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Screening for Drought Tolerance through Root Morphology and Yield Characters of Groundnut (*Arachis hypogaea L.*) Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Drought affects the rainfed groundnut (*Arachis hypogaea* L.) at different phases of development and it is the serious threats on groundnut productivity causing losses than any other abiotic factor under rainfed agriculture. In the world's semiarid regions, groundnut accounts for 90% of worldwide production. Drought mainly affects the pace and pattern of nutrient and water intake from the soil, affecting the architecture of the groundnut root system. Plant selections with desirable root trait have been a major focus in developing drought resistant Groundnut cultivars. In 2019, 60 groundnut genotypes were cultivated in root block design with two different soil water treatments, as well as in the field during the year under same circumstances. The purpose of this study was to see how different groundnut cultivars fared in terms of yield, yield contributing features, root characters, and their relationships with drought tolerance. Drought resistant genotypes had thicker roots, larger roots, and a deeper root system than susceptible genotypes. Recent series in

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groundnut genotypes of 60 numbers were sown during kharif 2019 (july-september) under rainfed condition (It includes life irrigation and rainfall received during cropping season). Groundnut genotypes were semi spreading with the duration of 110-120 days. Observation on root morphological character viz., roots length, root volume after 20 days of stress imposition of the crop and yield parameters were observed at the harvest. Among the 60 genotypes, 20 genotypes (VG 17008, VG 17046, VG 18005, VG 18102, VG 18077, VG 19572, VG 19709, VG 18111, VG19561, VG19576, VG 19620, VG 19681, VG 19688 etc.,) similarly, yield character were observed for 60 genotypes and all the genotypes given above recorded higher value in Total number of pods per plant, Number of double seeded pods per plant, Pod yield per plant, Harvest index and Total dry matter production. The methods used in this study identified correlation between yield character and root characters. Groundnut genotypes by assessing yield metrics and their relationship with root trait. These findings lay the groundwork for future study aimed at deciphering the molecular pathways underpinning Groundnut drought resistance.

Keywords: Root length (RL); root volume (RV); total dry matter production (TDMP); pod yield per plant (PYPP); total number of double seeded pods (TDSP); and total number of pods Per plant (TNPP); drought.

1. INTRODUCTION

Groundnut (*Arachis hypogea* L.) is an important oilseed crop because the seed contains 44–56 percent oil, which is used as a staple oil in most parts of the country, as well as 22–30 percent protein on a dry seed basis [1] making it the second most important protein source after soybean. Groundnut is primarily a rainfed crop that is grown on 19.3 million hectares of land in 82 countries. Arid and semi-arid regions account for more than half of the producing area. Abiotic stresses, such as heat and drought, are significant environmental conditions that frequently limit crop development and output [2 and 3]. Drought accompanied by high temperature are projected to become more common in the near future [4], highlighting the need to explore crop responses to combined heat and drought stresses [5]. Estimated increment in mean temperature of 1.3–6 °C in future climates [6] as well as an increase in the variability of temperature, will exacerbate these problems [7]. Due to climate change, groundnut is frequently subjected to drought and high temperature stresses of various durations and intensities. In India, groundnut yields varied from 550 to 1100 kg/ha in different years, resulting in total production ranging from 4.3 to 9.6 million tons. Drought stress has emerged as a serious issue in around 45 percent of agricultural areas and one of the most significant global constraints to productivity [8 and 9]. The rise and fall in the yield and production coincided with the percentage deviation from the mean annual rainfall along with temperature [10]. Breeding of crop for sustaining adverse climatic condition has been increased a lot nowadays but they concerned more towards the aboveground plant parts (forage, seed or grain production) for the generation of food, feed and fibre. Breeding of improved cultivars that can tolerate a variety of abiotic stress conditions such as drought, flooding high temperature is most promising thing. The methodologies include selection for improved plant growth characteristics such as grain or biomass yield, seed production, leaf surface area, the number of tillers and there should be a consideration towards “root breeding” which means the identification of the underground root traits that enable a them to modify themselves more efficiently towards the utilization of water and nutrients resources in different environments.

