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Research article

Responses of physiological and morphological characteristics in Thai pummelo cultivars (*Citrus maxima* (Burm.) Merr.) under drought stress condition

Saowanee Kongsri[a,†], Anyamanee Auvuchanon[b,†], Nopporn Jaroonchon[b,*]

[a] *Division Crop Production and Technology, Faculty of Science and Technology, Nakhon Pathom Rajabhat University, Nakhon Pathom 73000, Thailand*

[b] *Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand*

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Abstract

Importance of the work: Drought is a production problem in Thailand for pummelo and the trees of this fruit have not been evaluated under drought stress.
Objectives: To evaluate drought tolerance in nine Thai pummelo cultivars: Thongdee, Khaonamphueng, Khaoyai, Tubtimsiam, Takoy, Khaopan, Khaotaengga, Khaohom and Khaokrun.
Materials & Methods: Air-layered pummelo trees (10 plants/cultivar) under drought stress were measured for their physiological and morphological responses (photosynthesis, stomatal conductance and transpiration rate, leaf greenness, relative water content, leaf water potential, proline content, total non-structural carbohydrate (TNC) and plant biomass). All cultivars were grouped based on principal component analysis (PCA).
Results: Most parameters in all cultivars decreased after the plants were subjected to drought stress for 12 wk. The plant biomass and TNC decreased, while there were also decreases in the photosynthetic rate, stomatal conductance and transpiration rate to 79.4%, 87.3% and 71.6%, respectively, while leaf greenness decreased by 8.1%. The relative water content decreased by 9.6–37.3%, while the proline content increased 1.4–2.3 times. All cultivars under the control treatment had leaf water potential values from -2.2 to -3.2 MPa and these values decreased to be in the range from -3.5 to -9.0 MPa under drought stress. The measurement results of physiological and morphological parameters and from the PCA were used to classify Khaokrun and Khaotaengga as tolerant pummelo cultivars, and Khaopan, Tubtimsiam and Khaonamphueng as moderately tolerant cultivars, while Takoy, Khaoyai and Thongdee were classified as susceptible cultivars.
Main finding: The results could be used to manage pummelo orchards by selecting tolerant cultivars in drought risk areas. Further studies are required to develop drought-tolerant pummelo rootstock.

† Equal contribution.
* Corresponding author.
E-mail address: fagrnoj@ku.ac.th (N. Jaroonchon)

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Introduction

In 2019, “El Niño” caused extensive drought in Thailand, with severe water deficiency that affected the agricultural production of rice, cassava, sugarcane and fruit crops (Sowcharoensuk, 2020). To solve this problem, an alternative crop production method was suggested. However, this was only successful with high-water consumption annual crops, such as rice production in the summer season; it did not solve the drought problems associated with fruit trees and other perennial crops.

Pummelo is one of the top-10 economic fruit crops in Thailand that is widely grown throughout the country, especially in Nakhon Pathom, Samut Songkhram, Phichit, Chai Nat, Kanchanaburi, Prachinburi and Nakhon Si Thammarat provinces (Office of Agricultural Economics, 2018). However, drought stress impacts pummelo production in upland areas, such as Phichit, Chai Nat, Prachinburi and Kanchanaburi provinces. In 2020, the Takoy and Khaotaengga cultivars grown in Phichit and Chai Nat Provinces were affected by drought stress. The trees showed severe dehydration with leaf wilting and defoliation, while some died (Ejan, 2020; MGR online, 2020). Water shortage resulted in reduced yields and poor quality pummelo fruit in many areas, including Prachinburi and Kanchanaburi provinces (Thaich8, 2017; Prachinburi Land Development Station, 2020). Other important pummelo production areas in Thailand are Nakhon Pathom and Samut Songkhram provinces near the Tha Chin and Mae Klong Rivers, respectively. The planting system used in these areas is a Chinese raised bed surrounded by water that helps to reduce the problem of water shortage. However, this area is impacted by climate change with drought stress in the early summer season and also salt stress from seawater diffusing to underground water and the Tha Chin and Mae Klong Rivers that are important water sources for agriculture (Thairath, 2016; Bureau of Agriculture Commodities Promotion and Management, 2022). Therefore, this area had a problem with water deficit affected by high salinity from the seawater.

Drought stress affects physiological characteristics in citrus, where the initial response of plants under drought stress is stomatal closure, rapidly followed by reductions in water potential, net CO_2 assimilation, photosynthesis and transpiration (Brakke and Allen, 1995; Garcia-Sanchez et al., 2007; Rodriguez-Gamir et al., 2010). In addition, drought stress inhibits plant growth, resulting in fruit reductions in yield, size and quality that result in economic losses in citrus orchards (Gonzalez-Altozano and Castel, 1999; Romero et al., 2006).

