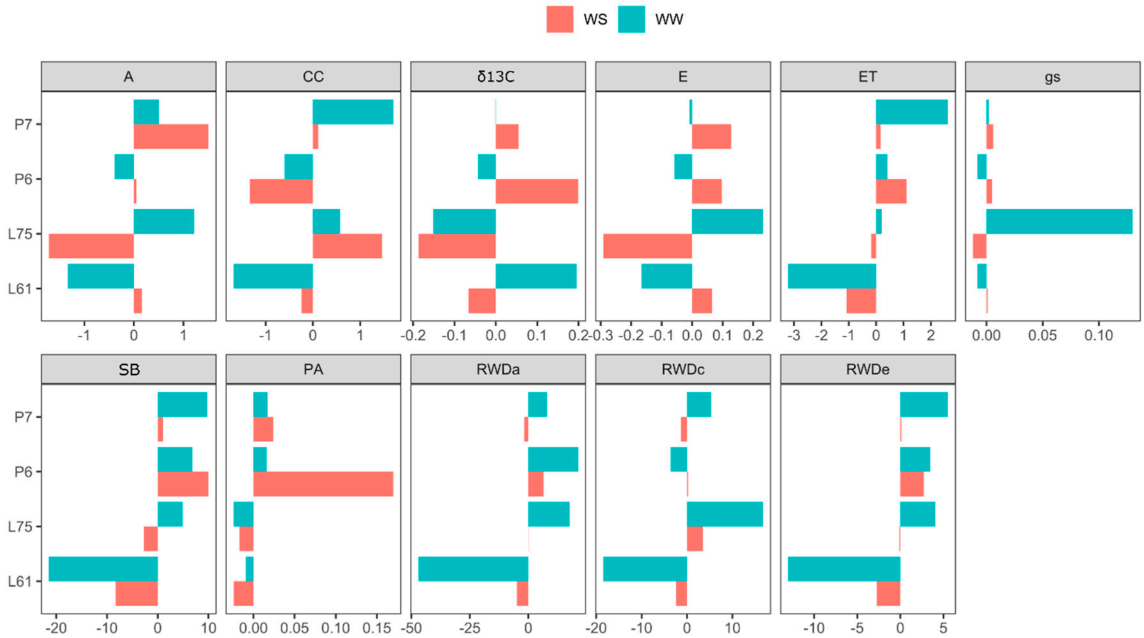

Figure 3. Estimates of the effects of general combining ability (GCA) on morphological traits, water status, and root traits in popcorn lines under water stress (WS) and well-watered (WW) conditions. WS: water stress; WW: well-watered; SB: shoot biomass; PA: whole plant area; CC: chlorophyll concentration (Dualex index); $\delta^{13}C$: carbon isotope discrimination; A: net CO_2 assimilation rate; g_s: stomatal conductance; E: transpiration; ET: cumulative plant transpiration; RWD_a: root weight density of the section a (0–30 cm); RWD_c: root weight density of the section c (60–90 cm); RWD_e: root weight density of the section e (120–150 cm).

For the traits related to water status, the parents L75 and P7 were the ones that presented the highest positive estimates of the effects of GCA in WW for A, while in WS, the parents P7 and L61 stood out. For gs, the parent L75 presented the highest positive estimate of the effect of GC in WW, while in WS, P7 and P6 stood out (Figure 3). For E, the parent L75 in the WW condition and P7 in the WS were the ones that presented the highest positive estimates of the effects of GCA. The highest positive estimates of the effects of GCA for ET were observed at P7 and P6 in both WC (Figure 3).

Regarding root traits, for RWDa, the P6 parent presented the highest positive estimates of the GCA effect, independent of WC. In RWDc, L75 stood out, as it expressed the highest positive estimates of the effects of GCA in both WCs. In RWDe, the parents P7 and P6 were the ones that presented the highest positive estimates of the effects of GCA in WW, while in WS, this occurred for the parent P6 (Figure 3).

