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Phenotypic Dissection of Drought Tolerance in Virginia and Carolinas within a Recombinant Inbred Line Population Involving a Spanish and a Virginia-Type Peanut Lines

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Abstract: Peanut (*Arachis hypogaea* L.) is a rainfed crop grown in both tropical and subtropical agro-climatic regions of the world where drought causes around 20% yield losses per year. In the United States, annual losses caused by drought are around $50 million. The objective of this research was to (1) identify genetic variation for the normalized difference vegetation index (NDVI), canopy temperature depression (CTD), relative chlorophyll content by SPAD reading (SCMR), CO_2 assimilation rate, and wilting among recombinant inbred lines (RILs) derived from two diverse parents N08086olJCT and ICGV 86015, to (2) determine if the physiological traits can be used for expediting selection for drought tolerance, and (3) experimental validation to identify lines with improved yield under water-limited conditions. Initially, 337 lines were phenotyped under rainfed production and a selected subset of 52 RILs were tested under rainout shelters, where drought was imposed for eight weeks during the midseason (July and August). We found that under induced drought, pod yield was negatively correlated with wilting and CTD, i.e., cooler canopy and high yield correlated positively with the NDVI and SPAD. These traits could be used to select genotypes with high yields under drought stress. RILs #73, #56, #60, and #31 performed better in terms of yield under both irrigated and drought conditions compared to check varieties Bailey, a popular high-yielding commercial cultivar, and GP-NC WS 17, a drought-tolerant germplasm.

Keywords: peanut; RIL; drought tolerance; pod yield

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1. Introduction

Peanut (*Arachis hypogaea* L.) is planted on 31.6 million hectares worldwide with a production of 53.6 million tons [1]. In the United States, peanuts are grown on 619,000 ha across eleven states under three geographical regions: Southeast (Alabama, Florida, Georgia, and Mississippi), the Southwest (Oklahoma, New Mexico, and Texas), and Virginia–Carolina (VC; North Carolina, South Carolina, and Virginia) [2]. In 2021, the U.S. produced $1.5 billion worth of peanuts, production in the VC region accounted for 15% of this total [3]. Peanut is widely grown as a rainfed crop in tropical and subtropical agro-climatic regions of the world, which are characterized by inconsistent rainfall patterns and reduced water availability at critical growth stages. Water deficiency along with high temperatures in the U.S. peanut belt can impact the growth and development of the crop, and reduce yield by approximately 70% [4–6]. The economic losses to the U.S. peanut industry due to drought are estimated at around $50 million per year [7]. The VC region grows over 90% of peanut acreage under rainfed production with abundant precipitation ranging between 965 and 1270 mm each year. However, the uneven distribution of rainfall (Figure 1) significantly limits peanut yield and quality in this region [8]. Similarly, extreme temperature predictions suggest yield reductions of up to 11% for each degree Celsius increase in temperature during the growing season [9]. In addition, the soils of the VC region are shallow and sandy with low water holding capacity [8]. Therefore, peanut is subjected to drought stress without irrigation in the VC region. Drought directly affects the transpiration rate, and thus photosynthesis, which results in a reduction in biomass accumulation and yield [10,11]. One of the solutions to reduce the impact of drought is to breed for drought-tolerant peanut genotypes to improve yield under water-limited conditions [11–13].

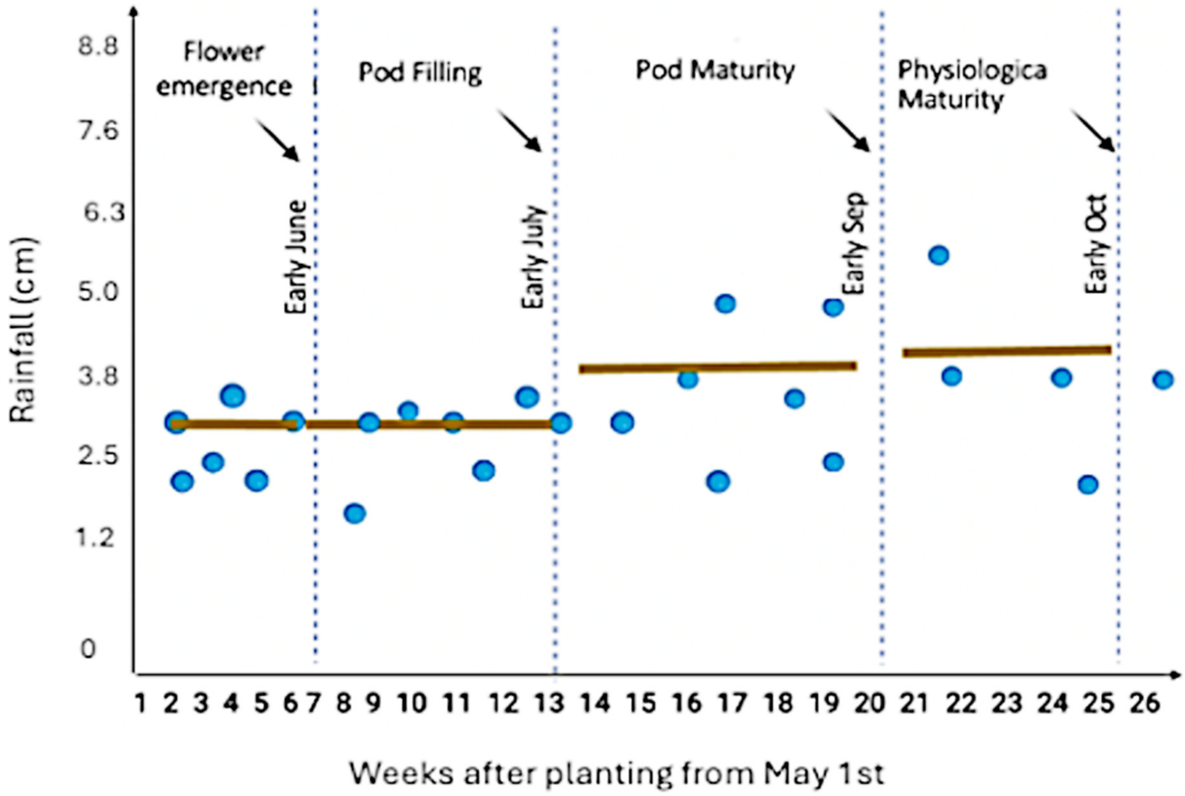


Figure 1. Average (20 years) annual rainfall patterns during peanut development in the VC growing region. Sequential weeks after a 1 May planting are shown on the *x*-axis. Weekly accumulated rainfall in centimeters (cm) is on the *y*-axis. The horizontal solid bar shows the optimum amount of rainfall required weekly at different growth stages to yield maximum. The vertical bars divide the growing season into different growth stages beginning from flowering to physiological maturity. These blue dots show the average rainfall in that week in the last 20 years.