A plant’s response to drought stress is related to adaptive mechanisms in its leaves or roots, including increasing the solute concentration by osmotic adjustment, reducing stomatal conductance and transpiration, accelerating leaf shedding and increasing the root density and depth (Castel and Fereres, 1982; Torrecillas et al., 1988; Zhang and Davies, 1989). Proline and other types of betaines act as osmotic adjustment solutes to maintain leaf turgor pressure for stomatal opening in citrus leaves (Nolte et al., 1997; Hare et al., 1998; Garcia-Sanchez et al., 2007). Shafqat et al. (2021) reported that Brazilian sour orange and Gadha dahi (*C. aurantium* L.) showed highest tolerance to severe drought because they maintained plant growth and plant water status, whereas Rangpur Poona nucellar (*C. limonia*) and Sunki × bentake were drought-sensitive rootstock. The citrus hybrid Forner-Alcaide no. 5 was considered to be a drought-tolerant rootstock since it showed better osmotic adjustment, water potential, stomatal conductance, transpiration rate and net CO_2 input than its parents *Cleopatra mandarin* and *Poncirus trifoliate* (Rodriguez-Gamir et al., 2010). The ability of tolerance to environmental stress in the form of drought stress also depends on both the cultivar and the production procedure. However, Thai commercial and local pummelo cultivars and their rootstocks have not been evaluated for drought stress. Pummelo propagation in Thailand is performed by the air-layering method, whereby the new plant uses its own roots. Therefore, the objectives of the current study were to assess the drought response of pummelo which will provide useful information for further development of drought-tolerant rootstock as an alternative future propagation method for pummelo.

Materials and Methods

Plant material preparation

Four commercial Thai pummelo cultivars; Thongdee (T), Khaonamphueng (K), Khaotaengga (KT) and Takoy (TK) and five local cultivars; Khaoyai (KY), Tubtimsiam (TS), Khaopan (KP), Khaohom (KH) and Khaokrun (KK) were propagated using the air shoot layering method and evaluated under drought stress. All the plants were grown in pots (7.6 L in size) in a greenhouse with the growing medium consisting of a ratio of soil-to-coconut coir-to-husk-to-sand-to-manure of 1: 1: 1: 1: 0.5 under greenhouse for 3 mth. Ten plants of each variety with uniform size were selected for the experiment.

Drought stress

The experiment was conducted between February and April, 2021. Each pot was weighed every week in the morning during experiment to calculate daily evapotranspiration (ET). Watering was done every other day whereby 100% of ET was set as well-watered treatment (control); and pummelo cultivars under drought stress treatment were supplied with 75% of ET for 2 wk, then 50% of ET for 1 mth, followed by 25% of ET for 1 mth and then no watering until the pummelo tree showed susceptible symptoms (wilting and defoliation) of severe drought stress (at age 2 wk). Physiological and morphological parameter values were averaged from five replications (trees) in control and drought treatment.

Photosynthetic rate, stomatal conductance, transpiration rate and leaf greenness measurements

The photosynthetic rate, stomatal conductance and transpiration rate were determined using an LCi portable infrared gas analyzer (ADC BioScience Ltd.; Hoddesdon, UK). The atmospheric pressure was 0.1 MPa and the ambient CO_2 concentration was in the range 333–355 parts per million. Measurements were performed at the 3rd–5th leaf position from the shoot tip of fully expanded plant leaves in the morning (0900–1100 hours) at an air temperature in the range 35−40 °C. Data were collected every 2 wk until the end of the experiment. Leaf greenness was measured at the same shoot position with a chlorophyll meter (SPAD model 502; Minolta Corporation Ltd.; Osaka, Japan) at the end of the experiment.

Leaf water potential, relative water content and proline content measurements

The leaf water potential and relative water content (RWC) measurements were collected after 12 wk of treatment between 0900 hours and 1100 hours. The leaf water potential was determined using a Scholander Pressure Chamber (model 600; PMS Instrument Company; Albany, OR, USA) at the 4th leaf position from the shoot tip. The RWC estimation was conducted at the 3rd leaf position from the shoot tip. A leaf disk (area 0.79 cm^2) was cut using a cork borer. The disk was weighed fresh (FW), then put into water, stored in the dark for 24 hr and reweighed to determine the full turgor weight (TW). Then, the leaf disk was dried at 70 °C and reweighed to determine the dry weight (DW). The RWC was calculated based on Equation 1:

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100 \qquad (1)$$

Leaf-free proline extraction and estimation were conducted according to the procedures described by Bates et al. (1973). A leaf sample (0.05 g) at the 3rd leaf position from the shoot tip was homogenized with 10 mL of 3% sulfosalicylic acid and then centrifuged at 3,000 revolutions per minute for 10 min. The supernatant was reacted with acid ninhydrin and then boiled at 100 °C for 1 hr. The reaction was terminated in an ice bath for 10 min and extracted with 4 ml toluene. The absorbance at 520 nm was determined using proline as a standard.