Root characteristics are believed to be a significant component of the dehydration postponement mechanism because they help to the regulation of plant development and the extraction of water and nutrients from deeper layers, among the many qualities that contribute to enhanced stress tolerance. Roots detect and respond to abiotic and biotic stresses, and they use signalling channels to connect with aboveground plant sections. Root shape and physiology influence the growth and development of aboveground plant organs through altering mineral nutrient transfer or the transit of a variety of organic signalling molecules like as hormones, proteins, and RNAs from the root to the shoot. Reduced water availability (drought) is a major abiotic stress that causes large crop losses [7], thus plant roots use morphological plasticity to adapt to and respond to soil moisture levels [6]. Identifying root characteristics that boost the capability of soil foraging for water and maintain productivity during periods of reduced water availability are examples of research fields with practical significance. Because various scientific studies have been conducted on distinct root features targeted for plant improvement under drought and nutrient limitation circumstances. Several architectural elements that contribute to drought resistance have been identified [11]. If there is water in the profile, a deep and thick root system will mine water from the deep horizon of the soil and react to evaporative demand, and is one of the most common features contributing to drought avoidance, at least in highland settings.

Despite the well-known importance of roots, very little breeding for root traits has been done due to a lack of a reliable, quick, and cost-effective screening methodology for phenotyping root traits in multiple environments [12]. The effectiveness of various rooting characters must be established. Despite the presence of significant GxE interactions, quantitative trait loci (QTL) for rooting depth, root volume, and root thickness (diameter) have been identified in rice [12]. Primary root length, primary root diameter, primary root weight, and adventitious seminal root weight QTL in maize have been mapped [13]. Unfortunately, large-scale, precise, and cost-effective phenotyping appears to be the restriction for QTL discovery of key root characteristics. The goals of this study were (i) assess the relevance of root traits for seed yield in groundnut under receding soil moisture and high temperature; (ii) identify an alternative procedure to screen groundnut genotypes with efficient roots under receding soil moisture and high temperature; and (iii) evaluate the variability of root traits under progressively receding soil moisture conditions and high temperature. In groundnut significant genetic variation has been observed in various root traits [14]. No information is available on the individual and combined effects of heat and drought stress on the root growth and in turn its effect on yield, which formed the reason for this study. A better understanding of root systems is critical to crop improvement in water-limited environments. Considering these aspects, the present study was undertaken with 60 groundnut genotypes and were screened for drought tolerance and high temperature tolerance to study the performance of groundnut genotypes for yield, yield contributing characters, root characters and their association with drought and high temperature tolerance.

2. MATERIALS AND METHODS

2.1 Plant Materials

2.2 Experimental Design

The experiments were conducted at regional research station at vriddhachalam cuddalore district, during the dry season 2019 (from March to June). The experiment was set up in a 2 × 11 factorial in randomized complete block design (RCBD) with three replicates with two water regimes (100 % field capacity and 50 % field capacity) measured using theta probe (ML3). Stress was given at pre flowering stage. A field trial was conducted in RBD replicated thrice to screen the genetic efficiency of 60 groundnut genotypes for their drought tolerance capacity. Sowing was adopted with a spacing of 30 x10 cm and the crop was raised to maturity.

2.3 Root Block Structure Trial

In 2019, two container-based studies were conducted. Root block design was used to cultivate the groundnut plants. The plots are raised above ground level to imitate a trench wall. Trench walls are created by digging the soil close to a plant in such a way that the root systems are exposed. The single trench wall plot's exterior measurements are 8 feet long and 3 feet wide. The plot has a height of 4 feet. Cement blocks are used to build the walls. Three sides of the trench are surrounded by walls, while one side is closed by a removable sliding door.

2.4 Field Trial

Two field trials were conducted during the post rainy season, in the month of March 2019 and 2020, in a Red sandy loam. Slightly acidic to alkaline in pH, Poor in water holding capacity, low in N, medium in P and K and micronutrient zinc and boron. The water holding capacity of this field in 1.9”/ft. The bulk density is 1.35 g cm-3 for the 0–15 cm soil layer and 1.45 cm-3 for the 105–120 cm soil layer, while the accessible soil water till 120 cm depth was 165 mm 26. Before sowing, 18 kilograms N per acre and 20 kg P per acre were applied prior to sowing. Plants were successively cultivated under rain-fed circumstances. Hand weeding was used to keep the plots free of weeds, and thorough protection against the Gram pod borer was implemented (*Helicoverpa armigera*). In 2019, root growth and