Peanut is identified with different drought tolerance mechanisms including reduced deep and dense roots [14,15], high water-use efficiency (WUE) [16], reduced leaf area [17], quick transpiration reduction [13,18,19], early stomatal closure [20], and maintenance of vegetative growth [11] or photosynthesis [21]. Remarkable progress has been made through integrated approaches encompassing physiology and productivity in understanding the intrinsic mechanisms of drought tolerance in peanut [22]. However, the selection criteria for drought tolerance on traits like root density, WUE, and water-use calculation based on the carbon isotope discrimination technique require elaborative phenotyping and is very labor intensive, which limits the number of genotypes that can be analyzed [23]. Hence, more easily measurable correlated traits can be used on a large number of genotypes. Some of these traits are the normalized difference vegetation index (NDVI), canopy temperature depression (CTD), relative chlorophyll content by SPAD reading (SCMR), canopy height (HT), plant width (WD), wilting, and CO_2 assimilation rate as predictors of plant performance under drought stress [24–26]. NDVI was used as a screening tool for the stay-green trait, drought tolerance in wheat, with a significant relationship (r^2 = 0.5, $p < 0.0001$) between NDVI and grain yield [27]. In soybean, NDVI was used to differentiate between high- and low-grain yield genotypes, and it was concluded that NDVI can be used as a screening tool for genotypes with high-grain yield under irrigated conditions [28]. The agronomic characteristics of peanut including plant cover and yield were also associated with NDVI, for which NDVI was recommended for use as a screening tool for high yield in peanut. CTD has been recognized as an indicator of the overall plant water status and plant response to environmental stress [29–32]. Regarding positive and high values of CTD, i.e., cooler canopies when CTD = Ta − Tc, where Tc is canopy temperature and Ta represents the ambient air temperature that has been used as a selection criterion to improve drought and heat tolerance [33] and has been associated with yield increase among wheat cultivars. SPAD chlorophyll meter reading (SCMR) has been recognized as an important water stress tolerance trait contributing to yield variation under drought stress in peanut. The relative chlorophyll content (SPAD readings) quantitatively measures chlorophyll levels. A reduction in SPAD readings indicates a decline in photosynthetic activity due to drought stress. Under water stress, low cellular turgor pressure causes leaves to lose structural integrity resulting in leaf folding, rolling, or dropping [34]. Wilting can be visually assessed and associated with water stress in plants, and studies have shown a direct association between reduced leaf water potential under drought and wilting severity [35]. In peanut, wilting was negatively associated with the CO_2 assimilation rate and yield under drought [26]. Thus, visual wilting assessment is an important tool for quickly quantifying moisture stress in peanut across large populations [5,24,25,35,36]. Using these traits in combination, instead of relying on a single trait, may facilitate the identification of genotypes with improved drought tolerance. Genetic variability among peanut genotypes for root traits, specific leaf area (SLA), and SPAD was reported to identify drought-tolerant lines with high pod yield under stress [37]. The variation in transpiration efficiency (TE, g biomass/kg water transpired) was evaluated in peanut RILs using surrogate traits like SLA and SCMR, as TE is an important source of yield variation under drought. TE was negatively associated with SLA (r^2 = 0.15) and positively associated with SCMR during stress (r^2 = 0.17).

The perpetual nature of the RIL population enabling them to be evaluated in replicated trials over different locations and years provides advantages in terms of phenotypic and genotypic data reproducibility. It is also crucial at the same time to recognize the significance of using a segregating population like RILs derived from crosses between genetically diverse parents leading to a wide range of genetic variation among offspring. By analyzing these phenotypic variations within the population and further correlating them with genetic markers enables us to find genomic regions responsible for drought tolerance.

The present study was aimed at phenotyping of the RIL population for the identification of genomic variations among RILs for NDVI, CTD, SPAD, wilting, CO_2 assimilation rate under rainfed conditions, and experimental validation under drought stress among a set of lines for these measured traits.

2. Materials and Methods

2.1. Plant Material and Experimental Site

A recombinant inbred line (RIL) population of 337 individual peanut genotypes (lines) generated from a cross between 'N08086olJCT' and 'PI 585005 (ICGV 86015)' was grown at the Virginia Tech Tidewater Agricultural Research and Extension Center (TAREC), in Suffolk, VA (36.66° N, 76.73° W) and Peanut Belt Research Station, in Lewiston-Woodville, NC (36.13° N, 77.17° W) between 2018 and 2021. PI 585005 is a drought-tolerant Spanish-type line [18] from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, whereas N08086olJCT is a Virginia-type, large-seeded, high yielding, and less drought-tolerant line from the North Carolina State University. These parents were selected based on their contrasting traits for drought tolerance.

2.2. Experimental Design under Rainfed Production

Parents and RILs were phenotyped across two years (2018 and 2019) under rainfed conditions at both locations. RILs were planted in two-row plots of 3.05 m long × 0.9 m wide at Suffolk, VA, and single-row plots at Lewiston, NC using a randomized complete block design (RCBD) with two replications. The seeding rate was 13 seeds m^2 with a 2.13 m alley between each replicate.

2.3. Induced Drought Experiment

From the total of 337 RILs, a selected subset of 52 RIL lines was used in a rainout shelter experiment. In this experiment, cultivar 'Bailey' and germplasm 'GP-NC WS 17' were used as checks. Bailey is a high-yielding commercial cultivar, whereas GP-NC WS 17 is a drought-tolerant line with a stay-green characteristic [38]. The study was conducted for two growing seasons (2020–2021) at TAREC, Suffolk, VA. The subset (n = 52) was planted in single-row plots of 1.52 m long $\times$ 0.9 m wide at a rate of 13 seeds m^2 in an RCBD with both checks in three replications. All plots were irrigated before planting to provide uniform germination. Irrigation treatments were designed as two regimes: Irrigated (full irrigation, open field plots) and drought (rain-shelter covered plots). To satisfy the minimum water needs for high yield, irrigated plots received at least 600 mm of total water from rainfall and irrigation, throughout the growing season [39] . A lateral pull boom cart sprinkler irrigation system (E1025 Reel Rain, Amadas Ind., Suffolk, VA, USA) was used to irrigate the plots. Rain shelter plots were subjected to drought stress for eight weeks starting at 60 days after planting (DAP), which coincides with the critical stage of peanut for drought stress, i.e., reproductive stage from flowering to pod filling. Irrigation was completely withheld from plots that were covered with the rainout shelters for 6 weeks, after which the shelters were removed, and plots received rainfall until harvest. Except for the irrigation, agronomic practices were performed for all plots as recommended by the Virginia Peanut Production Guide [8]. Fluopyram and imidacloprid at 1.24 kg ai/ha were applied in-furrow at planting for tobacco thrips (*Frankliniella fusca*) control. Thrip control was maintained during vegetative growth by foliar application with 0.58 kg ai/ha acephate before flowering when an average of 25% threshold leaflet damage was observed. Biweekly applications of fungicides were started in late June to control foliar and soil-borne diseases using 0.15 kg ai/ha chlorothalonil, 1.26 kg ai/ha prothioconazole, and 0.21 kg ai/ha tebuconazole. Fluazinam at a rate of 0.52 kg ai/ha was used in late August. Calcium sulfate was applied at 1350 kg/ha rate at flowering. Boron liquid fertilizer was applied pre-plant and at the beginning of pod set at a rate of 1.62 kg ai/ha. In late July and August, 1.12 kg ai/ha manganese was also applied as a fertilizer. Pre-emergence and post-emergence weed control was applied when weeds were observed, and all plots received the same herbicide treatments each year. Daily weather, including air temperature and relative humidity (RH), was recorded using an on-site weather station (WatchDog 2000 Series Weather Station) starting from 1 May until end-September. Soil moisture data were collected every other week starting after 4 weeks after planting (WAP) at 10 cm, 20 cm, 30 cm, and 40 cm depth under irrigated and rain shelters in plots where Bailey and GP-NC WS 17 were grown. Soil moisture information was extracted using a Delta-T HH2 moisture meter (Delta-T Devices Ltd., Cambridge, UK).

2.4. Phenotyping of the RIL Population

The normalized difference vegetation index (NDVI) was measured for each plot on progressive weeks starting 5 weeks after planting (WAP) at different growth stages using a handheld GreenSeeker Crop Sensor (Trimble Ag., Sunnyvale, CA, USA) until physiological maturity. The measurements were taken at a height of 50 cm above and perpendicular to the canopy on sunny and wind-free days by scanning the hand-held GreenSeeker over both rows of each plot, from end to end of each row, and the average of both rows NDVI was recorded. The NDVI values were derived from the formula of red: near-infrared ratio [NDVI = (NIR − RED)/(NIR + RED)], where RED and NIR are the amounts of red and near-infrared light, respectively [40] .