Shoot and root biomass, leaf area and leaf number measurements

At the end of the experiment, all pummelo trees were collected and the shoot and root fresh weights were measured for each plant. The shoots and roots were dried at 70 °C for 72 hr to determine DW. All leaves on each plant were measured for leaf area using a portable leaf area meter (AM350; ADC BioScience Ltd.; Hoddesdon, UK) and the number of leaves per tree was also recorded.

Total non-structural carbohydrate analysis

Leaf samples were collected for total non-structural carbohydrate (TNC) analysis. All the samples were washed with tap water, then rinsed with distilled water, dried in a hot-air oven at 70 °C for 72 hr and ground in a mill grinder machine (SM 100; Retsch; Haan, Germany). The dried samples were extracted with 0.2 N H_2SO_4 and TNC was determined by Nelson's reducing sugar procedure method (Hodge and Hofreiter, 1962).

Statistical analysis

The photosynthetic rate, stomatal conductance, transpiration rate, leaf greenness, leaf water potential, relative water content and proline content were subjected to 2 way-analysis of variance, with cultivars and water treatments (control and drought) as the factors. Shoot and root biomass, leaf area, leaf number and TNC were subjected to an independent sample t-test. Shoot and root biomass, leaf area, leaf number and TNC percentage were subjected to analysis of variance. Fisher's least significant difference test was used to discriminate the means of the variables with the SPSS software (version 16;

SPSS Inc.; Chicago, IL, USA). Pearson's correlation coefficient was calculated for phenotypic correlation testing. All dependent variables were used to calculate the principal component analysis (PCA). All tests were considered significant when $p < 0.05$.

Results and Discussion

Nine commercial and local pummelo cultivars were assessed for drought tolerance. After 12 wk, some pummelo cultivars presented a highly sensitive response to drought stress with severe leaf wilting and defoliation. These symptoms were used as indicators to terminate the experiment and to estimate the physiological and morphological parameters of all cultivars.

Shoot and root growth measurements

After 12 wk of drought stress, the shoot fresh and dry weight, number of leaves, leaf area, TNC and fresh and dry weight of root decreased by 30.4%, 27.4%, 27.9%, 24.8%, 13.1%, 32.8% and 38.7%, respectively, compared to the control (Table 1). The drought stress reduced plant growth and development by impacting stomatal closure, rapidly resulting in reductions in the water potential, respiration, net CO_2 assimilation, photosynthesis and enzyme activity. In addition, the turgor pressure of plant cells decreased, causing cell wilt and reduced cell expansion (Ma et al., 2020). Karimi et al. (2018) reported that water conservation of plant leaves under drought stress was regulated by reducing leaf expansion and leaf number as an adaptive response to reducing water loss, while TNC decreased by 13.1% compared to the control. Adams et al. (2013) also found that TNC concentrations decreased in *Pinus edulis* under drought stress. TNC is an important parameter under drought stress that indicates the C balance in plants between photosynthetic carbon assimilation and metabolic carbon demand (Chapin et al., 1990). The percentage reduction in the roots was higher than in the shoots, which differed from Alvarez et al. (2020), who reported that drought stress significantly decreased shoot growth, including leaf area, number of leaves, plant height and trunk diameter in almond rootstock but had little effect on root growth. Disparities in these results may have been caused by the different seedling types, as it appeared that almond grafted on rootstock adapted well under drought stress compared to air layered pummelo seedlings.

Comparison of shoot and root growth under drought stress

The fresh and dry weights of the plant and root, number of leaves, leaf area and TNC were presented as percentages and compared with the control values to determine any differences in growth for each pummelo cultivar (Table 2).

Of the tested cultivars, KT and KK had higher percentages of shoot fresh weight (82.9% and 78.4%, respectively) compared to the control, while KT and K had higher shoot dry weights (80.5% and 79.3%, respectively). The T cultivar had the lowest percentages of shoot fresh and dry weight (59.3% and 67.3%, respectively), while TS had the highest leaf area (90.7%) and KY and T had the lowest two values (66.8% and 64.4%, respectively). KT, K and KK had the three highest TNC percentages (87.3%, 87.5% and 85.9%, respectively), while KY and T had the two lowest (68.6% and 64.7%, respectively), as shown in Table 2.

TS and KK had the two highest percentages of root fresh weight (83.0% and 80.1%, respectively) compared to the control, while T and KY recorded the two lowest percentages of root fresh weight (60.7% and 60.3%, respectively). For root dry weight, KK had the highest percentage (78.1%), while T had the lowest (56.5%), as shown in Table 2.