3.4. Effects of Specific Combining Ability and Estimates of Means of the Hybrids under Different Water Conditions

Regarding SCA estimates, among the morphological traits, it is observed that for SB, the superior hybrids were P6 × L75 (23.47) in WW, and P6 × L75 (12.91) and L61 × L75 (10.38) in WS (Figure 4). As for the means in WW, the hybrids that presented the highest estimates were P6 × L75 (137.65 g), L75 × P6 (137.31 g), and L75 × P7 (130.75 g); in WS, they were P7 × P6 (95.32 g) and L75 × P6 (94.47 g) (Figure 5). It is worth noting that the hybrids that presented the highest SCA estimates were the same ones that showed the highest averages. For PA, the highest SCA estimates were observed in L61 × L75 in WW (0.04) and WS (0.05), while P6 × L61 (0.04) excelled in both WC (Figure 4). For PA, the hybrids that presented the highest mean values were P6 × P7 (0.69 m^2), L61 × P6 (0.68 m^2), and P7 × L61 (0.65 m^2) in the WW water condition; in turn, in the WS condition, the hybrids with the highest estimates of averages were P7 × L75 (0.56 m^2) and L75 × P6 (0.51 m^2) (Figure 5).

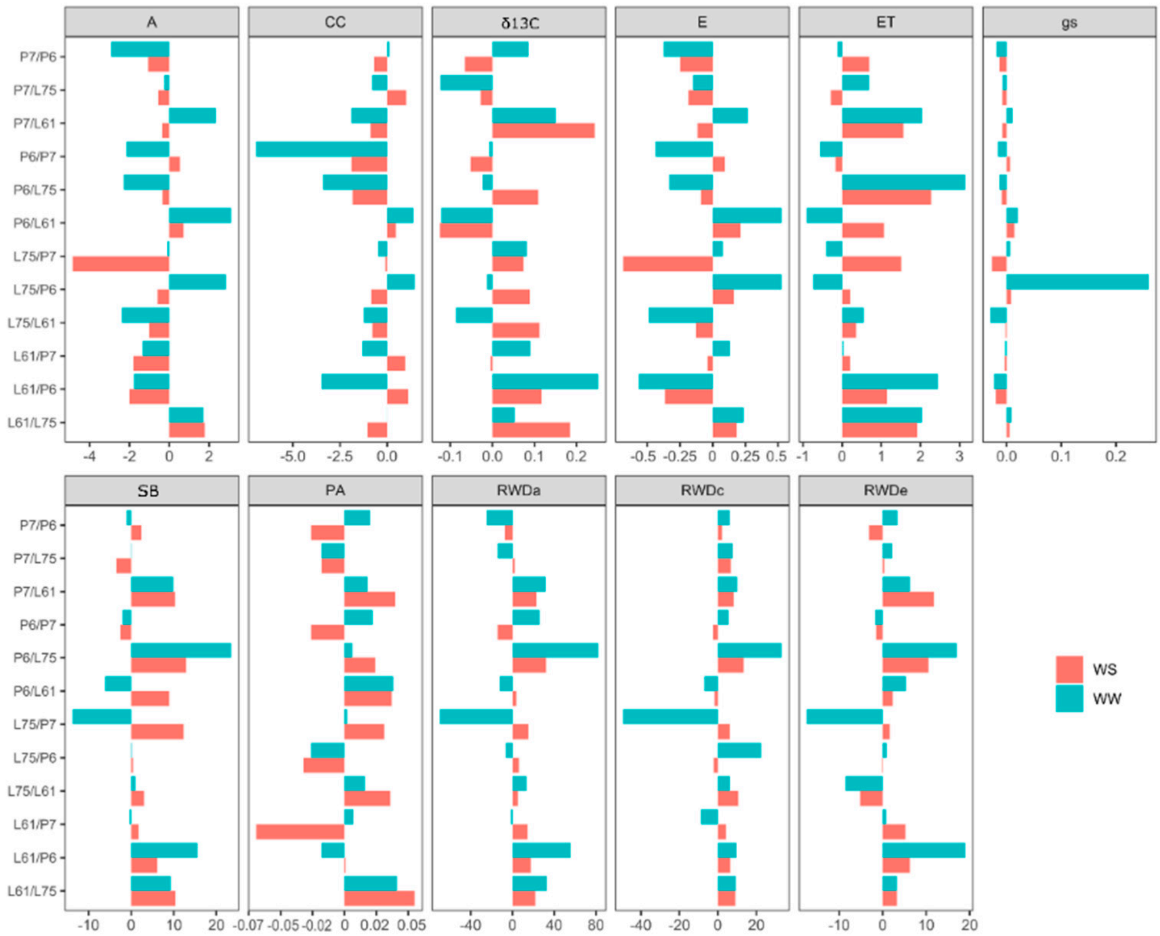

Figure 4. Estimates of specific combining ability (SCA) effects for morphological traits, water status, and root traits in popcorn hybrids under water stress (WS) and well-watered (WW) conditions. WS: water stress; WW: well-watered; SB: shoot biomass; PA: whole plant area; CC: chlorophyll concentration (Dualex index); δ^{13}C: carbon isotope discrimination; A: net CO_2 assimilation