Canopy temperature depression (CTD) of each plot was measured with a hand-held AGRI-THERM II™ infra-red thermometer (Agri-Therm Model 6110L, Everest Interscience

Inc., Tucson, AZ, USA). CTD was measured twice over a random spot on each row, at approximately 50 cm above the canopy and at a 45° angle. CTD values from both rows were averaged to calculate plot CTD. CTD was measured on sunny days with minimal wind. CTD was measured starting from 5 WAP until physiological maturity throughout the growing season. The "diff" option was selected to obtain the CTD value calculated by subtracting the canopy temperature (Tc) from the ambient air temperature (Ta), i.e., CTD = (Tc − Ta).

Plant height (HT) was manually measured weekly, between 4 WAP and 8 WAP. Two plants were randomly selected within each row per plot, and height of each plant was determined from the base of the main stem to the newest leaf. The canopy height of each plot was obtained by averaging the value of plant height from two rows. Similarly, lateral branching or plant width (WD) was randomly taken from each row and the two rows of a plot were averaged for lateral growth of the plot.

Leaf wilting was visually scored using a 0–5 rating scale. A score of 0 represented a potentially healthy plant with no visible wilting or leaf drooping symptoms; 1 represented some terminal and newer leaves folded but overall the plant looked healthy; 2 represented upper leaves almost all folded with visible signs of wilting, lower and older leaves started to fold; 3 represented wilting and drooping of all leaves on the plant and bare ground started to become visible; 4 represented all leaves were wilted and some started to change color, bare ground was prominently visible, some leaves dried and crisped; 5 represented all leaves were severely wilted and color of all leaves was light green to yellow, bare ground was fully visible, more than 50% of leaves were crisp and dry, plant was almost physiologically dead [26]. Leaf wilting was measured at different growth stages and drought severities starting from 6 WAP until physiological maturity.

The LI6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) was used for the measurement of CO_2 assimilation rate. Two LICOR readings were taken per plot from the newest fully developed leaf on the main stem. The chamber environment was set at 2000 µmols of light, 50% relative humidity, 400 ppm of CO_2, 500 µmol s^{-1} flow rate, and 10,000 rpm mixing fan speed. The chamber temperature was maintained at ambient temperature. The leaf was clipped for 60–90 s in the chamber until the readings stabilized.

Plots were dug from mid-September to early October at physiological maturity [41] with a KMC two-row digger. Pods were combined after a few days of windrow drying with a Hobbs peanut combine (Model 325A). Pod yield was calculated from plot weight and adjusted to 7% seed moisture.

2.5. Statistical Analysis

Phenotypic evaluations of the RIL population (n = 337) under rainfed condition for different traits were performed using generalized linear models (GLM) in R using the package 'agricolae'. For the selected subset (n = 52), we fitted linear mixed models wherein RILs were considered as fixed effects while year and replication were included as random effects as implemented in the 'lme4' package in R [42]. Pearson's correlation was performed separately for irrigated and drought conditions for different traits and yields using package 'corrplot' in R studio [43]. All the data were analyzed and visualized in R Studio in R 4.1.2 (R Core Team, Boston, MA, USA, 2021).

3. Results

3.1. Subsection Population Wide Phenotypic Variation among RILs under Rainfed Condition

Analysis of variance (ANOVA) showed significant variation among RILs at $p \leq 0.01\%$ for pod yield, NDVI, and CTD at the full pod stage, and wilting at the beginning of peg development (Table 1). The NDVI values ranged from 0.20 to 0.93, whereas for CTD and wilting, the values ranged from −6.0 to 0.6 and 1.5 to 3.6, respectively, under rainfed conditions (Table 1).

Table 1. Mean squares, minimum, maximum, and mean of NDVI, CTD, wilting, and pod yield of 337 RILs that were tested under rainfed conditions in 2019.

SOV	DF	NDVI	CTD	Wilting	Yield
RILs	336	0.02 **	0.89 *	0.14 **	3,874,631.03 **
Rep	1	0.02 ns	54.17 *	0.01 ns	663,610,350.1 **
Residuals	335	0.01	0.74	0.1	2,908,793.7
Min.		0.20	−6.0	1.5	317
Max.		0.93	0.6	3.6	9944
Mean		0.83	−3.1	2.7	5167

SOV = Source of variation, DF = degrees of freedom, ns = non-significant, Significant at $p \leq 0.01$ (**) and $p \leq 0.05$ (*).

3.2. Experimentally Induced Phenotypic Variation among a Selected Subset of RILs under Irrigated and Drought Conditions

A linear mixed model was applied to the phenotypic data using 52 RILs and the two checks, cultivar Bailey and germplasm GP-NC WS 17. Genotypes were treated as fixed effects, whereas replications nested within years were fitted as random effects. Principal component analysis (PCA) was carried out to create a PCA biplot of phenotypic measurements. Pearson's correlation matrix was used to measure correlation among phenotypes.

Analysis of variance of 52 RIL for 8 phenotypic traits, evaluated under drought and irrigated conditions, is presented in Table 2. The ANOVA revealed significant differences at $p < 0.05$ under drought stress for NDVI, CTD, SPAD, and yield, and at $p < 0.001$ for HT. Under irrigated conditions, significant differences were identified for NDVI, HT, WD, and yield. Under irrigation, line #42 performed significantly better than Bailey for pod yield when Bailey was used as the check. Under drought, lines #33, #4, #71, and #76 performed better using GP-NC WS 17 as the check (Table 3 and Figure 2a,b). Under both drought and irrigated conditions, RILs that performed significantly ($p < 0.005$) better for NDVI relative to both checks were #11, #136, #154, #188, #220, #35, #38, #44, #56, and #60 (Table 3). For CTD, RILs #131, #146, and #215 were significantly better than Bailey under irrigated conditions; in contrast, under drought, there was not a single RIL with a CTD value greater than the check variety GP-NC WS 17 (Table 3). For leaf wilting, we identified line #69 as performing significantly better than 'Bailey' under irrigated conditions. For plant height, under both irrigation regimes, lines # 110 #135, #154, #165, #166, #188, #201, #220, #280, #31, #1, #44, #69, #8, and #92 were higher than respective checks. (Table 3). The RILs performing better ($p < 0.1$) for SPAD under irrigated conditions were #42 and #154; however, we did not notice any differences among lines under drought conditions (Table 3). In contrast to SPAD, we found lines (#2, #56, 59, #77, and #90) significantly ($p < 0.01$) different for the CO_2 assimilation rate under drought (Table 3).

Table 2. Analysis of variance showing means square values of 52 RILs for 8 phenotypic traits across two years evaluated under drought (rain shelter) and irrigated conditions.

Trait	Drought	Irrigated
NDVI	0.002167 **	0.0004828 ***
CTD	0.001041 **	0.06608
Wilting	0.1085	0.1279
SPAD	0.006784 **	0.5721
HT	0.0000001483 ***	0.0000002 ***
WD	0.01146 *	0.0000002 ***
Photosynthesis	0.4455	0.4512
Yield	0.01257 *	0.02046 *

Significant at $p \leq 0.001$ (***); $p \leq 0.01$ (**) and $p \leq 0.05$ (*).