Table 1 Mean ±SD values of shoot fresh and dry weights, leaf number, leaf area, total non-structural carbohydrate (TNC) and root fresh and dry weights of nine pummelo cultivars after 12 wk drought stress compared to control

Parameter	Control	Drought	Reduction (%)	t-test
Shoot fresh weight (g)	183.3±64.7	127.6±41.4	30.4	**
Shoot dry weight (g)	69.8±23.8	50.7±17.1	27.4	**
Leaf number (leaves/tree)	91.0±38.3	65.6±26.5	27.9	**
Leaf area (cm^2)	2,008.1±640.3	1,510.7±525.7	24.8	**
TNC (mg/g)	148.0±49.0	128.6±39.0	13.1	**
Root fresh weight (g)	78.3±21.0	52.6±12.8	32.8	**
Root dry weight (g)	28.1±13.5	17.2±6.8	38.7	**

** highly significant ($p < 0.01$) difference

Table 2 Percentage (relative to control) of shoot fresh weight (SFW), shoot dry weight (SDW), leaf number (LN), leaf area (LA), total non-structural carbohydrate (TNC), root fresh weight (RFW) and root dry weight (RDW) of nine pummelo cultivars after 12 wk drought stress

Cultivar	SFW	SDW	LN	LA	TNC	RFW	RDW
T	59.3±7.8^{b}	67.3±11.2^{c}	67.8±12.4	64.4±17.5^{b}	64.7±23.7^{c}	60.7±19.1^{c}	56.5±26.2^{c}
K	72.1±18.6^{ab}	79.3±26.3^{a}	70.0±11.9	77.7±25.9^{ab}	87.5±25.8^{a}	77.5±22.6^{ab}	74.3±12.5^{ab}
KY	69.8±10.1^{ab}	68.9±7.3^{bc}	69.0±18.1	66.8±2.8^{b}	68.6±15.9^{c}	60.3±4.9^{c}	59.9±12.3^{bc}
TS	76.1±13.5^{ab}	74.4±9.4^{ab}	70.5±21.7	90.7±7.1^{a}	74.6±31.4^{b}	83.0±8.2^{a}	73.6±11.9^{ab}
TK	72.9±22.4^{ab}	73.3±14.8^{ab}	75.0±10.7	76.9±23.1^{ab}	70.6±13.1^{bc}	62.0±8.4^{bc}	58.3±5.4^{bc}
KP	69.4±17.0^{ab}	75.3±13.5^{ab}	73.6±8.6	74.9±3.7^{ab}	82.8±6.7^{ab}	69.4±4.4^{b}	70.7±14.1^{ab}
KT	82.9±6.5^{a}	80.5±12.0^{a}	80.7±13.0	79.0±9.7^{ab}	87.3±6.3^{a}	78.9±15.9^{ab}	61.1±18.3^{b}
KH	74.0±7.5^{ab}	70.7±15.2^{bc}	77.8±14.7	78.7±18.1^{ab}	74.3±35.4^{b}	62.8±16.7^{bc}	63.1±16.2^{b}
KK	78.4±10.3^{a}	73.0±15.1^{ab}	80.0±7.1	79.6±5.2^{ab}	85.9±10.7^{a}	80.1±10.5^{a}	78.1±28.4^{a}
F-test	*	*	ns	*	*	*	*

T = Thongdee; K = Khaonamphueng; KY = Khaoyai; TS = Tubtimsiam; TK = Takoy; KP = Khaopan; KT = Khaotaengga; KH = Khaohom; KK = Khaokrun cultivars;
Mean ± SD in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different;
ns = non-significant ($p \geq 0.05$); * = significant ($p < 0.05$).

Photosynthetic rate, stomatal conductance, transpiration rate and leaf greenness measurements

The effect was investigated of drought stress on the photosynthetic rate, stomatal conductance and transpiration rate of pummelo. The results showed no interactions between drought stress and pummelo cultivars for the photosynthetic rate, stomatal conductance or transpiration rate parameters. After 2 wk of low-level drought stress at 75% of ET compared to 100% of ET, the photosynthetic rate, stomatal conductance and transpiration rate decreased by 8.1%, 16.4% and 11.5%, respectively (Figs. 1A, 1C and 1E). When the period of drought stress was longer and more intensified, pummelo showed high stress responses causing the photosynthetic rate, stomatal conductance and transpiration rate to decrease to 79.4%, 87.3% and 71.6%, respectively, at 12 wk of drought stress. These reductions related to stomatal closure controlled by abscisic acid (ABA) which is rapidly produced during drought stress (Gomez-Cadenas et al., 1996; Rodriguez-Gamir et al., 2010). ABA is generated by citrus roots and transported to leaves as root signaling to induce stomatal closure under drought stress (Davies and Zhang, 1991; Gomez-Cadenas et al., 1996; Osakabe et al., 2014). Wei et al. (2018) noted that two citrus cultivars (Sanhuhongju and Sanhuhuahong) showed decreased rates of photosynthesis, stomatal conductance and transportation under drought stress; however, Sanhuhongju showed less reduction or greater tolerance than Sanhuhuahong, indicating that the different cultivars responded diversely to drought stress.

