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Relationship between physiological and root traits of peanut genotypes under terminal drought stress

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Abstract Drought occurring at all growth phases of peanut (*Arachis hypogaea* L.) reduced pod yield and affected the physiological traits. All peanut accessions increased the percentage of root length density (%RLD) in response to terminal drought, but pod yield was greatly reduced. The increases in RLD varied among peanut genotypes. ICGV 98348 and Tifton 8 were the most resistant accessions based on RLD and pod yield. Pod yield was positively correlated with RLD and relative water content. The results would provide a better understanding on the responses of peanut to terminal drought to improve selection efficiency of peanut breeding for drought resistance.

Keywords: Pod filling stage, Water stress, Groundnut, %RLD

Introduction

Drought at the end of growth phase or terminal drought is the main production problem of peanut (*Arachishypogaea* L.) in the rainfed areas. The terminated drought affected to reduce yield are reported by Girdthai *et al.* (2010), Junjittakarn *et al.* (2014) and Koolachart *et al.* (2013). The association of root growth and water uptake in response to terminal drought reveal the mechanism underlying drought avoidance. According to Songsri *et al.* (2009) stated that the development of root system under drought stress is associated with water use efficiency. The similar results were also reported in many crop species such as sugarcane (Jangpromma *et al.*, 2012) and wheat (Gesimba *et al.*, 2004). Root traits of peanut grown under drought might be used as selection tools for improving drought resistance.

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Peanut can be acclimatized by early-season drought if the drought events are not too severe (Puangbut *et al.*, 2009). Drought stress occurs at the middle of growth phases reduces relative water content and stomatal conductance in peanut, but it increases root length density (Junjittakarn *et al.*, 2016). In a previous investigation, the pod yield of peanut was not correlated with root traits under terminal drought, but the relationship was positive and significant in specific genotypes (Junjittakarn *et al.*, 2014). Previous studies raised the question to the authors that peanut genotypes might respond differently to terminal drought for root traits and pod yield. It is also promising to use physiological traits to assist in peanut selection for drought resistance.

Previous research has not provided a clear or conclusive answer to the question. The goals of this research were to investigate the effects of terminal drought on root traits and physiological traits of peanut and to examine the relationships between root traits and other physiological traits.

Materials and methods

Experimental site and design

The experiment was set up in a split-plot design with four replications. Main plots had two water regimes consisting of field capacity (FC) and 1/3 available water (1/3 AW) applied to the crop at R7 to R8 growth phases (Boote, 1982). Subplots arranged into five peanut accessions including ICGV 98348, ICGV 98324, ICGV 98308, Tainan 9 and Tifton 8 with different in drought resistance levels (Girdthai *et al.*, 2010). The treatment was carried out during October 2012 to March 2013 at the Agronomy farm of Khon Kaen University, Thailand (16°28′N, 102°48′E, 200 m above mean sea level).

Experimental details and crop management

The soil in the experimental fields was a Yasothon series, which is characterized by low soil fertility and sand texture. The values of water holding capacity were low at both field capacity (FC) (10.64%) and 1/3 available water (1/3 AW) (6.34%). The soil was ploughed three times for soil preparation. Forty plots were prepared for planting, and the plot size is 5×5 m. Peanut seeds were planted in the flat soil (without ridge) at a spacing of 50×20 cm. The used fungicide was Captan which applied to the seeds to control soil-borne diseases and provided good germination of the seeds. The seeds were planted at high rate (3-4 seeds/hill), and the seedlings were later thinned to one seedling per hill at 14 days after planting (DAP). Alachlor was sprayed as a pre-emergence herbicide soon after planting. The soil was inoculated with rhizobium (*Bradyrhizobium* sp.) at 1 DAP. The details for crop management was follwed the method of Koolachart *et al.* (2013).

Water management

Subsoil drip irrigation was available for water regimes. The spacing of each drip lines were 50 cm and installed at 10 cm soil depth. After planting, surface irrigation was supplied uniformly at the FC level to the experiment at the depth of 60 cm. The fully-irrigated treatments, soil moisture was maintained at FC until harvest. Water supply for stress treatments was terminated at the R5 (beginning seed) growth phase, and the soil moisture content was allowed to reduce gradually to reach 1/3 AW at the R7 (beginning maturity) growth phase. The soil moisture level of 1/3 AW was derived from the simulation of pan evaporation which collected data for 20 years. The soil moisture content at 1/3 AW was maintained uniformly from R7 to harvest. The replenished water to each plot was calculated according to the method described by Doorenbos and Pruitt (1992). The detailed method of irrigation is followed Koolachart *et al.* (2013).