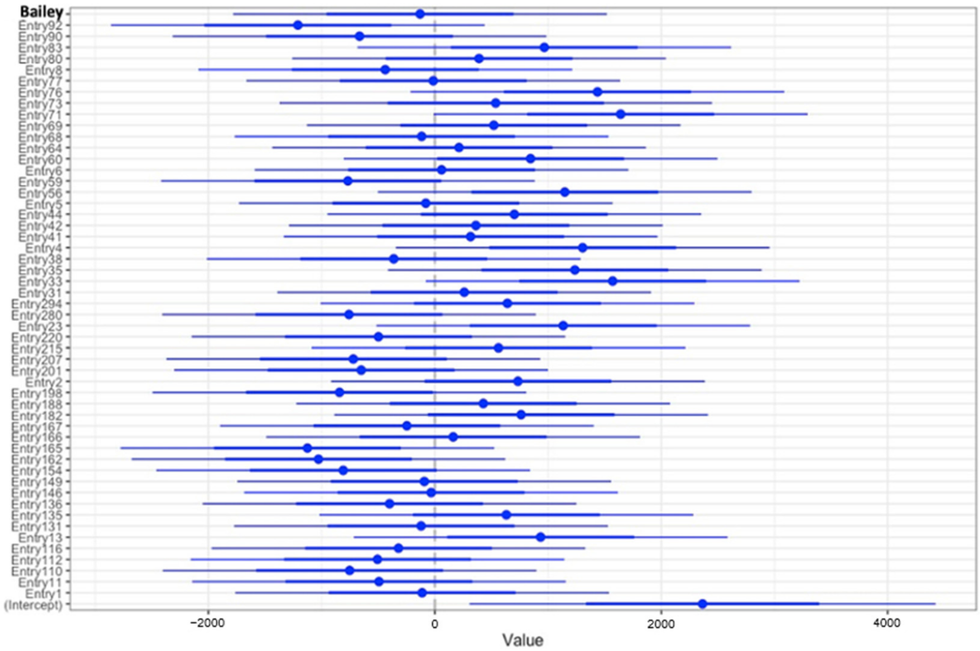
Table 3. Regression coefficient estimates for RILs along with checks under drought and irrigated conditions for NDVI, CTD, HT, and SPAD.

RIL Lines	NDVI [z]		CTD [y]		HT [x]		SPAD [w]	
	Drought	**Irrigated**	**Drought**	**Irrigated**	**Drought**	**Irrigated**	**Drought**	**Irrigated**
Line1	0.030	0.038 *	0.256		2.640 ***		−1.400	
	(0.023)	(0.022)	(0.394)		(0.824)		(2.853)	
Line11	0.072 ***	0.042 *	−0.098	−0.262	4.220 ***	2.640 ***	0.800	2.190
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line110	0.058 **	−0.016	−0.004	0.170	4.060 ***	2.200 ***	−3.660	2.470
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line112	0.072 ***	0.026	0.346	−0.150	3.360 ***	0.400	0.320	−4.030
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line16	0.028	0.022	0.218	−0.052	2.880 ***	0.420	−3.440	−2.610
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line13	0.018	0.032	0.026	−0.194	1.580 *	0.740	−3.760	1.180
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line131	0.014	0.020	0.288	0.544 *	1.240	−0.560	−2.680	−3.550
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line135	0.020	0.042 *	0.154	−0.092	2.560 ***	2.200 ***	−0.900	0.930
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line136	0.056 **	0.040 *	0.112	−0.138	4.260 ***	3.060 ***	−2.260	−1.230
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line146	0.016	−0.006	0.020	0.614 **	1.200	−0.380	3.720	−1.790
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line149	0.006	0.004	0.414	0.130	2.060 **	0.600	−2.480	−2.570
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line154	0.056 **	0.044 **	−0.716 *	0.112	4.800 ***	3.580 ***	−3.400	−6.390 *
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line162	0.042 *	0.018	0.624	0.426	0.660	−0.840	−4.360	−3.930
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line165	0.032	0.026	−0.072	0.098	2.860 ***	2.700 ***	−3.480	−0.270
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line166	0.060 ***	0.028	−0.280	0.096	4.520 ***	1.800 **	−1.120	−3.750
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line167	−0.008	0.002	0.386	0.144	0.820	−0.920	−1.220	−1.890
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line182	0.056 **	0.008	−0.088	−0.110	1.420 *	−0.260	2.680	−2.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line188	0.078 ***	0.044 **	−0.076	−0.326	4.000 ***	2.260 ***	−5.180 *	−5.110
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line198	0.012	0.036	0.638	0.064	0.960	0.100	1.600	−1.850
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line2	0.048 **	0.014	0.322	0.000	1.800 **	−0.500	4.980 *	−2.590
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line201	0.040 *	0.006	0.140	0.256	3.260 ***	1.740 **	−0.940	−6.250 *
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line207	0.054 **	0.018	0.202	0.344	1.420 *	−0.700	0.780	−3.750
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line215	0.040 *	−0.008	−0.406	0.664 **	2.620 ***	0.820	−2.960	−1.090
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line220	0.056 **	0.064 ***	−0.106	−0.018	4.600 ***	3.140 ***	1.820	−4.790
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line23	0.026	0.034	−0.254	0.386	1.780 **	0.900	3.040	−1.490
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line280	0.086 ***	0.056 **	−0.772 *	−0.144	5.660 ***	3.340 ***	−4.060	−2.310
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line294	0.006	−0.010	−0.080	0.320	2.220 ***	0.540	−1.040	−4.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line31	0.018	−0.018	−0.540	0.262	1.080	−0.660	1.680	−0.610
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)

