



Wilting index and root morphological characteristics used as drought-tolerance variety selection at the seedling stage in soybean (*Glycine max* L.)

Guifeng Wang¹ · Qizheng Zhou¹ · Mengdi He¹ · Xuanbo Zhong¹ · Guixiang Tang¹ 

Received: 10 October 2018 / Accepted: 12 April 2020 / Published online: 18 April 2020
© Springer Nature B.V. 2020

Abstract

Drought stress is a common issue that affects agriculture production. The soybean plant is one of the drought-sensitive crops that could lose the yield of up to 40% under severe drought years. Improvements in drought tolerance have been one of the main objectives of breeding programs in soybean. In this study, 62 soybeans [*Glycine max* (L.) Merri.] germplasms including landraces and elite cultivars were evaluated by analyzing canopy and root morphological characteristics at seedling stage under polyethylene glycol (2.5 M PEG 8000, – 0.54 MPa) simulated drought stress. The results showed that the wilting index of sixty-two soybean germplasms showed significant polymorphism differences after 4 days PEG treatment. The lowest wilting index was 2.75 in Tiefeng 31, while the largest reached 9 in Fengdou 93 and Songzidou. The wilting index was positively correlated with plant height, electrolyte leakage, and negatively correlated with a dry weight of the above-ground part, stomatal conductance, and transpiration rate by regression analysis. The root system architecture (RSA) study showed root-canopy ratio, root length, number of the lateral roots, root surface area, and root volume were significantly negatively correlated with the wilting index and positively correlated with the root volume. Based on these results, sixty-two soybean germplasms were comprehensively evaluated for drought-tolerant cultivar by the principal component analysis (PCA). The results showed that Tiefeng 31 was the most drought-tolerant elite cultivar. However, Fengdou 93 was the most drought-sensitive elite variety. The drought tolerance screening results were consistent with the wilting index and RSA analysis, especially the root length. Both droughts tolerant and sensitive elite cultivar could be further used to breed drought-tolerant germplasms and to clarify the drought tolerance mechanism in soybean.

Keywords PEG-simulated drought stress · Above-ground part characteristics · Root system architecture

Introduction

Global warming affects not only temperature but also rainfall size, intensity, frequency, and distribution. Additionally, climate change will also reduce the predictability of rainfall and lead to increased drought and flooding stress (Douglas et al. 2008). Drought stress is a major global issue that adversely affects plant growth and limits crop productivity. Yield losses of significant crops due to the fluctuating weather will be challenging to meet the food requirements

of the world's growing population in 2050 (Foley et al. 2011). Soybean is one of the most abundant oil and plant protein crops in the world (Hasanah et al. 2015). It needs 450–700 mm of water during the growing season and considers as a drought-sensitive crop (Dogan et al. 2007). Drought stress exposure during flowering and pod stages could severely limit the yield and may reduce yield by approximately 20–40% in soybean (Thao and Tran 2012). Obtaining more water resources, absorbing more water, and reducing water loss are useful measures to improve drought stress in crops (Passioura 1983). Therefore, tremendous efforts have been placed on planting drought-tolerant soybean cultivars are an effective strategy for enhancing seed yield under drought stress (Tester and Langridge 2010). The acceptable policy is selecting the drought tolerance trait from soybean germplasms and using traditional breeding,

✉ Guixiang Tang
tanggx@zju.edu.cn

¹ Zhejiang Provincial Key Laboratory of Crop Genetic Resources, Institute of Crop Science, Zhejiang University, Hangzhou 310058, Zhejiang, China

which combines the desirable feature into elite cultivar via crossing and backcrossing process.