All nine pummelo cultivars were evaluated. The results showed that KK had the highest photosynthetic rate, stomatal conductance and transpiration rate, while T and TK had the two lowest photosynthetic rate. Furthermore, T, KY and TK had the three lowest values of stomatal conductance and T had the lowest transpiration rate (Figs. 1B, 1D and 1F).

Rodriguez-Gamir et al. (2010) reported that drought-tolerant citrus rootstock maintained the highest levels of water potential, stomatal conductance, transpiration rate and net CO_2 assimilation throughout the experiment when subjected to severe drought stress. Therefore, based on the photosynthetic rate, stomatal conductance and transpiration rate parameters, KK was classified as a drought-tolerant cultivar, while T, KY and TK were classified susceptible cultivars.

There was no leaf greenness interaction between drought stress and pummelo cultivar. Drought stress reduced the average pummelo leaf greenness by 8.1% compared to the control (Fig. 2A). The leaf greenness values for the different drought treatments (Fig. 2B) were used to assess the tolerance of each pummelo cultivar under drought stress. There were significant differences in leaf greenness among the nine cultivars in this experiment. The cultivars were classified into 2 groups. KP, KT, TS, KK, KH and K showed high values of leaf greenness, while TK, KY and T were low greenness cultivars (Fig. 2B). Shafqat et al. (2021) reported that plants with high leaf greenness under drought stress could be considered as tolerant cultivars. Thus, from visual assessments, KP, KT, TS, KK, KH and K were considered as tolerant cultivars, while TK, KY and T were the most sensitive and showed leaf shedding and ultimately death during drought stress (Fig. 5).

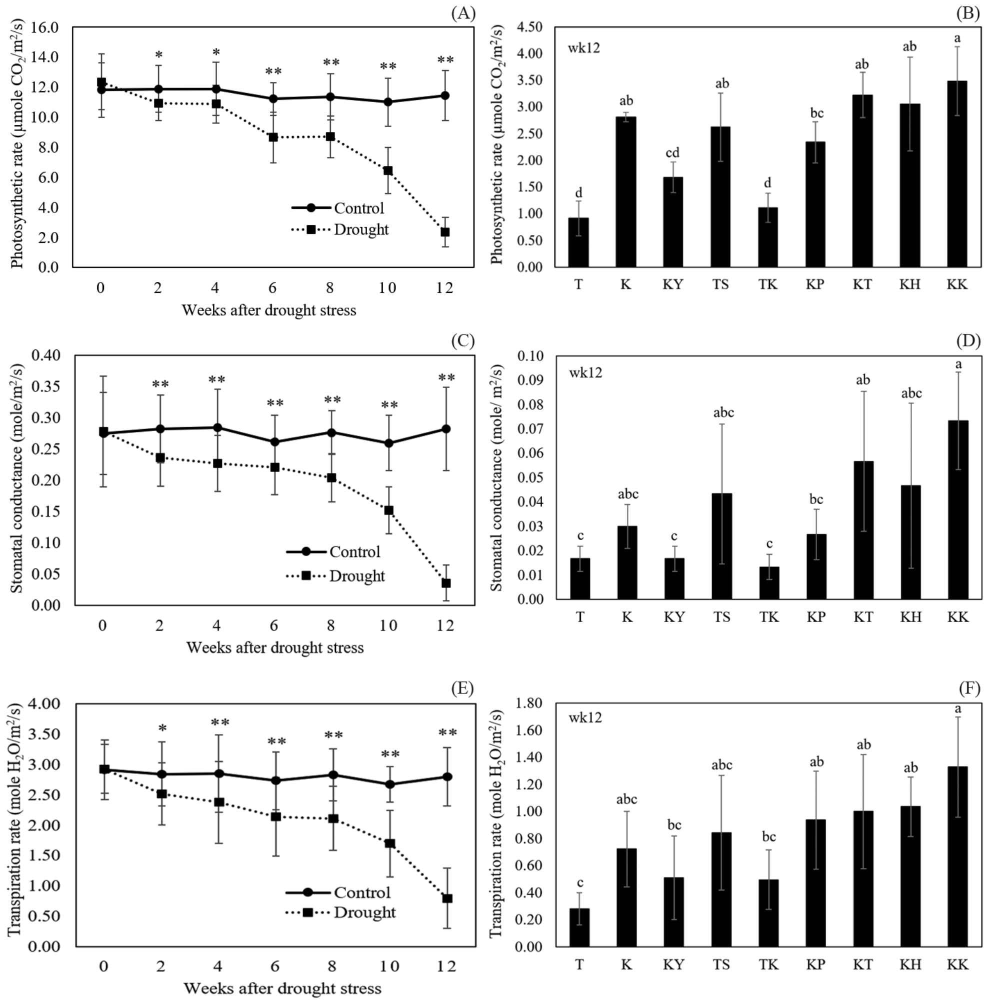

Fig. 1 Effect of drought stress on photosynthetic rate, stomatal conductance and transpiration rate of nine cultivars (A, C and E) and their parameters in each cultivar after 12 wk (B, D and F); * and ** indicate significant ($p < 0.05$) and highly significant difference ($p < 0.01$) between the plants received drought treatment and the control at each time point; bars represent means and error bars represent ±SD; Different lowercase letters above bars indicate significantly ($p < 0.05$) different ($n = 45$ in A, C and E and $n = 5$ in B, D and F) and T = Thongdee; K = Khaonamphueng; KY = Khaoyai; TS = Tubtimsiam; TK = Takoy; KP = Khaopan; KT = Khaotaengga; KH = Khaohom; and KK = Khaokrun cultivars