Soil moisture content and relative water content (RWC)

Soil moisture content was measured at 7-day intervals from of the experiment to harvest using the neutron probe method (Type I.H. II SER. N◦NO 152, Ambe Diccot Instruments Co. Ltd., England). Soil moisture content was recorded from 0 to 100 cm of the soil profile in an aluminum tube, which was installed in each plot. Data were read at 30, 60 and 90 cm of the soil profile.

Relative water content was measured at harvest from five leaflets of the plants in each subplot. The leaflets were collected, kept in sealable plastic bags, put in an insulated cooler and transported to the laboratory. Data were recorded for leaf fresh weight within 2 hours after the leaves were detached from the plants. The leaflets were then immersed into distilled water in a dim light room at 25 ℃. After 8 hours of imbibition. Data were recorded for saturated leaf weight, and the leaflets which were oven-dried at 80 ℃ for 48 hours until the weights become constant to determine leaf dry weights. RWC was calculated according to the method described in Gonzalez and Gonzalez-Vilar (2001) as the following equation:

$$\text{RWC} = [(\text{fresh weight} - \text{dry weight})/ (\text{turgid weight} - \text{dry weight})] * 100.$$

SPAD chlorophyll meter reading (SCMR), stomatal conductance (SC), specific leaf area (SLA) and pod yield (PY)

SCMR was measured by using a handheld portable chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan) between 10 -12 AM from five leaflets of each genotype at harvest. The data for stomatal conductance were recorded by using a Porometer-AP4 (Delta-T Devices, Cambridge, UK) from five plants in each subplot during 11-12 AM. SLA was recorded at harvest by using the following equation;

$$\text{SLA} = \text{leaf area } (\text{cm}^2)/\text{leaf dry weight (g)}.$$

The data were recorded for pod yield at harvest from five plants in each subplot. The plants in each plot were dug from the soil and the pods were separated from the plants. The pods were air-dried in a shelter and pod dry weight was recorded at 8% moisture.

Root traits

The auger method was used for determining root length density percentage (% RLD) at harvest (Koolachart *et al.,* 2013). The roots in each subplot were collected from 0 to 90 cm of the soil profile, and the soil core was divided into six layers. Root samples were recovered from the soil as much as possible, and root length was analyzed using the Winrhizo program. The data for % RLD in lower soil layer were recorded by combining root length density at the third to sixth layers (The first and second layers were assigned as upper soil layer.). Root samples were oven-dried at 80 °C for 48 hours until constant weights were reached to determine root dry weight (RDW).

Data analysis

Data were statistically analyzed according to the experimental design using STATISTIX 8 (Statistix 8, 2003). Significant differences among means of peanut genotypes were determined by the least significant difference (LSD) at 0.01 and 0.05 probability levels (Gomez and Gomez, 1984). The differences between FC and 1/3 AW were determined by T-test.

Results

Analysis of variance

Water regimes were significantly different ($P \leq 0.01$ and 0.05) for %RLD, RDW, RWC, and pod yield, but not significantly difference for SCMR, SC and

SLA (Table 1). Peanut genotypes were significantly different ($P \leq 0.01$) for most traits except for RWC. The interactions between water treatment and genotype were significant ($P \leq 0.01$ and 0.05) for SC and SLA, whereas the interactions for %RLD, RDW, RWC, SCMR and pod yield were not significant.