It is essential to assess the drought tolerance in soybean germplasms. Experiments have been conducted to evaluate the soybean drought tolerance by seed yield reduction, drought tolerance index, and wilting index (Bousslama and Schapaugh 1984; Liu 1986). The first visible symptom of soil water deficit is canopy wilting in soybean (Charlson et al. 2009). The sign of canopy wilting included leaves fading, upper canopy curling, leaf angles movement, and petioles shrinking (Oosterhuis et al. 1985). However, the time of onset and severity of canopy wilting in response to water deficit were different in soybean germplasms (Sloane et al. 1990). The slow-wilting genotype PI 416937 from Japan and PI 471938 from Nepal had been identified among soybean germplasms (King et al. 2009), and the progeny of these genotypes had shown to have high yield under water deficit conditions (Bagherzadi et al. 2017). Slow wilting was the main factor for drought-tolerant soybean to increase the yield (Sinclair et al. 2010). The slow-wilting genotypes reportedly had a high capacity allowing high CO₂ assimilations even with partial stomata closure (Sadok et al. 2012), increased transpiration rate under air vapor pressure deficit (Fletcher et al. 2007; Pathan et al. 2014; Bagherzadi et al. 2017). Compared to the fast-wilting genotypes, the slow-wilting genotypes generally had less radiation use efficiency and improved water use efficiency. Still, the stomatal conductance or canopy temperature had no difference (Ries et al. 2012). Soybean plants could withstand drought by root length elongation and prolonged nitrogen fixation besides slow canopy wilting (Sinclair et al. 2008).

Plant roots are the first tissues to percept the moisture change in the soil. The root system architecture (RSA) plays a crucial role in determining crop productivity in water-limited environments (Bengough et al. 2011). Root system architecture (RSA) refers to the shape and physical space of the roots. More deep-seated taproot and more lateral roots could absorb more water from deep soil layer and avoid drought stress in most of a dicotyledonous root system (Gilbert et al. 2011; Battisti and Sentelhas 2017; Ye et al. 2018). The drought-tolerant landrace (PI 416937) had a slow canopy wilting index and more fibrous root morphology (Hudak and Patterson 1996). Pantalone et al. (1996) also found the slow-wilting genotype showed a highly prolific root system and increased lateral root growth. Besides, the soybean plants can adapt to drought by developing a longer taproot under water-deficit environment (Manavalan et al. 2009). Root morphology parameters were used to classify the different cultivars in conferring drought tolerance traits in soybean. The drought-sensitive variety had a shallow root and root angle < 40°, and the drought-escaping cultivar had a deep root and root angle > 60° (Fenta et al. 2014). Some researchers had been studied the influence of drought on

the growth of soybean crops, especially on leaves. However, fewer studies are focusing on combination above-ground part and root system architecture for screening drought-tolerant cultivars in soybean. Therefore, the objectives in this study will be (i) to evaluate differential wilting index and other physiological characteristics among soybean genotypes after polyethylene glycol (PEG) -simulated drought stress at the seedling stage; (ii) to determine the root morphological characteristics among soybean genotypes after PEG-simulated drought stress; (iii) to explore the relationship between the wilting index and root morphological characteristics; (iv) to identify some varieties more tolerant or more sensitive to the drought stress and select the drought-tolerant varieties for further soybean drought tolerance breeding program.

Materials and methods

Plant materials

62 soybean (*Glycine max* L. Merri.) germplasms including landraces and elite cultivars, comprising of 29 spring-type, 11 summer-type, and 22 autumn-type, which were obtained from Oil crops Research Institute, Chinese Academy of Agricultural Sciences (Wuhan, China), were evaluated in this research. The full list of germplasms is given in Table 1.