rate; *gs*: stomatal conductance; E: transpiration; ET: cumulative plant transpiration; RWD_a: root weight density of the section a (0–30 cm); RWD_c: root weight density of the section c (60–90 cm); RWD_e: root weight density of the section e (120–150 cm).

Regarding SCA for the CC trait, the highest estimates were observed in L75 × P6 (1.46) in WW and L61 × P6 (1.11) in WS (Figure 4). For this same trait, hybrids L61 × P6, L61 × P7 and L75 × P7 expressed the highest averages in WW, corresponding to values of 34.45, 31.70, and 31.04, respectively; in WS, the highest values were presented by the hybrids L61 × P6 (30.74) and L75 × P7 (28.65) (Figure 5).

For the water status traits, in particular δ^{13}C, the highest SCA estimates occurred for the combinations L61 × P6 (0.25) in WW and P7 × L61 (0.27) in WS (Figure 4). The hybrid with the lowest mean estimate for δ^{13}C was L75 × L61 (−11.87) in WW and P7 × L61 (−12.84) in WS (Figure 6). For trait A, higher SCA estimates were observed in P6 × L61 (3.11) in WW and L61 × L75 in WS (1.77) (Figure 4). Higher mean values of A in WW occurred for the combinations L61 × L75 (33.06 µmol CO_2 m^{-2} s^{-1}), L61 × P7 (32.33 µmol CO_2 m^{-2} s^{-1}), and L61 × P6 (32.25 µmol CO_2 m^{-2} $s{-1}$); whereas in WS, the combinations with the highest mean estimates were P6 × L75 (28.2 µmol CO_2 m^{-2} s^{-1}) and P7 × L61 (27.27 µmol CO_2 m^{-2} s^{-1}) (Figure 6). Regarding SCA for gs, the highest estimate was observed for L75 × P6 (0.26) in WW (Figure 4).