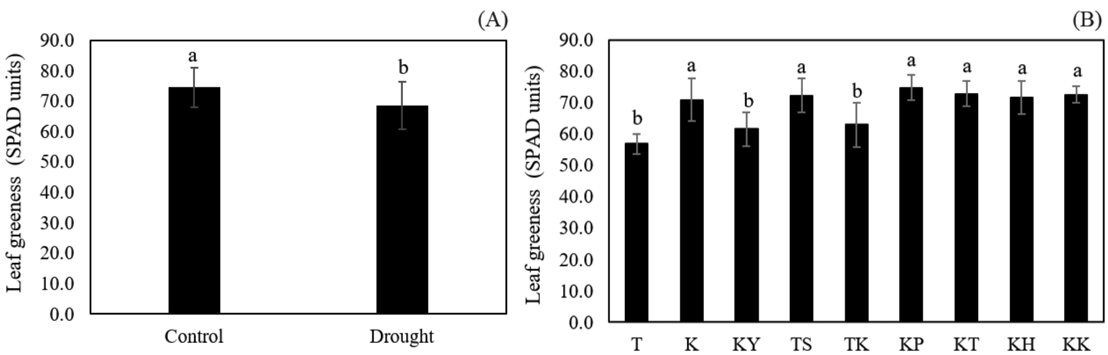

Fig. 2 (A) Effect of drought stress on leaf greenness of nine cultivars; (B) effect of cultivar on leaf greenness under drought stress treatment only at 12 wk; bars are means and error bars represent ±SD; different lowercase letters above bars indicate significantly ($p < 0.05$) different ($n = 45$ in A and $n = 5$ in B) and T = Thongdee; K = Khaonamphueng; KY = Khaoyai; TS = Tubtimsiam; TK = Takoy; KP = Khaopan; KT = Khaotaengga; KH = Khaohom; and KK = Khaokrun cultivars

Mafakheri et al. (2010) reported that drought stress decreased the total chlorophyll content in chickpea during both the vegetative and flowering stages compared to the control. Shafqat et al. (2021) studied the effect of drought stress on 10 different citrus rootstocks and found that chlorophyll a, chlorophyll b and carotenoids were reduced by drought stress. The tolerant cultivars (Brazilian sour orange and Gada dahi) had the highest chlorophyll contents, while Savage citrange was the most sensitive, with defoliation and subsequent death.

Relative water content, leaf water potential and proline content measurements

Under the control treatment, all cultivars presented high values of relative water content while drought stress reduced the relative water content by 9.6–37.3% (Fig. 3A). The leaf water potential of all cultivars under the control treatment had values in the range -2.2 to -3.2 MPa that decreased to -3.5 to -9.0 MPa under drought stress. (Fig. 3B). Karimi et al. (2015) reported that under drought stress, tolerant plants maintain their relative water content and water potential by reducing water loss and regulating osmotic potential, as was also reported for olive trees (Karimi et al., 2018). The level of drought tolerance in pummelo trees can be measured by the relative water content and leaf water potential values. In the current study, cultivar T had the lowest values under drought stress (Fig. 3A and B) and was considered a susceptible cultivar. Proline is an osmotic adjustment solute that can maintain water content in pummelo (Nolte et al., 1997; Perez-Perez et al., 2007). The proline contents in the plants were higher under drought stress than in the control by 1.4–2.3 times. The cultivars KY, TK, KP and KT had the four lowest values of proline content under well-watered conditions, while T, KY and KH had the highest three values under drought stress (Fig. 3C). Cultivars T and KY presented severe leaf wilting and defoliation (susceptible group), while KH presented no symptoms. The pummelo cultivars responded to drought stress by accumulating more proline than the control. However, in the current study, the proline content was not used as a selective index to classify drought-tolerant and susceptible cultivars.

Phenotypic correlation

Pearson's correlation coefficients (r) among the 14 phenotypic characteristics based on the 9 pummelo cultivars under drought stress are shown in Table 3. There were strong positive correlation coefficients with significant differences at $p \le 0.01$ ($0.77 \le r \le 0.92$) between: the photosynthesis rate and stomatal conductance, transpiration rate, leaf greenness, relative water content, water potential, shoot fresh weight and TNC; between stomatal conductance and transpiration rate; between transpiration rate and leaf greenness, relative water content, water potential and leaf number; between leaf greenness and relative water content, water potential and TNC; between relative water content and water potential, TNC and root dry weight; between shoot dry weight and TNC; and between root fresh weight and root dry weight.