Table 1. Soil moisture and weather data

Month	Temperature (^{o}C)			Relative humidity (%)			Evaporation (mm)	Rainfall (mm)
	Max	Min	Mean	Max	Min	Mean		
March	39.6	29.0	34.3	79	50	64	134.0	00.3
April	46.2	31.2	38.0	86	64	75	96.5	00.1
May	45.7	33.0	39.5	91	69	80	105.4	00.8
June	39.2	29.5	34.2	89	67	78	102.0	00.2
Total							**109.47**	**0.35**

yield evaluation trials were undertaken at various locations for the purpose of screening root character. A separate root block design was built to examine the root character of 60 genotype. Both field and root block design were sown in the same date on 23th march 2019. The trials for root excavation and yield evaluation were grown in adjacent areas of the same location. The individual plot size was 1.5 m wide 4.0 m long with 33.3 plants m^{-2} on a broad-bed furrow in an RBD with three replications. At the end of the growing season, the root properties of each pot were assessed. To remove soil and debris, root samples of each genotype in the root block structure were manually washed on a wire mesh screen with tap water. The Gia root software V.2004a was used to analyze root samples. Root length (RL), and Root volume (RV) per sample. Root samples were oven-dried at 80 °C for 48 h or until constant weight and root dry weight (RDW) was determined. At the final harvest, total number of pod per plant, pod yield per plant, were obtained from 10 plants in each block of each variety. Biomass included total shoot and root, and pod yield per plant was also calculated along with the Harvest index (HI). For estimating total dry matter production, 10 plants were randomly pulled out with intact root system at the end of each stages in each treatments and weighed after drying the plants at 80ºC for 48 hours. TDMP (Total dry matter production) measured using a weighing balance and mean was calculated

Large physiological datasets obtained from plants grown under drought and well-watered circumstances are still difficult to evaluate and comprehend. For such assessments, a variety of methodologies and statistical models have been offered. In phenotypic screening for drought tolerance, correlation analysis, PCA, and clustering are considered to be good methods for analysing the correlations between the parameters and their principal components [15, 16].

2.5 Soil and Weather Data Collection

Red sandy loam soil was used for pot culture experiment. Soil texture was sandy loam (sand 51.6 %, silt 34.7 % and clay 11.5 %), bulk density (1.42 g cc^{-1}), particle density (2.51 g cc^{-1}), pH 6.69, EC 0.32 dS m^{-1}, water holding capacity at field capacity and available water were 18.0 % and 10.0 % respectively. Soil moisture was measured at planting using the gravimetric method. Rainfall, relative humidity (RH), maximum and minimum air temperature, evaporation (E0), and solar radiation were measured daily by a meteorological station 50 meters away from the study field from planting to harvest. The experiment was carried out in a transparent roofed open-sided greenhouse. Soil moisture was measured at planting using the gravimetric method.

Rainfall, relative humidity (RH), maximum and minimum air temperature, evaporation (E0), and solar radiation (Table 1) were measured daily by a meteorological station 50 meters away from the study field from planting to harvest.

3. RESULT AND DISCUSSION

Analysis of variance revealed significant differences among 60 genotypes for all the characters studied. The mean performance of genotypes for yield and root characters were given in Table 1. The genotypes VG 18005 had recorded the maximum pods yield per plant of 52.80 gm and found to be drought tolerant along with optimum and better root performance. The increase in yield in this genotype is due to more number of pods per plant (pod yield per plant) and higher double seeded pods per plant. Whereas, the genotype VG 17019 found to be highly susceptible to drought and recorded the lowest yield of 3.97 gm. The yield reduction in this genotype is due to reduction in spikelet fertility. Root length increased during drought compared to control condition and it ranged from

17 to 60 cm (Fig. 1). Drought resistant entries had recorded higher root length than the susceptible genotypes. Namuco et al. [17] reported that the drought tolerant varieties have thick and elongated root system than the susceptible ones. Highest rooting depth was recorded in VG 17008, VG 17046, VG 18005, VG 19709, and VG 19732. Similar findings of deep root system for drought resistant varieties were also reported by [18]. Similarly high variations were observed for root volume. There was a significant variation in root length and volume between genotypes and treatments (Fig. 1). The commencement, branching orientation, and turnover of new roots are all influenced by soil temperature [19, 20] found that an increase in root length and volume during a water shortage and after recovery was associated with greater drought tolerance. They also stated that the rationale for this rise was due to higher partitioning to root mass, which resulted in a propensity for reduced allocation to other portions of the plant, potentially conserving water. Rapid root growth into the surrounding soil would provide an adaptation advantage in terms of better utilizing the soil water. In groundnut, similar findings by [21, and 22] are in agreement with the current study.