Drought stress trial

Drought stress trial was conducted at the walk-in growth chamber in the Institute of Crop Science, Zhejiang University, Zijinggang campus, Hangzhou, China (30° 17' 55" N, 120° 5' 12" E) in 2017 and 2018. The day/night temperature was about 25 °C/22 °C, the light length was 14 h/10 h (day/night), and the relative air humidity was about 57%. Sixteen seeds of each germplasm were sowed on the 19 × 13 × 12 cm germinating box containing 1.3 kg sterilized river sand with a 130 ml half-length Hoagland nutrient solution (Hoagland and Arnon 1938). Plants were watered 100 ml ddH₂O daily until the beginning of PEG simulated drought stress. Nine days after sowing, the seedlings were thinned to ten. Fourteen days after sowing when the first trifoliolate leaf was coming out, PEG simulated drought stress was imposed by watering plants with 100 ml 2.5 M PEG 8000 (ψ -0.54 MPa) solution and watering with 100 ml ddH₂O as control. Each treatment for each germplasm was repeated three times at least 30 plants.

Physiological traits of above-ground part assessment

Four days after PEG-simulated drought stress treatment, the treated and control plants were analyzed for wilting canopy

Table 1 The list of soybean germplasms including landraces and elite cultivars used in this study

Germplasm	Ecological type	Germplasm	Ecological type
Yangyan dou	Spring	Zhonghuang 71	Summer
Tiegan 1	Spring	Zhonghuang 74	Summer
Ludou 8	Spring	Heihe 1	Spring
Yudou 19	Spring	Daimi dou	Autumn
Yudou 15	Spring	Xiamen Tengzi	Autumn
Yudou 20	Spring	Shaxian green bean	Autumn
Liaodou 14	Spring	Shaxin dou	Autumn
Tiefeng 31	Spring	Tonghua Pingdingxiang	Autumn
Hedou 13	Spring	Chihuang dou 2	Autumn
Dongnong 43	Spring	Wuyanwo	Autumn
Fengshou 19	Spring	Qionglai Huangmaozi	Autumn
Jilin 33	Spring	Qiyuehuang	Autumn
kennong 19	Spring	Baimaozao Douzi	Autumn
Suinong 10	Spring	Xihuangdou 9	Autumn
Heihe 19	Spring	Erjizaodou-2	Autumn
Kenjiandou 4	Spring	Qiyuehuang 1	Autumn
Jindou 42	Summer	Longchuan Huangniumao	Autumn
Jimidou 2	Spring	Lianjiangpo Huangdou	Autumn
Qihuang 35	Summer	Bozhi dou	Autumn
L-6	Summer	Songzi dou	Autumn
Jinda Zaohuang 2	Summer	Baomuji	Autumn
Jidou 21	Summer	Lvhuangdou	Autumn
Jiyu 403	Spring	2,340,322	Autumn
Jiyu 501	Spring	Liuyuehuang	Spring
Jihe 2	Spring	Pengshan Huangkezi 3	Autumn
Jihe 3	Spring	Dahuangdou 2	Autumn
Changnong 26	Spring	Madaiqingdou 2	Autumn
Jinong 27	Spring	Zheng 92116	Summer
Jinong 29	Spring	Zhonghuang 203	Summer
Yannong 12	Spring	Fendou 93	Spring
Zhonghuang 63	Summer	Zhonghuang 66	Summer

index, stomatal conductance, transpiration rate, electrolyte leakage, and root morphological characteristics.

Canopy wilting index

Four days after PEG treatment, the leaf wilting was recorded according to Pathan et al. (2014). The leaf wilting was rated visually on a scale of 0 to 4. The wilting scale of 0 represented no wilting. 1 represented the cotyledon wilting. 2 represented the wilting of both the cotyledonary and unifoliolate leaf. 3 represented the cotyledonary, unifoliolate, and the first trifoliolate leaf wilting together. 4 represented the whole plant wilting (Fig. 1). The wilting index was calculated by formula as follows:



Fig. 1 The wilting phenotype of leaves after 3 days of PEG simulated drought stress in soybean

$$\sum (\text{grade} \times \text{plants of each grade}) / \text{total plants}$$

Grade represented the degree of wilting scale, e.g., 0, 1, 2, 3, 4.