Table 1. Mean squares for percent root length density in the soil depth of 30-90 cm (% RLD), root dry weight (RDW), relative water content (RWC), stomatal conductance (SC), specific leaf area (SLA) and pod yield (g/plant) at harvest of five peanut genotypes planted under field capacity (FC) and 1/3 available water (1/3 AW)

SOV	df	% RLD	RDW	RWC	SCMR	SC	SLA	Pod yield
Rep	3	7.01	1.74	0.77	8.83	1641.2	109.8	34.44
Water (W)	1	1944.35**	9.35*	245.47**	2.63^{ns}	42.0^{ns}	1.87^{ns}	812.61**
Error (a)	3	34.76	0.37	0.57	3.54	2705.6	402.19	3.09
Genotype (G)	4	119.00**	17.63**	1.12^{ns}	159.29**	57604.6**	5209.77**	142.21**
W×G	4	12.08^{ns}	0.98^{ns}	2.21^{ns}	3.19^{ns}	3.965.7**	968.03*	12.49^{ns}
Error (b)	24	21.27	2.23	3.41	12.16	3701.4	268.67	8.43
CV (%)		17.70	25.7	1.9	8.9	34.7	8.5	12.35

*Significant at $P \leq 0.05$, **Significant at $P \leq 0.01$, ns = non-significant.

Soil moisture content

A large difference in soil moisture content between water regimes was found at 30 cm below the soil surface. A small difference was found at 60 cm of the soil profile, and water regimes were not different at 90 cm (Figure 1).

Physiological traits

Differences among peanut genotypes were significant differed ($P \leq 0.01$) for SCMR under FC and drought stress conditions (Table 2). Tainan 9 was significantly ($P \leq 0.01$) lowest for SCMR under FC and drought stress conditions, whereas other genotypes were similar for these traits. ICGV 98308 grown under drought stress condition was significantly lower ($P \leq 0.05$) for SCMR than that grown under FC condition.

Significant differences ($P \leq 0.01$) among peanut genotypes were found for stomatal conductance (SC) under well-watered conditions, but they were not significantly different under 1/3 AW condition. ICGV 98348 was highest (373.18 mmol m^{-2} s^{-1}) for SC under FC condition, whereas ICGV 98324 was the lowest (37.98 mmol m^{-2} s^{-1}). ICGV 98324 and Tainan 9 grown under drought had significantly higher ($P \leq 0.05$ for ICGV 98324 and $P \leq 0.01$ for

Tainan 9) SC than those grown under FC, indicating that terminal drought increased SC in these genotypes.

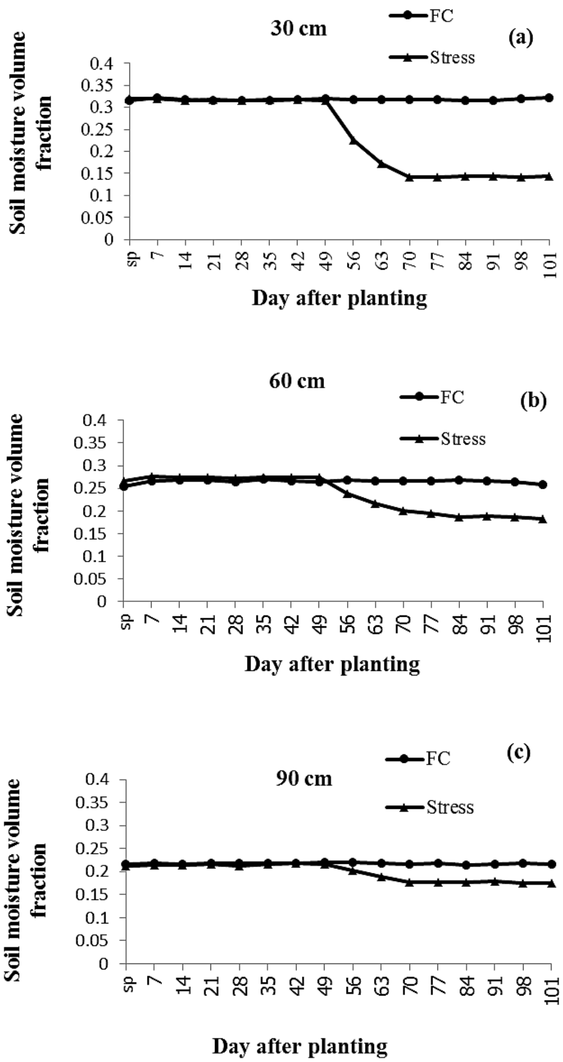

Figure 1. Volumetric soil moisture (fraction) under two water regimes including well-watered (FC) and terminal drought (1/3 available water:1/3 AW) conditions during October-February 2012/13 at 30 cm (a), 60 cm (b), and 90 cm (c) of the soil levels

Significant differences (P≤0.01) for SLA were observed among peanut genotypes under FC and drought. Tainan 9 was highest for SLA under FC and drought. A significant difference (P ≤0.01) in SLA of peanut grown under FC and drought was found in ICGV98308 only.