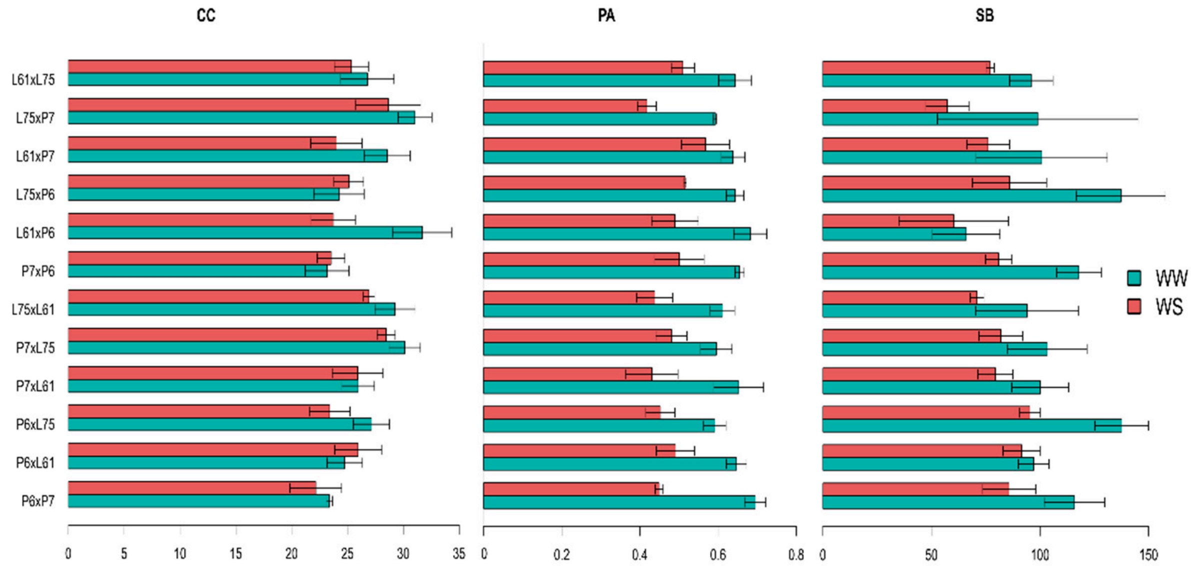

Figure 5. Estimates of the means of morphological traits of popcorn hybrids evaluated under water stress (WS) and well-watered (WW) conditions. WS: water stress; WW: well-watered; SB: shoot biomass (g); PA: whole plant area (m^2); CC: chlorophyll concentration (Dualex index).