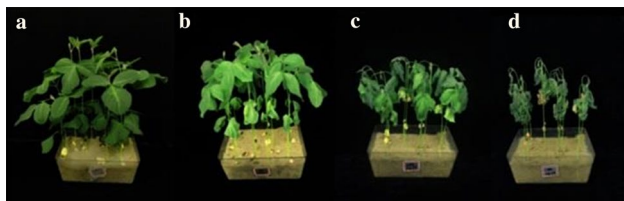


Fig. 2 The seedling growth after 0 day, 1 day, 3 day, and 5 day PEG treatment in soybean

Stomatal conductance, transpiration rate, and electrolyte leakage

The stomatal conductance (S_c) and transpiration rate (T_r) were measured with Li-6400 portable photosynthesis system (Li-Cor Inc. USA) at constant CO_2 of $380 \mu mol$ and light intensity of $1200 \mu mol m^{-2} s^{-1}$ at the time of 09:00–11:00 when the first trifoliolate leaf fully expanded. Measurement was conducted from 10 plants of each genotype exposed under drought and control treatments. The highest and lowest values were removed during analyses, and the remaining data were averaged.

The electrolyte leakage of leaves was measured according to the manufacturer's protocol using the Lei-magnetic conductivity meter (DDS-307, China). Three biological replicates of each germplasm were measured.

Root system architecture analysis

Four days after PEG-simulated drought stress, the above-ground part from five plants, which were selected randomly

was used to determine the dry weight, and the roots were washed thoroughly using tap water and making the root flat in the plexiglass tray for scanning the root system architecture by EPSON Perfection V850 Pro. Root system architecture parameters such as root length (mm), number of lateral roots, root diameter (mm), root surface area (mm^2), and root volume (mm^3) were obtained through WinRHIZO software (Reagent Instrument, Quebec, Canada).

A comprehensive evaluation of drought resistance

The method of Yue et al. (2003) was adopted for evaluating drought-tolerance cultivars by Principal component analysis (PCA). Those indexes which are easy to measure and relevant to drought-tolerance, such as wilt index, root length, number of the lateral root, root diameter, root surface area and root volume, root-canopy ratio, were used as components of PCA. The component score of PCA is calculated by formula as follows:

$$\text{Principal component score } (F_n) = \text{factor } n \text{ score} \times \lambda_n$$

$$\text{The principal component synthesis score } (F) = \frac{\lambda_1}{(\lambda_1 + \dots + \lambda_n)} \times F_1 + \frac{\lambda_2}{(\lambda_1 + \dots + \lambda_n)} \times F_2 + \dots + \frac{\lambda_n}{(\lambda_1 + \dots + \lambda_n)} \times F_n$$

The λ represents the square root of the eigenvalue corresponding to the principal component.

Data analysis

All data were calculated as the relative reduction percentage, i.e., the data under non-drought stress minus the data under drought stress then divided by the data under non-drought stress. The data were analyzed by using SPSS 20.0 statistic