Table 2. SPAD chlorophyll meter reading (SCMR), stomatal conductance (SC) and specific leaf area (SLA) at harvest stage of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW)

Genotype	**SCMR**			**SC (mmol m^{-2} s^{-1})**			**SLA (cm^2 g^{-1})**			**RWC (%)**		
	FC	**1/3 AW**	**T-test[1/]**	**FC**	**1/3 AW**	**T -test**	**FC**	**1/3 AW**	**T-test**	**FC**	**1/3 AW**	**T-test**
ICGV 98348	40.53a[2/]	42.02a	ns	373.18a	206.98	ns	154.82d	161.15b	ns	95.63	91.71	*
ICGV 98324	41.08a	41.19a	ns	37.98c	88.95	*	206.02b	208.41a	ns	95.40	90.30	**
ICGV 98308	42.04a	40.52a	*	246.17b	176.96	ns	206.31b	169.07b	**	95.21	91.23	ns
Tainan 9	30.39b	31.85b	ns	50.96c	214.77	**	223.34a	227.66a	ns	96.00	89.51	**
Tifton 8	40.11a	41.14a	ns	172.62b	182.99	ns	172.27c	194.30ab	ns	95.51	90.25	**
Mean	38.83	39.34		176.18	174.13		192.55	192.12		95.55	90.60	
F-test	**	**		**	ns		**	**		ns	ns	
CV (%)	9.43	8.40		37.08	32.15		3.32	11.60		1.52	2.40	

[1/] T-test indicates the difference between water treatments.

[2/] Means in the same column followed by the same letter were not significantly different (at P < 0.05) by LSD.

*Significant at P ≤ 0.05, *Significant at P ≤ 0.01, ns = non-significant.

Variation in RWC among peanut genotypes was not significant differed under FC and drought. Terminal drought stress reduced RWC in all peanut genotypes. Most peanut genotypes grown under FC and those grown under drought were significantly different (P≤0.05 and P≤0.01) for RWC except ICGV 98308.

Root traits and pod yield

Variation in % RLD among peanut genotypes was significant differed (P≤0.01) under FC, but it was not significant under drought (Table 3). The significant (P≤0.01 and P≤0.05) increases in %RLD in response to terminal drought were observed in all peanut genotypes. ICGV98324 had the highest %RLD under FC condition and was also high under 1/3 AW condition, although the differences were not significant. Tifton8 had the lowest %RLD under FC condition, but it had the highest increase in %RLD under terminal drought.

The differences in RDW were not significantly differed among peanut genotypes under the FC condition, but they were significant (P≤0.05) under drought. Tifton 8 had the highest RDW under FC and drought, and other genotypes were significantly lower ($P \leq 0.05$) than Tifton 8 under drought. All peanut genotypes increased RDW under 1/3 AW condition, but the significant difference was observed in ICGV98348 only.

Table 3. Means for percent root length density (% RLD) in deeper soil layer (30-90 cm), root dry weight (RDW) and pod yield at harvest stage of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW)

Genotype	**% RLD**			**RDW (g plant^{-1})**			**Pod yield (g/plant)**		
	FC	**1/3 AW**	**T-test**[1]	**FC**	**1/3 AW**	**T-test**	**FC**	**1/3 AW**	**T-test**
ICGV 98348	22.21a[2]	38.74	*	4.08	5.64b	*	30.52ab	22.92a	*
ICGV 98324	22.98a	35.22	**	3.76	5.29b	ns	26.69bc	20.59a	**
ICGV 98308	20.72ab	31.97	**	6.47	6.60b	ns	22.89c	13.79b	*
Tainan 9	16.16bc	29.33	*	4.89	5.17b	ns	25.17c	15.69b	**
Tifton 8	13.35c	29.88	**	7.42	8.73a	ns	34.86a	22.07a	**
Mean	19.08	33.03		5.32	6.28		28.03	19.01	
F-test	**	ns		ns	**		*	**	
CV (%)	17.90	16.82		33.48	18.07		12.03	12.34	

[1] T-test indicates the difference between water treatments.