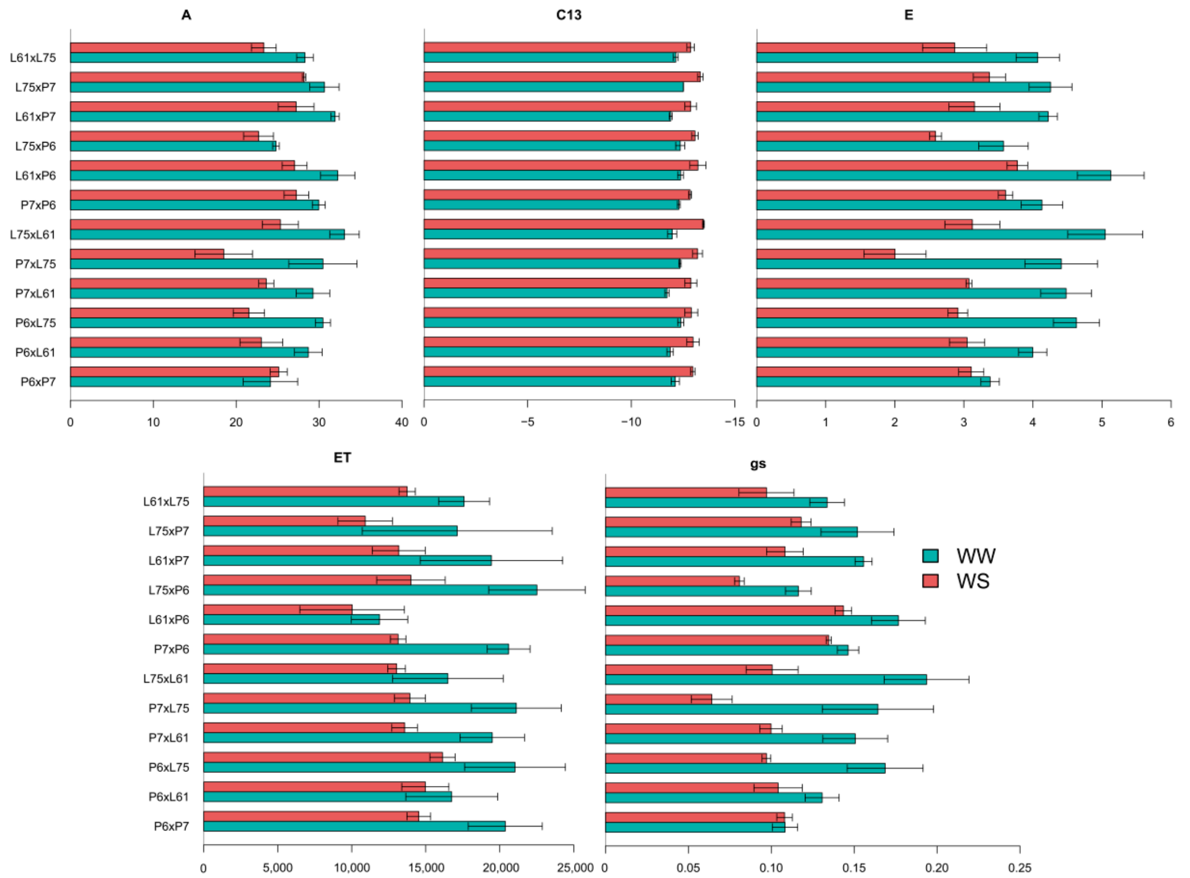

Figure 6. Estimates of the means of the water status of popcorn hybrids evaluated under water stress (WS) and well-watered (WW) conditions. WS: water stress; WW: well-watered; $\delta^{13}C$: carbon isotope discrimination; A: net CO_2 assimilation rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$); gs: stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$); E: transpiration ($mmol\ H_2O\ m^{-2}\ s^{-1}$); ET: cumulative plant transpiration ($dm^3\ plant^{-1}$).

As for the average for gs, the highest values were observed for L61 × L75 (0.19 mol H_2O m^{-2} s^{-1}) and L61 × P7 (0.18 mol H_2O m^{-2} s^{-1}) in WW; whereas in WS, higher values occurred for the hybrids L61 × P6 (0.14 mol H_2O m^{-2} s^{-1}) and P7 × L61 (0.13 mol H_2O m^{-2} s^{-1}) (Figure 6).

For E, the highest SCA estimates in both WCs were observed in the P6 × L61 hybrid, with estimates of 0.53 in WW and 0.21 in WS (Figure 4). The highest mean estimates in both WCs were observed in the hybrid L61 × P6, corresponding to 5.13 mmol H_2O m^{-2} s^{-1} in WW and 3.77 mmol H_2O m^{-2} s^{-1} in WS (Figure 6). The hybrid with the highest values of SCA estimates for ET was P6 × L75 in both WCs (Figure 4). The hybrid with the highest average in WW was L75 × P6, with a value of 22.51 dm^3 $plant^{-1}$, while in WS, it was P7 × P6, with an average of 16.13 dm^3 $plant^{-1}$ (Figure 6).

Regarding the analyzed root traits, in the three sections, RWDa, RWDc, and RWDe, the hybrid P6 × L75 stood out with the highest average estimates in WW and WS, with respective values of 335.85 cm and 177.18 cm, 151.27 cm and 43.27 cm, and 79.80 cm and 44.43 cm (Figure 7). The highest values were expressed in all root traits when submitted to the WW water condition.