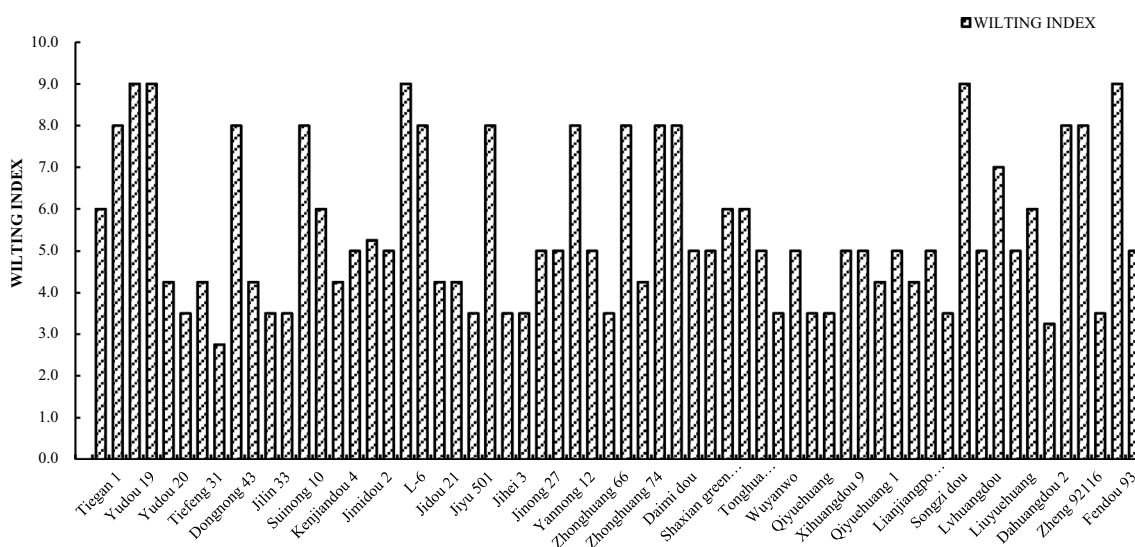


Fig. 3 The wilting indices of leaves in different soybean germplasm varieties after 4 days PEG-simulated drought stress at the seedling stage

Table 2 The list of above ground part traits in soybean germplasms

Germplasm	Plant height reduction %	Aboveground dry weight reduction %	Stomatal conductance reduction %	Transpiration rate reduction %	Wilting index
Tiefeng 31	3.43	4.44	92.81	88.68	2.75
Pengshan Huangkezi 3	40.80	46.90	87.43	76.54	3.25
Yudou 20	40.68	19.73	96.71	94.71	3.50
Fengshou 19	17.77	39.04	93.15	88.39	3.50
Jilin 33	3.08	38.73	88.42	78.81	3.50
Jiyu 403	53.24	41.91	94.41	87.09	3.50
Jihe 2	29.70	4.64	95.86	89.97	3.50
Jihe 3	35.31	39.52	95.50	93.15	3.50
Zhonghuang 63	37.55	3.03	97.58	94.75	3.50
Chihuang dou 2	11.32	37.63	94.92	91.06	3.50
Qionglai Huangmaozi	30.95	47.81	92.99	84.37	3.50
Qiyuehuang	49.89	36.37	96.13	92.17	3.50
Bozhi dou	17.78	6.51	93.05	88.98	3.50
Zheng 92116	47.50	41.95	92.00	88.20	3.50
Yudou 15	43.44	52.58	93.12	90.32	4.25
Liaodou 14	42.88	60.23	94.98	92.99	4.25
Dongnong 43	15.15	8.90	94.61	87.28	4.25
Heihe 19	22.59	50.65	93.55	88.54	4.25
Jinda Zaohuang 2	28.79	14.87	92.85	86.22	4.25
Jidou 21	47.49	41.72	93.96	88.41	4.25
Zhonghuang 71	19.60	14.71	97.42	92.19	4.25
Erjizaodou-2	37.80	19.02	96.82	92.24	4.25
Longchuan Huangniuma	39.24	53.16	92.16	89.36	4.25
Kenjiandou 4	14.29	40.00	93.01	90.81	5.00
Jimidou 2	39.68	44.82	96.25	92.49	5.00
Changnong 26	17.69	47.30	95.17	90.86	5.00
Jinong 27	33.69	37.68	97.69	94.16	5.00
Yannong 12	16.25	38.13	94.44	89.16	5.00
Daimi dou	43.59	59.07	93.66	86.71	5.00
Xiamen Tengzi	30.80	32.67	95.61	91.04	5.00
Tonghua Pingdingxiang	47.59	18.82	96.64	92.57	5.00
Wuyanwo	24.60	7.17	97.05	93.18	5.00
Baimaozao Douzi	31.44	9.64	94.99	89.45	5.00
Xihuandou 9	22.04	1.55	96.49	91.47	5.00
Qiyuehuang 1	45.70	21.04	96.56	93.30	5.00
Lianjiangpo Huangdou	44.90	32.85	95.64	90.02	5.00
Baomuji	49.60	34.18	95.91	90.88	5.00
2,340,322	48.59	30.77	95.87	91.47	5.00
Zhonghuang 203	34.62	43.91	91.93	90.06	5.00
Jidou 42	22.49	20.92	87.74	77.88	5.25
Yangyan dou	13.61	9.47	89.25	79.99	6.00
Suinong 10	52.41	57.34	94.88	89.39	6.00
Shaxian green bean	22.07	8.05	94.93	89.72	6.00
Shaxin dou	12.71	25.15	94.63	89.84	6.00
Liuyuehuang	38.44	50.27	97.84	94.96	6.00
Lvhuandou	23.59	28.24	95.79	90.62	7.00
Tiegan 1	31.37	7.57	97.06	93.28	8.00
Hedou 13	55.30	46.53	95.40	94.85	8.00
kennong 19	23.21	14.89	96.75	91.93	8.00