Drought resistant varieties like VG 18005, VG 18102, VG 18077, VG 19572, VG 19709, and VG 19732 had recorded highest root volume than the susceptible entries. Root depth was found positively correlated with root volume, and root thickness. This result is in agreement with earlier findings of [23] and they also observed significant positive correlation of root depth with root volume in drought condition [24&25]. According to Yogameenakshi et al. [26], root volume had a highly substantial and positive connection with grains per panicle and 1000 grain weight at both the genotypic and phenotypic levels. A well-developed root system will help the plant in maintaining high plant water status [27]. Maintaining a greater leaf water status during grain filling under receding soil moisture circumstances is critical for increased grain production. This signifies that deep-rooted cultivars have thick roots and are drought resistant. C. Kumar [28] studied the interrelationships and cause-effect linkages of grain yield and its component qualities in thirty rice genotypes and obtained consistent results demonstrating the importance of root character..

Chlorophyll content, root length, panicle per plant, spikelet fertility, and root volume all exhibited a substantial and positive relationship with grain output per plant, according to the findings. Groundnut has strong root properties that allow it retain yield even in the face of severe drought [29]. Groundnut with larger root systems may have a higher output under drought stress, but RD in deeper soil layers may have a greater impact on pod production and harvest index [25].

Deep rooting, and root distribution have been identified as drought adaptive traits [30] that can be used as selection criteria for drought resistance. Drought, on the other hand, increased Rooting depth in a peanut genotype's lower soil profile, according to Pandey et al. [31]. Rucker et al. [32] observed that several peanut genotypes with extensive root systems had high yield under drought circumstances under non-stress environments, suggesting that these genotypes contained drought avoidance traits.

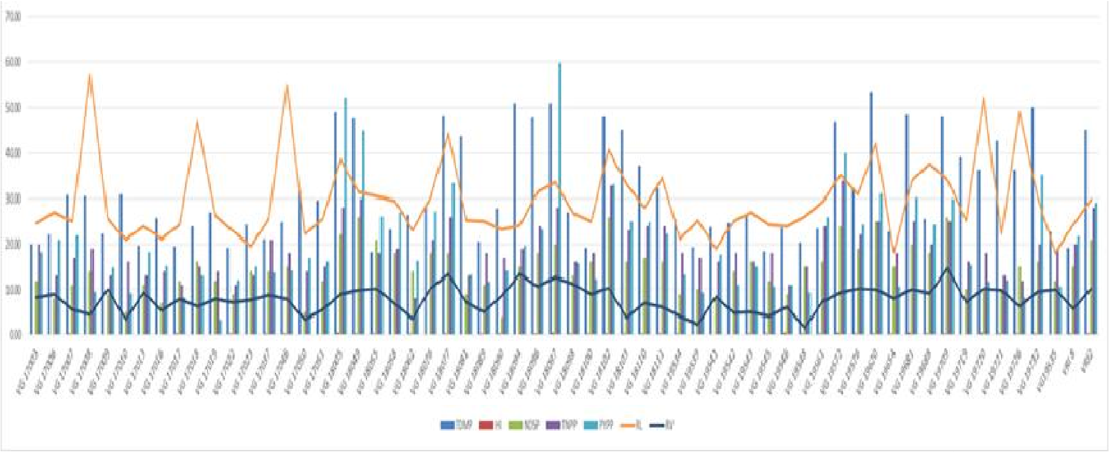

Fig. 1. Effect of drought on yield and root character

However, direct evaluation of deep roots and root spread of peanut genotypes under various water regimes to see how these traits respond to drought and high temperatures has not been adequately demonstrated. Reduction in TDMP due to elevated temperature was reported by [33]. In bean. A common adverse effect of drought stress on crop plants is the decrease in TDMP [34]. In this study, higher percent reduction of TDMP was observed in VG 17010, (45.39) and lowest in VG 18102 (34.76) in drought imposed plants. Nahar et al. [35] reported that drought severely reduce the biomass accumulation (Fig. 1). This is consistent with the current study, which found a greater decline in TDMP (Total dry matter production) during drought.

Optimum root length, root volume as they exhibited highly positive correlation with Harvest index and also a positive correlation among themselves. Having large root system helps in augmenting yield under drought. Selection based on root thickness and root depth is highly suitable for identifying varieties that can be used in groundnut improvement for drought tolerance.