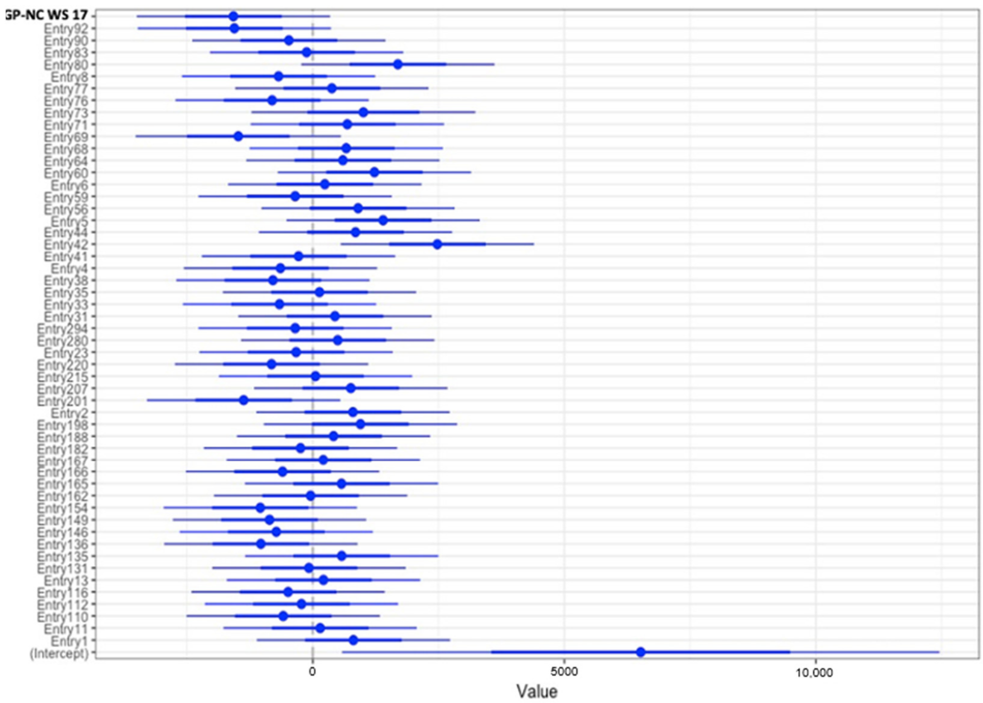
Table 3. *Cont.*

RIL Lines	NDVI ^{z}		CTD ^{y}		HT ^{x}		SPAD ^{w}	
	Drought	**Irrigated**	**Drought**	**Irrigated**	**Drought**	**Irrigated**	**Drought**	**Irrigated**
Line33	0.042 *	0.016	0.202	−0.148	3.240 ***	2.100 ***	−0.640	−3.490
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line35	0.058 **	0.046 **	−0.218	−0.108	3.040 ***	2.460 ***	−1.620	−2.150
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line38	0.062 ***	0.038 *	−0.382	0.156	2.480 ***	0.980	−0.640	−2.350
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line4	0.052 **	0.024	−0.456	−0.082	2.120 **	0.740	3.480	3.170
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line41	0.068 ***	0.030	0.192	0.268	3.000 ***	2.280 ***	1.040	−1.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line42	0.048 **	0.066 ***	−0.422	−0.212	2.320 ***	1.200	−0.640	−6.250 *
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line44	0.070 ***	0.052 **	−0.828 **	−0.138	3.560 ***	2.060 ***	1.620	0.630
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line5	0.046 **	0.018	−0.020	−0.084	2.020 **	−0.040	4.120	−3.710
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line56	0.040 *	0.048 **	−0.194	0.368	1.760 **	0.300	−1.180	2.690
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line59	0.042 *	0.016	0.292	0.278	2.140 ***	0.720	−3.020	−1.170
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line6	0.036	−0.008	0.278	0.070	1.640 **	0.560	0.400	−2.430
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line60	0.060 ***	0.048 **	−0.122	0.114	2.280 ***	0.740	−3.420	−1.810
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line64	0.074 ***	0.036	−0.108	0.160	3.380 ***	1.560 **	−0.720	0.090
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line68	0.072 ***	0.024	−0.398	−0.190	3.440 ***	1.800 **	4.300	−3.270
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line69	0.018	0.018	−0.448	0.052	3.580 ***	2.547 ***	1.020	−3.542
	(0.023)	(0.023)	(0.394)	(0.303)	(0.824)	(0.785)	(2.853)	(3.505)
Line71	0.036	0.024	−0.866 **	0.266	2.600 ***	−0.180	−5.320 *	0.930
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line73	0.093 ***	0.040	−0.245	0.419	4.135 ***	2.071 **	3.062	−4.531
	(0.027)	(0.025)	(0.457)	(0.330)	(0.954)	(0.856)	(3.302)	(3.822)
Line76	0.046 **	−0.008	−0.494	0.368	2.280 ***	−0.400	1.220	−2.550
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line77	0.056 **	0.020	−0.196	0.136	2.680 ***	−0.180	−1.380	−0.510
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line8	0.052 **	0.030	−0.234	0.038	3.320 ***	2.160 ***	1.240	−2.550
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line80	0.026	0.022	0.730 *	0.322	1.420 *	0.720	−2.120	−0.050
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line83	0.046 **	−0.004	−0.310	0.302	4.280 ***	1.140	0.320	1.970
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line90	0.048 **	0.026	0.746 *	0.232	0.780	−1.180	1.780	−2.450
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Line92	0.054 **	0.034	0.396	0.224	3.720 ***	1.980 ***	−3.320	−3.850
	(0.023)	(0.022)	(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
Bailey	0.040 *		−0.190	0.468	2.360 ***	−0.320	−3.840	−2.070
	(0.023)		(0.394)	(0.285)	(0.824)	(0.739)	(2.853)	(3.303)
		−0.058 ***		0.490 *		−2.380 ***		−0.730
		(0.022)		(0.285)		(0.739)		(3.303)
Constant	0.588 ***	0.627 ***	−0.773	−1.290 ***	6.382 ***	8.980 ***	40.182 ***	42.462 ***
	(0.018)	(0.020)	(0.930)	(0.311)	(0.842)	(0.750)	(2.918)	(2.782)

^{z} Normalized difference vegetation index, ^{y} Canopy temperature depression (CTD), ^{x} Canopy height, ^{w} SPAD, Standard errors in parenthesis, Significant at $p \leq 0.001$ (***); $p \leq 0.05$ (**) and $p \leq 0.1$ (*).



(**a**)



(**b**)

Figure 2. (**a**). Coefficient plot depicting pod yield for 52 RILs under irrigated conditions using Bailey as a check for comparison. Model-determined coefficients are along the *x*-axis and entry (RIL line or check) is along the *y*-axis. Dark horizontal blue lines through each point represent 95% confidence intervals and the dark blue dot represents the estimated mean values. Values are calculated from statistical regression models and are a direct reflection of parameter estimates. (**b**). Coefficient plot depicting pod yield for 52 RILs under drought conditions using GP-NC WS 17 as a check for comparison. Model-determined coefficients are along the *x*-axis and entry (RIL line or check) is along the *y*-axis. Dark horizontal blue lines through each point represent 95% confidence intervals and the dark blue dot represents the estimated mean values. Values are calculated from statistical regression models and are a direct reflection of parameter estimates.

3.3. Association among Physiological Traits for Drought and Irrigated Conditions

The correlation matrix between NDVI, CTD, and wilting at different growth stages or time points is shown in Figure 3. Correlations were weak under rainfed conditions between pod yield and NDVI (r = 0.03); CTD (r = −0.07); and wilting (r = −0.04). The association was significant and NDVI was inversely related to CTD, i.e., the higher the NDVI, the cooler the canopies. Pearson correlation among the traits for drought and irrigated conditions is summarized in Figures 3 and 4, respectively. The correlation under drought revealed that CTD and wilting were negatively correlated with pod yield (r = −0.42), whereas NDVI and HT were positively correlated (r = 0.65) with each other (Figure 4). Under irrigated conditions, NDVI and HT were negatively correlated with CTD (r = −0.52). Peanut WD exhibited a significant correlation with NDVI (r = 0.5).

The first three PCs with Eigenvalues greater than one accounted for 42.50% of the total phenotypic variability exhibited by the studied traits under drought-stressed conditions (Figure 5), whereas, under irrigated conditions, the first four PCs with eigenvalues greater than one account for 40% of the total phenotypic variability (Figure 6). The main contributing traits to PC1 and PC2 under drought conditions were NDVI, CTD, HT, and yield (Figure 7). Under irrigated conditions, NDVI, CTD, and HT contributed primarily to PC1 and PC2 (Figures 8 and 9).

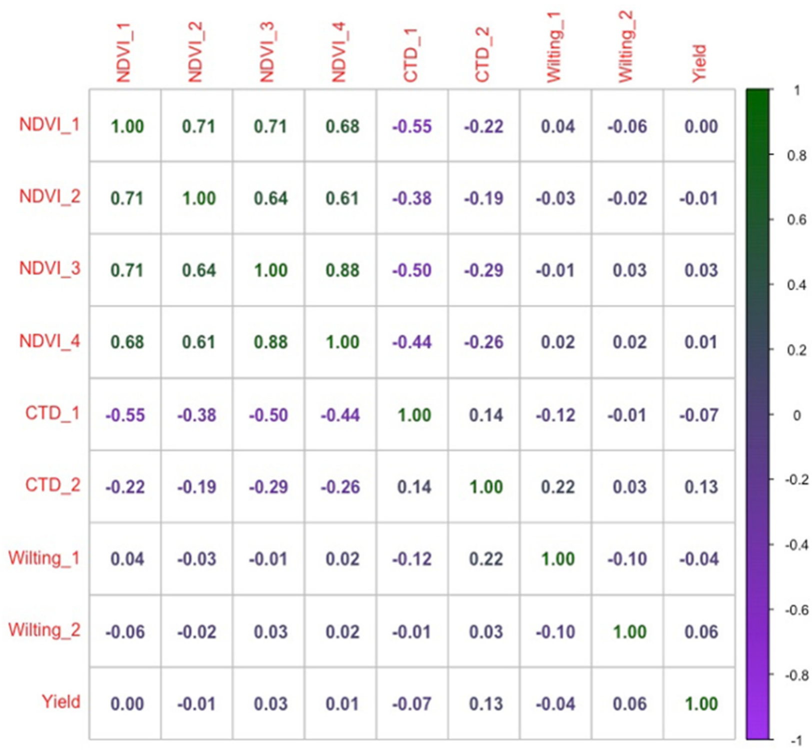


Figure 3. Correlation matrix of all measured parameters for drought tolerance across 337 RILs under rainfed field conditions. The vertical blue bar represents the range of correlation coefficients from −1 to +1 in a correlation matrix. It indicates the strength and direction of the relationship between different measured parameters for drought tolerance. Numbers in the boxes indicate the strength of the relationship as determined by Pearson's correlation coefficient (r; r > 0.113 denotes significance at 0.05 probability level). The numbers beside the NDVI, CTD, and wilting represent different dates where these were measured.