Table 2 (continued)

Germplasm	Plant height reduction %	Aboveground dry weight reduction %	Stomatal conductance reduction %	Transpiration rate reduction %	Wilting index
L-6	58.10	62.47	90.98	92.49	8.00
Jiyu 501	33.18	39.22	96.42	92.03	8.00
Jinong 29	38.54	40.90	93.73	87.90	8.00
Zhonghuang 66	19.09	23.52	92.29	88.41	8.00
Zhonghuang 74	19.60	6.00	95.84	95.88	8.00
Heihe 1	13.40	4.00	97.21	93.73	8.00
Dahuangdou 2	38.52	39.68	93.97	90.26	8.00
Madaiqingdou 2	46.03	40.49	93.81	91.06	8.00
Ludou 8	53.19	28.43	96.71	96.01	9.00
Yudou 19	28.98	45.39	97.30	94.23	9.00
Qihuang 35	25.82	53.72	95.44	90.79	9.00
Songzi dou	15.58	7.86	97.21	93.10	9.00
Fendou 93	47.67	65.94	95.35	93.60	9.00

software package (Chicago, USA), and the linear fitting was analyzed by OriginPro 9.0.0 (OriginLab Corporation, USA).

Results

PEG-simulated drought stress influence on the wilting index

We observed that the seedling wilting was more severe with the prolongation of PEG-induced drought stress (Fig. 2). The leaves were healthy before PEG simulated drought stress (Fig. 2a). The cotyledons and the unifoliolate leaf started wilting after 1d PEG simulated drought stress (Fig. 2b). The cotyledons, the unifoliolate leaf, and the first trifoliolate leaf became wilting after 3d PEG simulated drought stress (Fig. 2c). The whole plants showed complete wilting 5 d after PEG simulated drought stress (Fig. 2d). The wilting index of 62 soybean genotypes showed polymorphism (Fig. 3). From the Fig. 3, we found the lower wilting index were from Tiefeng 31, Pengshan Huangkezi-3, Jiyu 403, Jihei 3 and Dongnong 43 and the corresponding wilting index was 2.75, 3.25, 3.5, 3.5, and 4.25, respectively. The medium wilting indexes were 5, 5, 6, 6, and 7 respectively from Yannong 12, Zhonghuang 203, Shaxindou, Yangyan dou, and Lvhuangdou. The highest wilting indexes were 8, 9, 9, 9, and 9, respectively, which were from Madai Qingdou-2, Ludou 8, Fendou 93, Pine Bean, and Dahuangdou-2.