[2] Means in the same column followed by the same letter were not significantly different (at $P < 0.05$) by LSD.

*Significant at $P \leq 0.05$, **Significant at $P \leq 0.01$, ns = non-significant.

Significant variations ($P \leq 0.05$ and $P \leq 0.01$) in pod yield were observed among peanut genotypes grown under FC and drought. The highest pod yield was recorded in Tifton 8 grown under FC, and this genotype also had high pod yield under drought similar to that of ICGV 98348, which was highest, whereas ICGV 98308 had the lowest pod yield under FC and drought. Significant ($P \leq 0.05$ and $P \leq 0.01$) reductions in pod yield as affected by terminal drought were recorded in all peanut genotypes. Tifton 8 could maintain the highest pod yield under drought, and it was also the genotype with the highest yield reduction under drought.

Correlation between root traits, physiological traits and pod yield

The correlation coefficients among characters of peanut grown under drought are presented in Table 4. The correlation coefficients were both negative and positive. Among the traits under investigation, the large numbers of correlation coefficients were not significant except for some correlation coefficients. No trait under investigation was correlated with root dry weight. Positive and significant correlations were found between %RLD with relative water content (r=0.52**), %RLD with pod yield (r=0.50**), and relative water content with pod yield (r=.60**). Negative and significant correlations were found between specific leaf area with SCMR (r = -0.51**) and specific leaf area with stomatal conductance (r = -0.41**), but these physiological traits were not associated with root traits or pod yield.

Table 4. Correlation coefficients among percent root length density in the soil depth of 30-90 cm (% RLD), root dry weight (RDW), relative water content (RWC), SPAD chlorophyll meter reading (SCMR), stomatal conductance (SC), specific leaf area (SLA) and pod yield (PY) at harvest of five peanut genotypes

	% RLD	**RDW**	**RWC**	**SCMR**	**SC**	**SLA**
RDW	-0.04					
RWC	0.52**	-0.21				
SCMR	-0.18	0.15	0.09			
SC	0.04	-0.03	-0.02	0.21		
SLA	0.11	-0.07	-0.08	-0.51**	-0.41**	
PY	0.50**	-0.05	0.60**	0.20	0.09	-0.25

**Significant at P ≤ 0.01
Number without * indicates non-significance.

Discussion

The earlier research in our project has studied drought resistance in peanut focusing on early-season drought (Puangbut *et al.*, 2009; Thangthong *et al.*, 2017) and mid-season drought (Jongrungklang *et al.*, 2012). However, knowledge of late-season drought in peanut remains in conclusive. In this study, the authors selected five peanut genotypes with different drought resistance levels. The genotypes with ICGV numbers were previously identified as drought-resistant accessions by ICRISAT. Tifton 8 is a runner-type peanut with resistance to drought and large root system, while Tainan 9 is a released variety in Thailand that has been cultivated for many decades. The assumptions that must be tested in this study is that root traits and physiological traits might be useful to assist breeding programs of peanut for late-season drought resistance.

Pod yield

Yield is always important for crop breeding. In this study, late-season drought reduced pod yield in all peanut genotypes. However, variation in pod yield reduction was found among peanut genotypes. Tifton 8 had high pod yield under FC and drought conditions. Most genotypes with ICGV number grown under drought condition had high pod yield except ICGV 98308, which was similar to Tainan 9. Although Tifton 8 had the highest pod yield under drought, it had the highest reduction in pod yield. In previous investigation, Boontang *et al.* (2010) also found severe yield reduction as affected by terminal drought.

Our results supported previous investigations in other drought events. Under continuous long-term drought, high yielding genotypes should be of high yield potential under well-irrigated condition and low yield reduction under drought condition (Songsri *et al*., 2009).

The results might indicate that the genotypes with high yield potential are more advantageous because, although they have high yield reduction, the yield under drought is also high. The high yield under terminal drought in the ICGV accessions would be possible due to low yield reduction, although the yield potential of these accessions was not the highest.