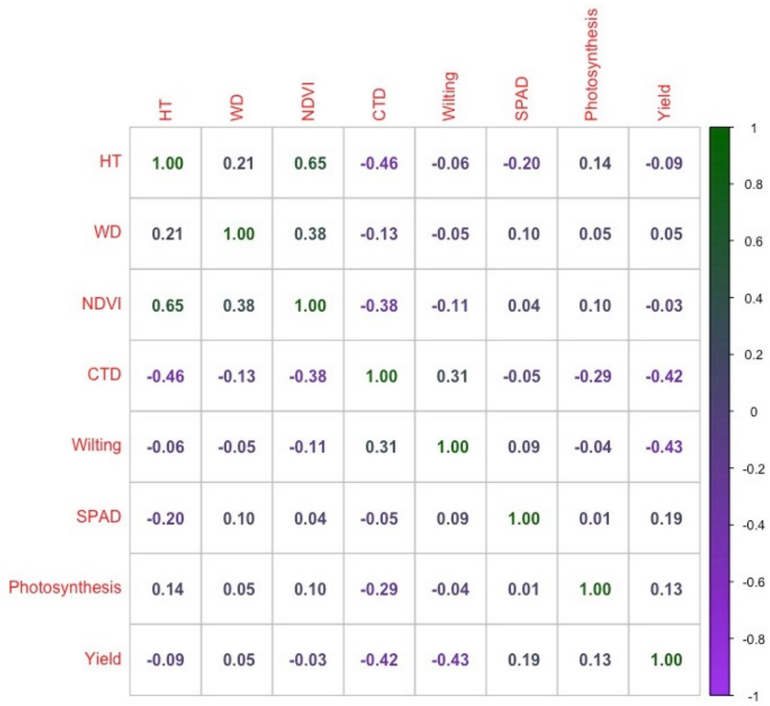


Figure 4. Correlation matrix of all measured parameters for drought tolerance on 52 RILs measured under drought (rain shelters) condition. The vertical blue bar represents the range of correlation coefficients from −1 to +1 in a correlation matrix. It indicates the strength and direction of the relationship between different measured parameters for drought tolerance. Numbers in the boxes indicate the strength of the relationship as determined by Pearson's correlation coefficient (r, r > 0.273 denotes significance at 0.05 probability level)). List of parameters measured: yield, photosynthesis, SPAD, wilting, CTD, NDVI, WD, and HT.

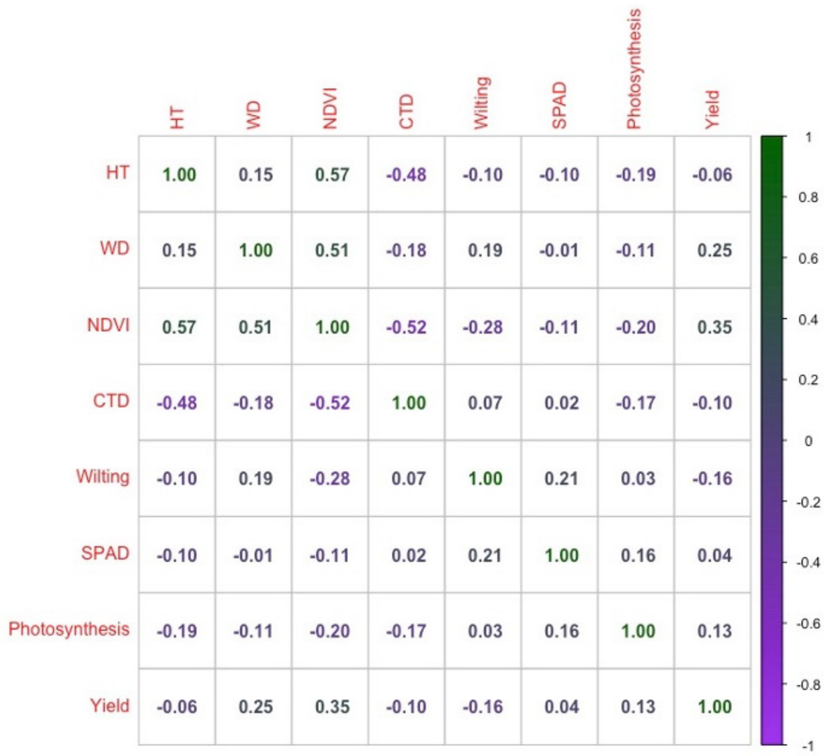


Figure 5. Correlation matrix of all measured parameters for drought tolerance on 52 RILs measured under irrigated condition. The number in the box indicates the strength of the relationship and correlation coefficient (r).

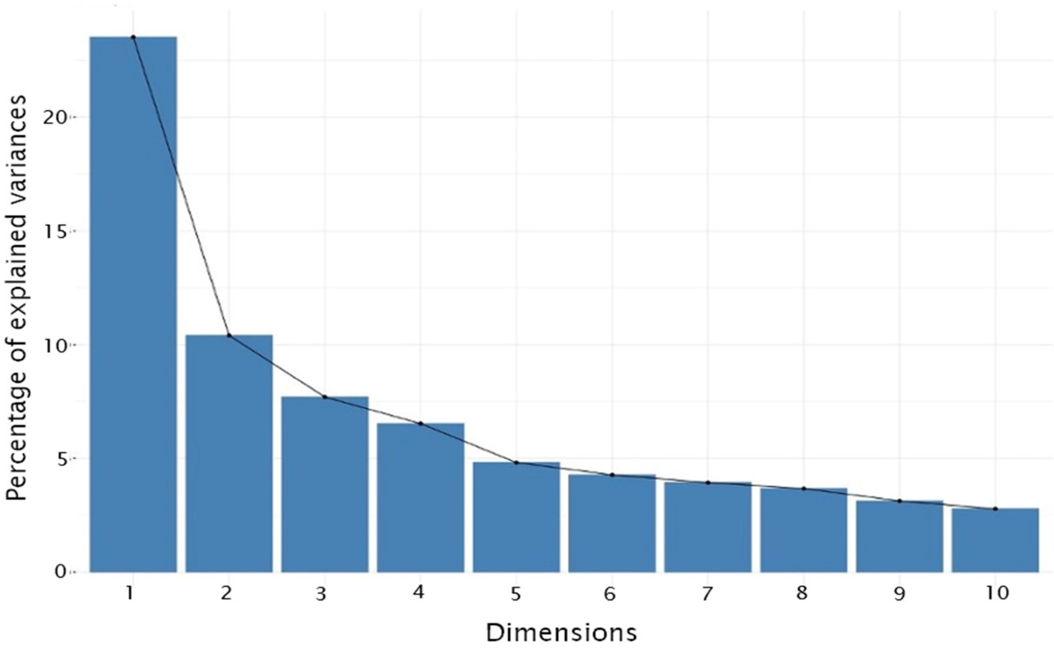


Figure 6. Scree plot of eigenvalues representing the percentage of variance explained by each principal component under drought-stress (rain shelters) conditions.