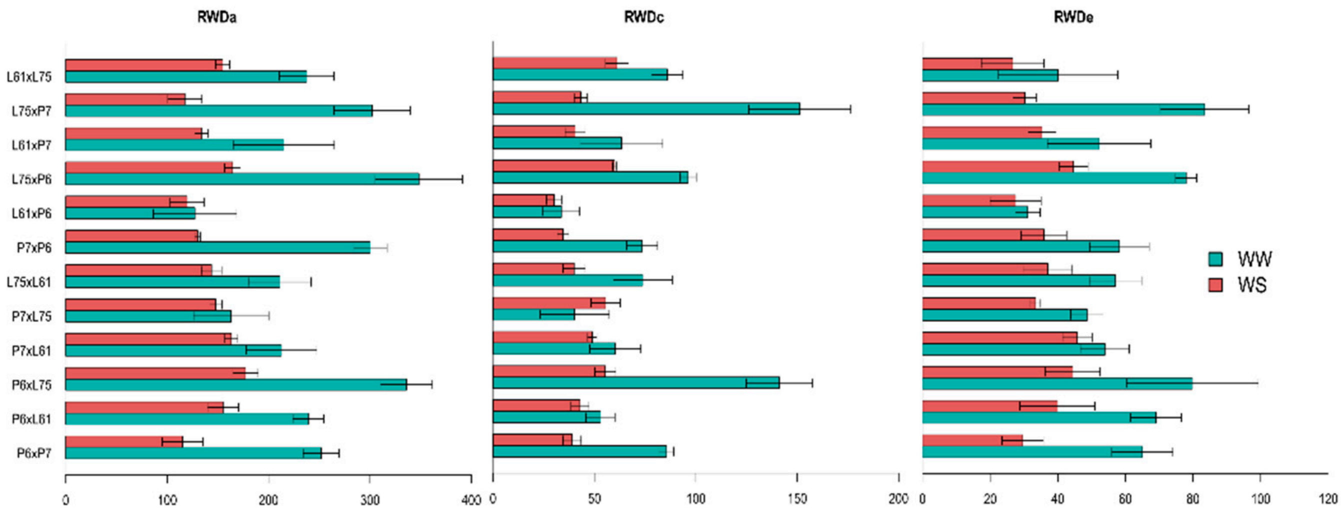

Figure 7. Estimates of means of root traits in popcorn hybrids evaluated under water stress (WS) and well-watered (WW) conditions. WS: water stress; WW: well-watered; RWD_a: root weight density of the section a (0–30 cm); RWD_c: root weight density of the section c (60–90 cm); RWD_e: root weight density of the section e (120–150 cm).

4. Discussion

4.1. Morphological Traits

As a result of the applied water deficit, significant reductions were observed in the average shoot biomass (SB) and whole plant area (PA). Similar results were found by Chairi et al. (2016) [4] when evaluating corn lines and hybrids. According to these authors, the higher accumulation of SB before flowering and the larger leaves resulted in a larger area of solar interception, thus favoring increases in CO_2 assimilation rates [11], a situation also observed in popcorn–corn hybrids evaluated in this study. Tollenaar et al. (2004) [11] cite some physiological mechanisms as responsible for the expression of heterosis, especially for grain yield, highlighting the larger leaf area, which favors higher light interception, providing dry matter accumulation in the pre-anthesis and the grain filling phase in the corn crop. Cairns et al. (2012) [6] observed a positive relationship between biomass and grain yield under water deficit conditions in corn hybrids, demonstrating that this trait is important in obtaining higher yields.

Effects of water restriction also caused a reduction in chlorophyll concentration (CC) (Dualex index), even with lower expression than SB and PA. Evaluating CC in corn plants under water deficit conditions in the soil, Cairns et al. (2012) [6] found reductions in the leaf green index (using SPAD index) in hybrids and lines, being less expressive in the hybrids. Water restrictions in the soil and air cause, in the short term, stomatal closure and, consequently, less CO_2 arrival at the Rubisco enzyme carboxylation sites. However, if this water-limiting condition in the plant is persistent, damage to the photochemical machinery occurs, including chlorophyll degradation, considered a non-stomatal effect [37]. In the long-term situation of the reduction in stomatal conductance and with the frequent arrival of solar radiation, reactive oxygen species are formed, inhibiting enzymes of the Calvin–Benson cycle [38], causing effects such as the degradation of photosynthetic components, especially chlorophyll, with non-stomatal limitation in carbon assimilation [39,40].

4.2. Water Status

As a response to water limitation, the genotypes exhibited more negative values of $\delta^{13}C$, indicating the occurrence of stomatal closure for a longer time (source) during plant growth and development. Kamphorst et al. (2022) [1], in a study with lines and hybrids of popcorn under drought conditions, described that hybrids presented more negative values of $\delta^{13}C$ when compared to their parents, which is indicative of better use of water by the hybrids. In addition, under WS conditions, equivalent results were observed for corn plants [3,4,41]. The authors mentioned above proposed that more negative values of $\delta^{13}C$ are essential indications of genotypic susceptibility to water deficit since, for corn (C4 metabolism), the higher intensity of signal negativity in the magnitudes of the $\delta^{13}C$ isotope indicates more frequent stomatal closure of leaves [3,26,33].

Maintaining 30% of the available water capacity for plant growth/development resulted in a more expressive reduction in E, and then in gs (stomatal limitations of photosynthesis). When submitted to soil water deficit, corn plants show a decline in stomatal conductance and transpiration rate, causing a consequent decrease in growth and yield due to the lower assimilation of CO_2 [8,10,42]. Considering that, in the present study, the CC (Dualex index) expressed lower values of proportional reductions when compared to gs and E, it is assumed that the declines observed in SB and PA may be related to stomatal and non-stomatal limitations in CO_2 assimilation. Despite the stomatal and non-stomatal limitations on A, in general, hybrids, compared to their parents, have higher gas exchange rates, regardless of the WC in which they were evaluated [3,4,11].