The result of the Correlation coefficient matrix revealed that all the variables in the model are positively correlated at 1 and 5 % level of significance (Table 2). The coefficient indicates that there is evidence of parameter overlap between TDMP, HI, and PYPP, as well as harvest index, root length, and root volume. Total dry matter production, harvest index, root length, and root volume had the most overlapping among these relationships, with coefficients of 0.863 and 0.805, respectively. (Fig. 3).

Results of the PCA revealed that the total variation in the data was found to be 80.11% (first compound) and all the parameters were positively correlated with themselves. First component shows total yield variation and exhibited a positive correlation with Harvest index, root length and root volume. The PC2 explained 19.24% of the total yield variation and had a higher positive correlation with root growth and harvest index. Therefore, PC1 and PC2 were named yield potential and stress susceptibility, respectively. Based on this criterion, stable genotypes possessed greater PC1 but lower PC2 values and vice versa [36]. The results of a biplot drawn based on the PC1 and PC2 data for the 60 Groundnut genotypes showed the six genotypes VG 18005, VG 18102, VG 18077, VG 19572, VG 19709, and VG 19732 closely located to the performing genotype under drought with high PC1 but low PC2 values. On the other hand, the majority of genotypes with low PC1 and high PC2 values were identified as susceptible genotypes. These included VG 17010, VG 17016, VG17018, VG17050, VG 19535 (Fig. 2). The principal compound (PC3, PC4, PC5, PC6, and PC7) has negligible variations of 3.64 (Table 3).

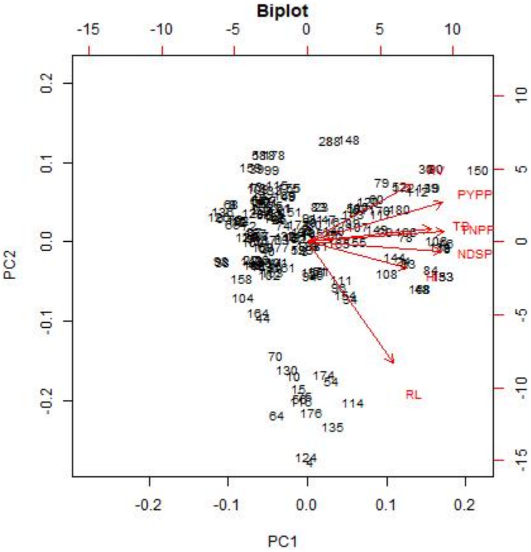

Fig. 2. Biplot drawn based on the first and second components obtained from principal component

Table 2. Correlation between Root trait and yield

Char	TD	HI	NDSP	TNPP	PYPP	RL	RV
TD	1						
HI	0.493*	1					
NDSP	0.533	0.516	1				
TNPP	0.619	0.495	0.769	1			
PYPP	0.652	0.518	0.698	0.771	1		
RL	0.373	0.341**	0.458	0.370	0.262*	1	
RV	0.485	0.388**	0.474	0.437	0.552*	0.205	1

Total Dry Matter Production (TDMP), Harvest index (HI), Pod yield Per Plant (PYPP), Total Number of Double Seeded Pods (TDSP), Total Number of Pods Per plant (TNPP), Root Length (RL), Root Volume (RV)

Table 3. Eigen value and vectors of principal component analysis for yield and root trait under drought

Principal compound	PC1	PC2	PC3	PC4	PC5	PC6
Proportion of Variance	0.57	0.12	0.09	0.09	0.07	0.03
Cumulative Proportion	0.57	0.69	0.78	0.87	0.94	0.97
EigenValues	4.00	0.84	0.65	0.62	0.47	0.23
TD	0.40	0.09	-0.14	0.13	-0.82	0.33
Harvest index	0.32	-0.19	0.92	-0.08	-0.01	0.08
NDSP	0.43	-0.06	-0.20	-0.21	0.47	0.51
TNPP	0.44	0.07	-0.20	-0.37	0.12	0.07
PYPP	0.43	0.27	-0.05	-0.28	-0.06	-0.74
Root length	0.27	-0.84	-0.22	0.30	0.01	-0.27
Root volume	0.33	0.40	0.04	0.79	0.29	-0.04