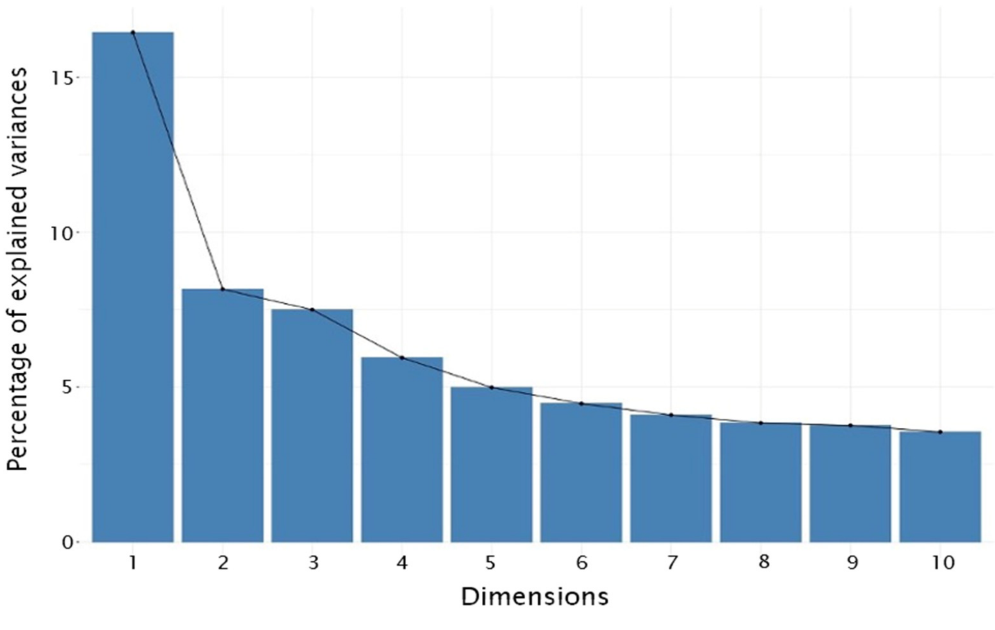


Figure 7. Scree plot of eigenvalues representing the percentage of variance explained by each principal component under irrigated conditions.

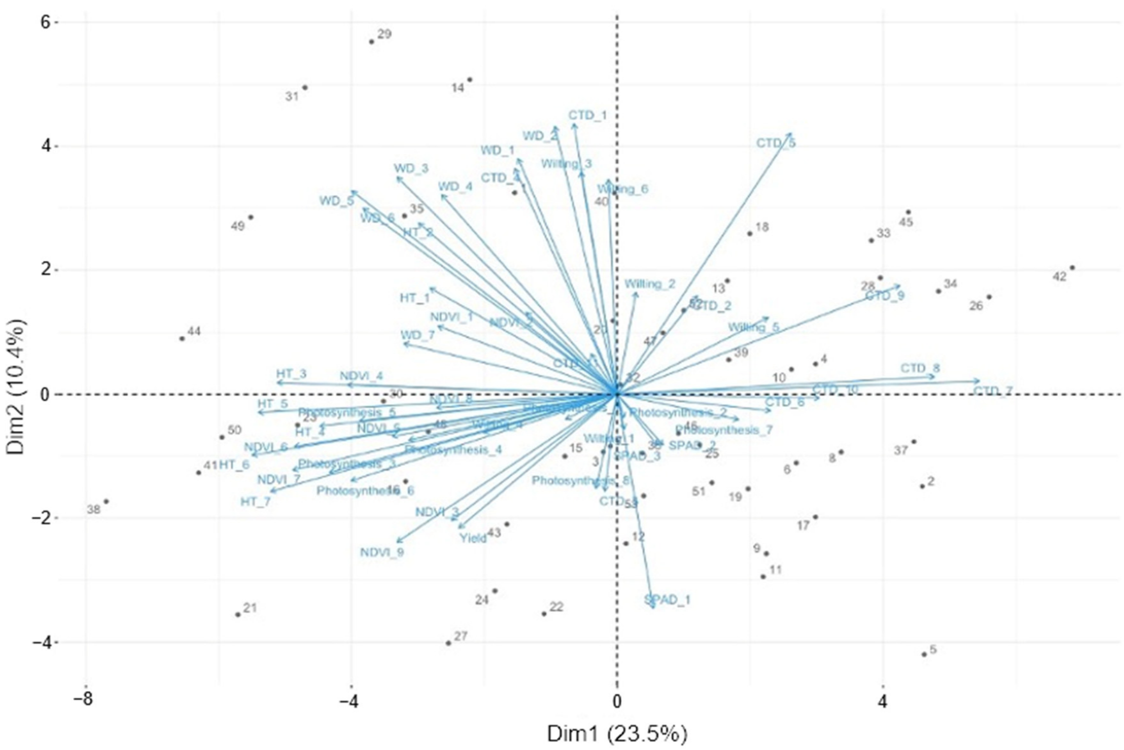


Figure 8. Principal component biplot of various phenotypic traits of 52 RILs evaluated under drought (rainout shelters) conditions. Traits farther from the center have higher variance, and the higher the angle between traits the lower the correlation.

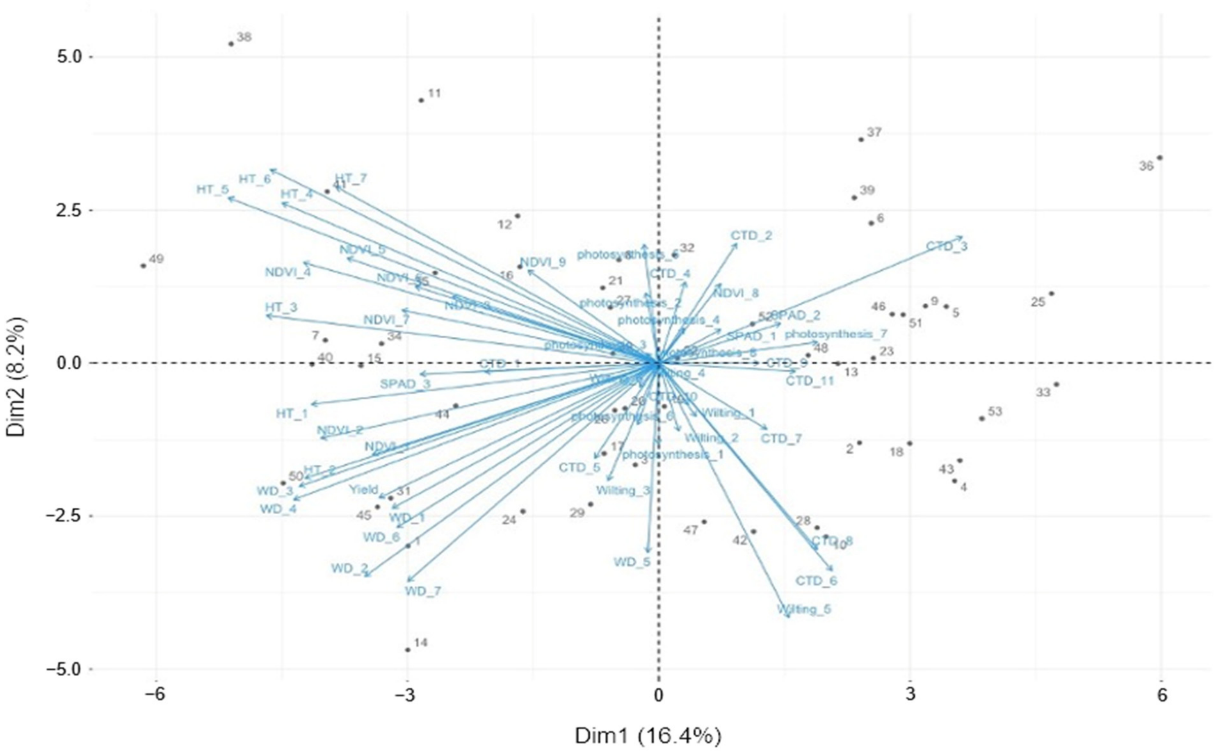


Figure 9. Principal component biplot of various phenotypic traits of 52 RILs evaluated under irrigated conditions. Traits farther from the center have higher variance, and the higher the angle between traits the lower the correlation.