PEG-simulated drought stress influence on above-ground growth

Drought stress can inhibit the growth of the above-ground part. Also, plant height, dry weight of the above-ground, stomatal conductance, and transpiration rate showed a significant down-regulation after PEG-simulated drought stress (Table 2). The wilting indexes were associated with plant height, above-ground part dry weight, electrolyte leakage, stomatal conductance and transpiration rate. The results of the linear analysis showed that the wilting index was positively correlated with plant height, and electrolyte leakage (Fig. 4a, c), and the correlation coefficients were 0.63 and 0.63, respectively. However, the wilting indexes were negatively correlated with a dry weight of the above-ground part, stomatal conductance, and transpiration rate (Fig. 4b–e), and the correlation coefficients were 0.72, 0.82, and 0.85, respectively.

PEG-simulated drought stress influenced on root system architecture

The PEG-simulated drought stress altered the root morphology of soybean plants (Fig. 5 and Table 3). From Fig. 5 and Table 3, we found the more extensive the wilting index, the shorter the root length, the fewer the number of lateral roots, and the smaller the root surface area and root volume. Furthermore, linear analysis between root morphological characteristics such as root length, lateral root number, root mean diameter, root surface area, root volume, and wilting index

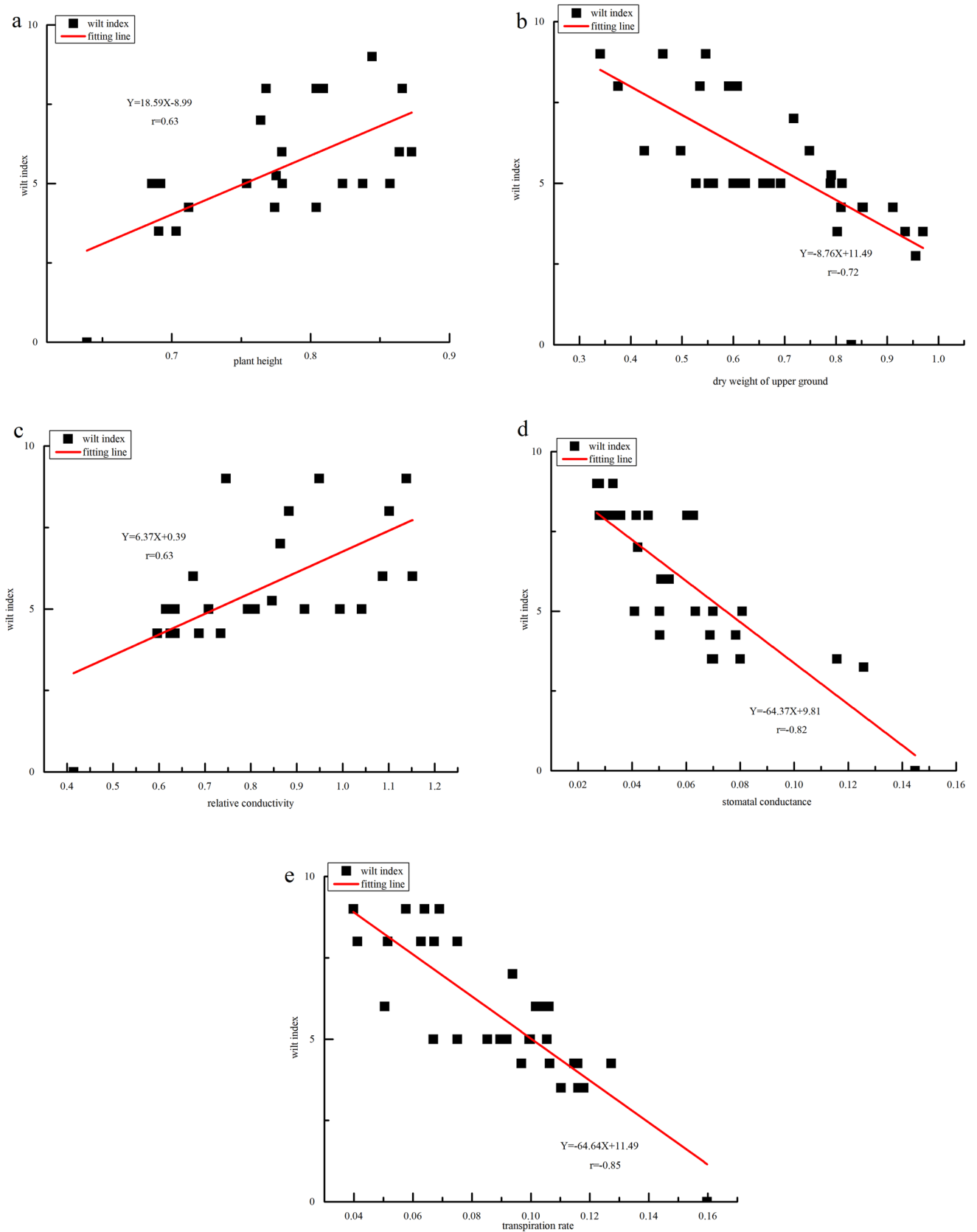


Fig. 4 Correlation analysis between the wilting index and plant height (a), shoot dry weight (b), electrolyte leakage (c), stomatal conductance (d), transpiration rate (e) in different soybean germplasm



Fig. 5 The representative images of root system architecture scanning of the different wilting index in five soybean germplasms. **a** Jihei 3, **b** Yudou15, **c** Yangyan dou, **d** Hedou 13, **e** Songzidou

in sixty-two soybean cultivars was evaluated. The results indicated that the root length, lateral root number, root surface area, and root volume were negatively correlated with the wilting index, and the correlation coefficients were 0.80, 0.82, 0.85, and 0.81, respectively. Meanwhile, the average root diameter was positively correlated with the wilting index, and the correlation coefficient was 0.86 (Fig. 6a–f).

A comprehensive evaluation of drought tolerance among soybean genotypes