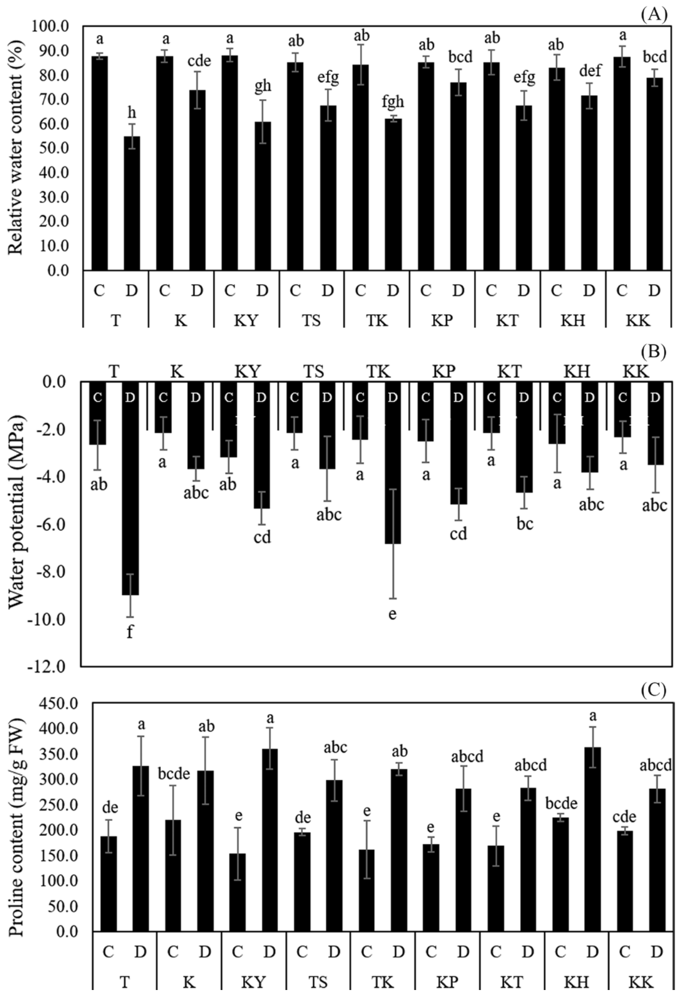

Fig. 3 Effects of drought stress and pummelo cultivar at 12 wk on: (A) relative water content; (B) water potential' (C) proline content, where x-axis letters are C for well watered (control) and D for drought treatment; bars are means and error bars represent ±SD; different lowercase letters above bars indicate significantly ($p < 0.05$) different ($n = 5$); FW = fresh weight; T = Thongdee; K = Khaonamphueng; KY = Khaoyai; TS = Tubtimsiam; TK = Takoy; KP = Khaopan; KT = Khaotaengga; KH = Khaohom; and KK = Khaokrun cultivars

Table 3 Phenotypic correlation between photosynthesis rate (A), stomatal conductance (G_s), transpiration rate (E), leaf greenness (SPAD), relative water content (RWC), water potential (Ψ), proline content (Pro), shoot fresh weight (SFW), shoot dry weight (SDW), leaf number (LN), leaf area (LA), total non-structural carbohydrate (TNC), root fresh weight (RFW) and root dry weight (RDW) of nine pummelo cultivars after 12 wk drought stress

Parameter	A	G_s	E	SPAD	RWC	Ψ	Pro	SFW	SDW	LN	LA	TNC	RFW
Gs	0.90**												
E	0.92**	0.90**											
SPAD	0.87**	0.68*	0.87**										
RWC	0.81**	0.62	0.86**	0.89**									
Ψ	0.89**	0.65 *	0.79**	0.83**	0.79**								
Pro	-0.37	-0.42	-0.47	-0.54	-0.47	-0.18							
SFW	0.77**	0.71*	0.75*	0.71*	0.52	0.75*	-0.41						
SDW	0.57	0.35	0.43	0.69*	0.52	0.52	-0.61	0.67*					
LN	0.66*	0.75*	0.78**	0.58	0.52	0.43	-0.38	0.77*	0.40				
LA	0.62	0.49	0.60	0.73*	0.52	0.71*	-0.43	0.72*	0.54	0.38			
TNC	0.81**	0.65*	0.74*	0.83**	0.82**	0.68*	-0.65	0.67*	0.85**	0.56	0.46		
RFW	0.73*	0.65*	0.63	0.72*	0.59	0.67*	-0.73*	0.67*	0.71*	0.32	0.76*	0.75*	
RDW	0.68*	0.51	0.67*	0.72*	0.84**	0.74*	-0.53	0.39	0.42	0.16	0.61	0.68*	0.78*