Total Dry Matter Production (TDMP), Harvest index (HI), Pod yield Per Plant (PYPP), Total Number of Double Seeded Pods (TDSP), Total Number of Pods Per plant (TNPP), Root Length (RL), Root Volume (RV)

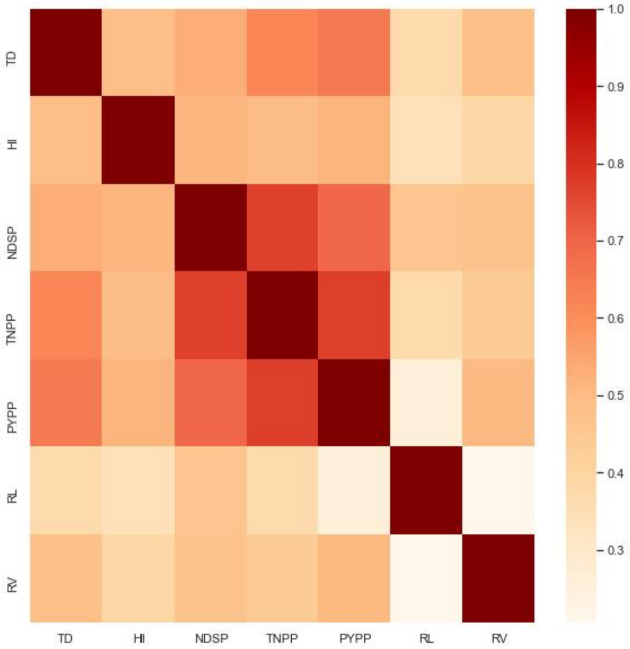

Fig. 3. Correlations heat map based on Root trait and Yield. Strong intensity of color shows the strong correlation

Total Dry Matter Production (TDMP), Harvest index (HI), Pod yield Per Plant (PYPP), Total Number of Double Seeded Pods (TDSP), Total Number of Pods Per plant (TNPP), Root Length (RL), Root Volume (RV).

Table 4. Summary of analysis of variance for the effects of treatments, lines, and the interaction

Char	TDMP	HI	NDSP	TNPP	PYPP	RL	RV
Treatment	**	**	NS	NS	*	**	**
Genotype	**	**	NS	*	*	**	*
TxG	**	**	*	*	*	**	**

*** Significant at P ≤ 0.01, NS nonsignificant at P ≤ 0.05*

Total Dry Matter Production (TDMP), Harvest index (HI), Pod yield Per Plant (PYPP), Total Number of Double Seeded Pods (TDSP), Total Number of Pods Per plant (TNPP), Root Length (RL), Root Volume (RV)