Due to the water deficit applied to the soil, there was a reduction in cumulative plant transpiration (ET) values, which was expected due to the lower water availability in the rhizosphere region. Chairi et al. (2016) [4] described that heterosis in corn seedlings is more pronounced as a result of the higher water consumption of the plant, regardless of the WC applied. Although not explored in the present study, the highest heterotic expression was also reported by Kamphorst et al. (2022) [1] in popcorn when there was an increase in the water supply.

4.3. Traits Related to the Length of the Roots

As a result of the water deficit applied, reductions on root weight density (RWD) were observed in all evaluated soil sections. Roots play a fundamental role in the adaptation of plants to environments with water restrictions, so it can be suggested that higher growth of the root system — expressed by higher RWD — can provide increases in grain yield [43,44], as the higher root growth resulted in higher performance in hybrid yields under water deficit conditions [43]. Plants under WS conditions tend to exhibit lower RWD estimates. There is a high metabolic cost to the process of soil exploration by the root system. This metabolic cost is required from the photosynthetic capacity of the plant, and under water restriction conditions, it can exceed the rate of photosynthesis/day/plant by 50% [45]. Proving this demand for photoassimilates, the results obtained in this study show a reduction in the net CO_2 assimilation rate due to water deficit (Table 1) in plants with lower RWD values. In related works in which different root depths were evaluated in the soil sections in corn and popcorn genotypes, the highest RWD values were observed in the hybrids [4,14,24]. In the case of popcorn, it is worth mentioning that the RWD values in the sections c and e were the ones that expressed the smallest proportional reductions, considering the comparison between the WS and WW conditions, which may indicate that the higher investment for the deep root growth is associated with drought adaptation [1]. Ali et al. (2016) [43] described highly positive correlations between grain yield and root biomass in corn hybrids.

4.4. Implications in Popcorn Breeding Programs for Water-Limited Soil Conditions

There is a higher influence of non-additive effects (related to the specific combining ability) on the control of shoot biomass (SB), whole plant area (PA), and chlorophyll concentration (CC) (Dualex index), independent of WC. In this case, the exploitation of heterosis is recommended as a strategy to obtain genotypic superiority [3,11] and a higher adaptive advantage in water-deficient environments [1,3]. Although the mean square of the specific combining ability (ϕ_s) was predominant for morphological traits, some of them were also significant for the general combining ability (ϕ_g) and the reciprocal effect (ϕ_{rc}), also indicating the influence of additive gene action, as well as the influence of the female parent on the expression of traits [46,47], even to a lesser extent. In general, the hybrids that best took advantage of the beneficial effects of allelic complementation, also combined with high averages, were P6 × L75 for SB, regardless of WC; P6 × P7 and L61 × P7 for AP in WW and WS, respectively; and L75 × P7 for CC in WS.

In general, the hybrids that best took advantage of the beneficial effects of allelic complementation, also combined with high averages, were P6 × L75 for SB, regardless of WC; P6 × P7 and L61 × P7 for AP in WW and WS, respectively; and L75 × P7 for CC in WS. The hybrid P7 × P6 showed the highest average in WS, thus being a promising genotype in taking advantage of the beneficial effects of allelic complementation; in WW, the L61 parent is a potential source of favorable alleles in breeding programs. These results reveal that different breeding strategies for WS and WW should be used to obtain gains for this isotopic trait related to better water use.

The traits related to gas exchange (A, gs, and E) seems to provide a stronger influence of the reciprocal effect (due to the cytoplasmic effect and nuclear genes of the maternal genotype) on the expression of these traits, especially in WS. The reciprocal effect in crosses is due to the action of genes originating from the mitochondria and chloroplasts (extrachromosomal effect) and of genes originating from the nucleus of the maternal parent (maternal effect) [46,47]. Therefore, the selection of the female parent is essential to obtain superior hybrids for traits related to gas exchange. The present study results show that the L75 parent, when used as female, was the one that provided the greatest increase in the estimates of the averages of A, gs, and E.

Concerning ET, similarly to what happens with the morphological traits, a stronger influence of non-additive effects related to the specific combining ability is assumed. The hybrids that best took advantage of the beneficial effects of allelic complementation were P6 × L75 in both WCs; L75 × P6 in WW; and P7 × P6 in WS; being, therefore, the combinations indicated as superior for cultivation under conditions of water limitation.