*, ** = significance ($p < 0.05$) and highly significance ($p < 0.01$), respectively

The strong phenotypic correlations between traits present an opportunity to improve overall efficiency by reducing the number of traits evaluated using an indirect selection procedure for traits that are hard to directly evaluate. As previously mentioned, the photosynthesis rate can be used as an indirect selection parameter for other traits, including stomatal conductance, transpiration rate, leaf greenness, relative water content, water potential, shoot fresh weight and TNC. Evaluation methods are difficult and require multi-steps, especially for relative water content and TNC parameters. Using the photosynthesis rate as an indirect selection evaluation parameter could save time and cost. The photosynthesis rate, stomatal conductance, transpiration rate, leaf greenness and relative water content should be used as a parameter index for screening for pummelo drought tolerance as they showed strong phenotypic correlation with other parameters and were correlated with the plant growth and biomass of tolerant pummelo cultivar. Furthermore, these parameters could readily indicate the response of pummelo to drought conditions and they can be readily estimated.

Principal component analysis

PCA provided a better understanding of the interaction between the nine pummelo cultivars under drought stress and the 14 measured parameters. The PCA scatterplot showed clear separation among the pummelo cultivars under drought stress and classified them into three groups with total variability of 77.27%, consisting of PC1 (67.32%) and PC2 (9.95%) (Fig. 4). The 1st group contained KK and KT, the 2nd group TS,

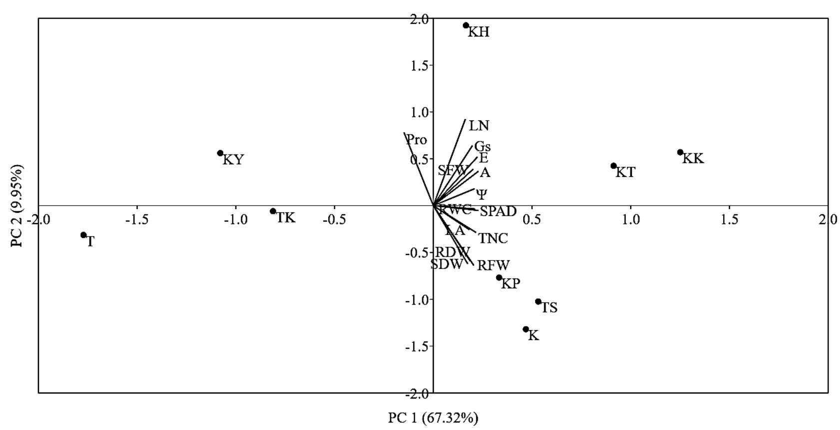

Fig. 4 Principal component (PC) analysis of both of physiological and morphological parameters estimated in nine pummelo cultivars under drought stress, where abbreviations are listed in Table 3 caption, T = Thongdee; K = Khaonamphueng; KY= Khaoyai; TS = Tubtimsiam; TK = Takoy; KP = Khaopan; KT = Khaotaengga; KH = Khaohom; and KK = Khaokrun cultivars

KP and K and the 3rd group TK, KY and T, while KH was an outgroup. The PCA plot showed that tolerant cultivars had high values for leaf number, stomatal conductance, transpiration rate, shoot fresh weight, photosynthesis rate, water potential, relative water content, leaf greenness, TNC and leaf area. The 1st group was classified as tolerant cultivars that presented with high values of physiological parameters (photosynthesis rate, stomatal conductance, transpiration rate, leaf greenness, water potential and relative water content) and high values of shoot and root growth parameters under drought stress (Figs. 1−3 and Table 2). The 2nd group was classified as moderately tolerant cultivars with high adaptation in root growth under drought stress, while the 3rd group was classified as susceptible cultivars that presented low physiological and morphological parameters, severe leaf wilting and defoliation. The susceptible group (T, KY and TK) had higher proline contents under drought stress than the tolerant group, with the exception of KH (Fig. 3C). Thus, the proline accumulation trend among the two groups was not clear. The visual symptoms, for both the tolerant and susceptible cultivars, are presented for two representatives in each group in Fig. 5.

Based on the responses to the physiological and morphological parameters and the PCA information under drought stress, the nine pummelo cultivars were classified into three groups: tolerant cultivars (KK and KT), moderately tolerant (KP, TS and K) cultivars and susceptible cultivars (TK, KY and T), while KH was not classified. This information can be used as a guideline for water management in pummelo orchards during drought stress, especially for susceptible cultivars, such as T, KY and TK. In addition, the current data could be used to develop pummelo drought-tolerant rootstock in the future.

Fig. 5 Effect of drought stress on pummelo tree growth on tolerant cultivars (KK and KT) and susceptible cultivars (KY and T), where C is well watered (control) and D is drought treatment at 12 wk and KK = Khaokrun; KT = Khaotaengga; KY = Khaoyai; and T = Thongdee cultivars

Conflict of Interest

The authors declare that there are no conflicts of interest.

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