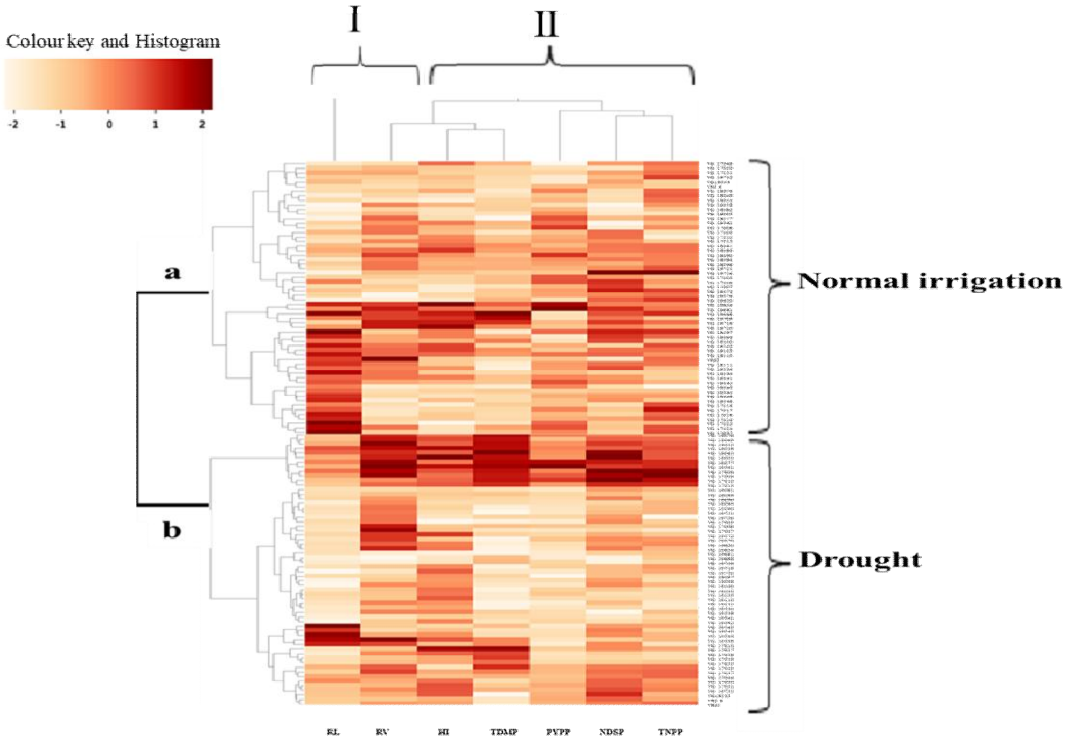

Fig. 4. Heatmap and hierarchical clustering for morphological and yield parameters under normal irrigation and drought stress conditions in 60 groundnut genotypes after 20 days of treatment. Clustering analysis of groundnut genotypes (left) revealed two major groupings, with group a representing 60 genotypes grown with normal irrigation and group b representing genotypes grown under drought conditions. The clustering analysis of various factors (top) revealed two significant groups: group I contains Root characteristics and group II includes the other five yield parameters, whereas group I important root features are associated with drought tolerance

Total Dry Matter Production (TDMP), Harvest index (HI), Pod yield Per Plant (PYPP), Total Number of Double Seeded Pods (TDSP), Total Number of Pods Per plant (TNPP), Root Length (RL), Root Volume (RV)

Drought responses in both well-watered and drought-stressed plants were measured using seven physiological parameters (TDMP, HI, NDSP, TNPP, PYPP, RL, and RV). It was observed that the effects of soil moisture regime and genotype, as well as the interaction between soil moisture and genotype, were significant ($p \leq 0.05$) for all parameters (Table 4). However, for each of the morphological parameters (plant height, LL, LW, and SL), the effects of soil moisture regime and the interaction between soil moisture and genotype were not significant ($p \leq 0.05$) (Table 4).

As shown in Fig. 4, the root trait and yield parameters of the 60 genotypes produced under either drought treatment or normal irrigation conditions (control) were employed for hierarchical (row) clustering. The 60 genotypes grouped into group a when produced under well-watered conditions, but the identical set of 60 genotypes clustered into group b when grown under drought conditions. This clear clustering demonstrates that in comparison to control conditions, drought stress treatment alters both the root and yield characters for each groundnut genotype. Hierarchical clustering analysis of the heatmap also indicated that the root and yield measurements could cluster the 60 genotypes into two distinct groups (top of Fig. 4 group I, II). The two root parameters, which reflect relative long-term response to abiotic stress mainly drought, were clustered together (top of Fig. 4, group I) and were consistently different between the control (Fig. 4, group a) and the drought treatment groups (Fig. 4, group b). Thus, morphological traits do not appear to closely correlate with drought tolerance in groundnut. The five yield parameters-Harvest index, TDMP, PYPP, TDSP, TNPP were clustered into group II, where all 60 genotypes showed decreased Harvest index, TDMP, PYPP, TDSP, and TNPP under drought treatment (Fig. 4). In general, all 60 genotypes showed increased Root length and root volume under drought treatment. Root length consistently increased under drought treatment in all 60 genotypes, while the root volume, a measurement of the density of the root, consistently increased in all 60 genotypes in response to drought treatment. A heat map is a visual method that can be used to explore complex associations between multiple parameters collected from various treatments. It is often useful to combine heatmap with hierarchical clustering, which is a way of arranging items in a hierarchy based on the distance or similarity between them. Despite its benefits, heatmap analysis (Fig. 4) could not clearly identify the significant differences between the genotypes in this study.

4. CONCLUSION

Drought avoidance is one of the methods that enables Groundnut to achieve high pod production and HI under drought conditions by altering root dispersion into deep soil. Optimal root length and root volume were shown to have a substantially positive link with the Harvest index, as well as a positive correlation among themselves. Breeding for yield stability under water-limited situations through deeper roots may enable the creation of superior groundnut cultivars in particular water-limited locations where water is accessible in deep soil. Having a big root system aids in increasing output during times of drought. Selection based on root thickness and root depth is highly suitable for identifying varieties that can be used in groundnut improvement for drought tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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