In terms of yield, the lines that performed better than checks under both water regimes were # 31, #56, #60, and #73 (Figure 10).

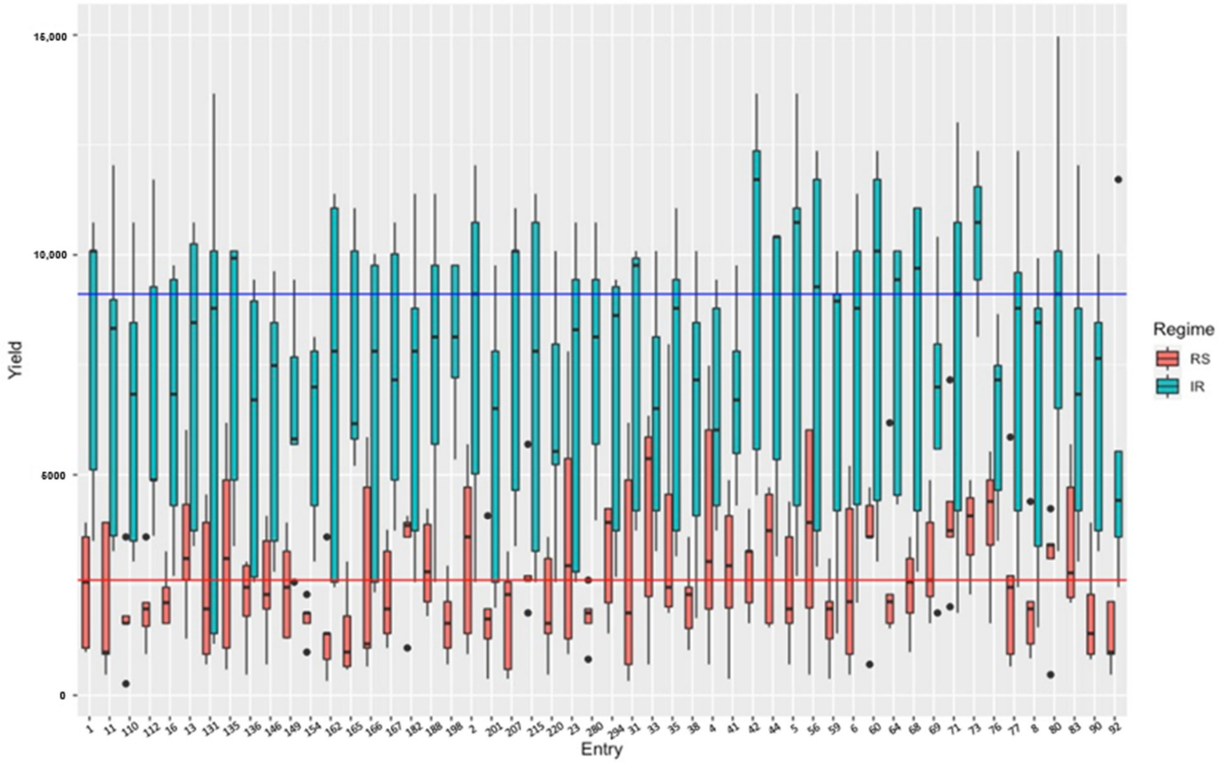


Figure 10. Boxplot indicating variation in pod yield (kg ha^{-1}) under irrigated and drought conditions for the years 2020 and 2021 for the selected subset (n = 52) lines. The red horizontal line represents the mean yield of GP-NC WS 17 as a check under drought. The blue line represents the mean yield of Bailey as a check under irrigated condition.

4. Discussion

Crop growth during the growing season and yield are dictated by the cumulative rates of photosynthesis and transpiration; however, both are reduced by drought stress. Previous studies reported that an increased CO_2 assimilation under water-limited conditions enhanced peanut growth and pod yield [16]. Parents (N08086olJCT × ICGV 86015) differed for transpiration traits under field and controlled conditions. ICGV 86015 is an early maturing, drought-tolerant Spanish line [22] with high TE under rapid drying of soil moisture [18]. Screening of all 337 lines under field conditions for direct measurements of photosynthesis, transpiration, and biomass is expensive and time-consuming. Therefore, NDVI, CTD, and SPAD, which are closely related to photosynthesis and transpiration were, instead, used to phenotype the RIL population for drought tolerance. NDVI is calculated from light reflectance by leaves in the red and near-infrared bands of the electromagnetic spectrum. Because both chlorophyll a and b absorb red light, higher NDVI values denote healthier leaves, i.e., healthy leaves absorb more in red wavelengths and less infra-red, therefore reflecting less in red and more in the near-infrared wavelengths than the unhealthy leaves. In diverse crops, CTD has been recognized as a key trait for evaluating plant response to low water use, high temperature, and other environmental stresses [33]. The current study evaluated the variation present among 337 RILs and a subset of lines (n = 52) for NDVI, CTD, and wilting for drought tolerance. Here, significant variation was found for NDVI under rainfed conditions, but not for CTD, wilting, or pod yield. These metrics were used to identify a subset of lines (N = 52) for experimental comparison under irrigated and drought-stressed conditions. Analysis of variance under drought stress revealed significant differences for NDVI, CTD, SPAD, and HT, whereas under irrigated conditions NDVI, HT, WD, and yield showed significant differences. Finally, we identified four RILs (#73, #56, #60, #31) that transgressed both check varieties in their respective conditions.

Developing high-yielding genotypes requires knowledge of the existing variability and degree of association between yield and the relative contribution of the yield-contributing traits [44]. The correlation of NDVI and pod yield under rainfed condition was insignificant, whereas, under drought conditions, the correlation was significant, ranging from 0.17 to 0.35. This might be due to the greater ability of NDVI to detect biomass differences between RILs under drought, where the canopy is not fully developed. These results agree with previous studies in wheat [45,46]. Higher CTD (positive) values under drought, i.e., the canopy was hotter than the air, might be due to decreased transpiration and increased respiration resulting from stomatal closure. There were no significant differences reported for wilting under both conditions. However, CTD showed differences under drought conditions and CTD was correlated with wilting. This shows that RILs with cooler canopies transpired more efficiently by using soil moisture and wilted less. These findings support previous studies in phenotyping drought-tolerant genotypes in peanuts [24]. The negative and strong correlations between CTD and wilting with pod yield revealed the significance of these traits in determining yield under drought conditions. SPAD showed significant variation among the selected subset of RILs under drought stress suggesting their sensitivity to detect differences among lines in response to drought tolerance. SPAD has been used as a selection trait for WUE in peanut [47]. The HT was highly significant and correlated with yield under irrigated and drought conditions. Reduction in canopy height under water-deficit conditions was attributed to a decrease in internodal length and was suggested to change dry matter partitioning and light interception [48–50].

In conclusion, our results concur with previous findings that NDVI, CTD, SPAD, and wilting are physiological traits related to peanut drought tolerance, even in a sub-humid environment, and that, in combination, these traits can be used for expedite selection for improved yield under drought in the VC region. We also found that certain RILs from the N08086olJCT × ICGV 86015 cross showed desirable recombination of traits related to yield improvement under water deficit. The selected RILs maintained high NDVI, low CTD, and wilting under water stress conditions, and enhanced yield. These RILs can be used to find genomic regions that are desirable for drought tolerance selection in the breeding programs in the VC region.

Author Contributions: M.B., D.C.H. and N.K. conceptualized the project. N.K. mainly accomplished the hypothesis and objective development, with valuable suggestions and comments from M.B., D.C.H. and J.C.D. N.K. collected and analyzed the data and wrote the manuscript. M.B. and D.C.H. provided a thorough review of the data analysis and manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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

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