Physiological traits

Physiological traits are related to the activities of plants to support growth and yield. Relative water content is a parameter indicating water status in plant. Stomatal conductance is related to gas exchange and photosynthesis, while a specific leaf area is related to both plant water status and photosynthesis. In this study terminal drought reduced relative water content in all peanut genotypes to the level that all peanut genotypes were the same for this trait. In a previous investigation, drought reduced relative water content due to stomatal closure. This mechanism is important for plant survival rather than maintaining productivity (Koolachart *et al.*, 2013). The results indicated that relative water content merely showed plant water status, but its usefulness as a surrogate trait for drought resistance is still in question because of its low variation.

In this study, drought had rather small effects on SCMR and specific leaf area as only one genotype grown under well-irrigated condition was different from that grown under drought condition. However, variations in SCMR and specific leaf area were found under both well-irrigated and drought conditions. These traits might be more useful than relative water content for drought resistance breeding of peanut. In previous study, SCMR was positively and significantly associated with pod yield, biomass and other agronomic traits, and SCMR under well-irrigated condition and SCMR under drought condition were well associated, indicating that the trait was rater stable across water regimes (Songsri *et al*., 2008).

Peanut genotypes responded differently to drought for stomatal conductance. Reductions in stomatal conductance were observed in ICGV 98348 and increases in this trait were observed in ICGV 98308, whereas Tifton

8 grown under well-irrigated condition and that grown under drought condition were not different. In case of mild drought stress, high stomatal conductance and highwater use efficiency were observed in Tifton 8. High stomatal conductance under mild drought stress promoted water use efficiency (Songsri *et al.,* 2013).

Root traits

The root of the plant functions as an anchor to support the above-ground parts. It also takes up water and nutrients. In this study, drought increased root dry weight in all peanut genotypes, and differences among peanut genotypes for this trait were significant under FC only. Selection for high root dry weight can be carried out under drought.

Similar to root dry weight, drought also increased root length density in all peanut genotypes, and differences in root length density among peanut genotypes were found under FC only. Selection based on actual values could be confounding, so it is advisable to select superior genotypes based on percent increase or percent reduction. In a previous study, the modification of root system in response to drought is an important plant mechanism of drought avoidance (Songsri *et al.,* 2009).

Correlations

It is interesting to note here that root length density and relative water content were positively correlated, and they were associated with pod yield, whereas root dry weight was not associated with any character. Therefore, root length density and relative water content should be more useful than root dry weight for the selection of peanut genotypes for resistance to terminal drought. ICGV 98348 had the highest %RLD under terminal drought, and it also had high pod yield. %RLD and RWC are promising traits for screening of peanut genotypes for drought resistance.

In a previous investigation, most root traits were not significantly correlated with pod yield under terminal drought except root length and root volume (Junjittakarn *et al.,* 2014). The authors also found that ICGV98348 and Tifton 8 with good root traits could maintain yield under terminal drought. Furthermore, root length density and root dry weight contributed to water extraction in some peanut genotypes under water deficit (Falalou *et al.,* 2018).

In our study, Tifton 8 had ability to maintain high yield under drought stress. This accession has been reported as a drought-resistant genotype (Koolachart *et al.,* 2013). The large root system in Tifton 8 may contribute to high yield under FC and take up sufficient water under drought. In this study, %RLD was positive and significant correlation was found between RWC and pod yield. The results indicated that drought promotes deep growth of root to acquire more water for maintaining yield.

The specific leaf area was negatively correlated with SCMR and stomatal conductance. However, specific leaf area, SCMR, and stomatal conductance were not associated with pod yield. According to Koolachart *et al.* (2013), high SCMR, high stomatal conductance, and low specific leaf area were desirable for maintaining high pod yield under drought in peanut. Drought reduced stomatal conductance but increased water use efficiency (Songsri *et al.*, 2013).

Previous results pointed towards the usefulness of these physiological traits in maintaining the pod yield of peanut under terminal drought. However, our results were somewhat disappointing as these traits were not correlated with pod yield. It seemed that these traits had very small contribution to pod yield under terminal drought. The timing of the evaluation might be the cause of differences in the results as peanut yield was accumulated from all growth phases until harvest.

This study reported the responses of peanut genotypes to terminal drought for pod yield, root traits and physiological traits. Pod yield of peanut under drought stress was closely related with root length density in the lower soil layer and relative water content, whereas root dry weight and other physiological traits were less important for maintaining pod yield. Therefore, root length density in the lower soil layer and relative water content might be useful as surrogate traits for terminal drought resistance in peanut.

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