Due to the prevalence of the quadratic effects of the specific combining ability (ϕ_s), it is recommended to explore heterosis to increase the root weight density (RWD) in any section of the soil, independent of WC, but mainly in the WS condition [21]. The hybrid L75 × P7 is recommended in experimental crops, especially for environments with water limitations, as it stood out with the highest average estimates, except in section a in WW. Nevertheless, the hybrid P6 × L75 in WS showed the highest average for section a, and high averages in the other sections, being the pair with the highest SCA estimate. In the WS condition, for the deeper sections of the root, the hybrid L75 × P6 also stood out for its highest averages and SCA estimates. Therefore, the combinations L75 × P7 in WW and P6 × L75 and L75 × P6 in WS are promising hybrids for obtaining superior gains in RWD. In addition to these, the hybrid L61 × L75 also showed promise in WS by expressing the highest average in the c-section and expressive values for the other sections. On the other hand, in the evaluation of a corn diallel under abiotic stresses (N deficit and water stress), Chun et al. (2005) [48] observed that for RWD, in particular, the heterosis values were significant under the distinct levels of N studied, which was attributed to the significant effects of general and specific combining ability, especially the latter. Based on this study and the results obtained here, it is emphasized that the use of hybrids is the feasible option to provide better root development under water deficit cultivation conditions.

In general, in this work, for the set of genotypes evaluated, it was found that both additive and dominance effects contributed to the expression of morphological, physiological, and water status traits, either under irrigation or stress conditions. However, as contributions to the popcorn breeding program in both water conditions, the dominance effects were more important in the expression of the evaluated traits and, for this reason, the heterosis exploration strategy is the most recommended to be applied, aiming at obtaining superior genotypes. To this end, the use of methodologies, such as the GEA, proposed by Cortés et al. (2018, 2019, 2021) [49–51], is an option that offers the possibility of genetic gains not only for greater tolerance to water deficit, but also for heat adaptation [50,52], favoring the identification of superior genotypes to multiple stresses; this therefore becomes a possible area of investigation in future studies with popcorn, with perspectives of generating promising results.

5. Conclusions

Based on the preponderance of non-additive genetic effects (associated with the quadratic components related to the specific combining ability) in the control of most morphological and root traits, especially in the condition of soil water deficit, it is recommended to explore heterosis for the success of breeding programs aimed at obtaining superior genotypes in environments with drought. In general, the hybrids that brought together high averages and favorable dominance deviations were P6 × L75, L61 × L75, L75 × P6, and L75 × P7. The P7 parent is considered a source of favorable alleles for traits A, CC, ET, SB, and RWDe. However, for water status traits, the correct choice of the female parent in the crossing block is essential, considering the maternal effect expressed on net CO_2 assimilation rate, stomatal conductance, and transpiration. In this sense, the parents L61 and L75 under well-watered (WW) conditions are considered promising in breeding programs developed through intrapopulation methods; under water stress (WS) conditions, the parents L75, P6, and P7 participated in combinations considered promising in the exploration of heterosis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071517/s1, Table S1: Summary of combined analysis of variance, for morphological traits, water status, and root traits of four parents and their hybrid combinations of popcorn grown under contrasting water conditions (WW and WS).

Author Contributions: Conceptualization, S.H.K.; methodology, M.M.C.; software, V.J.d.L.; formal analysis, F.N.V. and S.H.K.; investigation, M.M.C., S.H.K., V.J.d.L., J.T.L., K.F.M.S., U.A.d.O., D.L.L., J.L.P., G.F.P., H.D.V., E.C., J.G.d.O., R.F.D. and R.B.-S.; resources, A.T.d.A.J.; data curation, M.M.C.; writing—original draft preparation, F.N.V., M.M.C., S.H.K., E.C. and R.B.-S.; writing—review and editing, F.N.V., M.M.C., S.H.K., E.C. and R.B.-S.; supervision, A.T.d.A.J. and R.B.-S.; project administration, A.T.d.A.J.; funding acquisition, A.T.d.A.J. and R.B-S. All authors have read and agreed to the published version of the manuscript.

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