PCA comprehensively evaluated the 62 soybean germplasm for drought tolerance. The wilting index was positively correlated with the principal component, and R^2 was 0.997 (Fig. 7). Root length, lateral root number, root surface area, root volume, plant height, and shoot growth contributed more to drought tolerance in principal component 1. In contrast, stomatal conductance and transpiration rate contributed more to drought tolerance in principal component 2, and the root-canopy ratio contributed more to drought tolerance in principal component 3 (Table 4). The linear correlation analysis between the wilting index and 11 drought tolerance related factors showed that stomatal conductance, transpiration rate, root length, lateral root number, root surface area, root volume, and average root diameter were strongly correlated with the wilting index under PEG simulated drought stress.

Meanwhile, the results of the principal component analysis showed that the principal component scores of Tiefeng 31, Wuyanwo, and Jilin 33 were higher than Tonghua Pingdingxiang, Yudou 20, and Shaxindou. The scores of Fendou 93, Liuyuehuang and Suinong 10 were the lowest. Therefore, Tiefeng 31, Wuyanwo and Jilin 33 can be considered as drought-tolerant soybean germplasms. Besides, Fendou 93, Liuyuehuang and Suinong 10 can be considered as drought-sensitive germplasms.

Discussion

Soybean productivity is lower compared to cereal crops. How to increase the global soybean productivity to satisfy the demand for soybean products is a concern of many researchers and farmers. Given the importance of soybean to the world economy, it is imperative to select drought-tolerant germplasm to improve soybean yield under drought conditions. The results showed that the plant height, shoot growth, stomatal conductance, and transpiration rate of soybean varieties decreased after PEG-simulated drought stress. Generally, drought tolerance soybean germplasm has strong water retention and is slow-wilting than that of drought-sensitive germplasm. The wilting index is the most intuitive index that can be seen by naked eyes after drought stress. Researchers use the wilting index as an indicator of drought tolerance directly (Sloane et al. 1990). The wilting of different genotypes may be associated with soil water conservation. The slow wilting genotype PI 416937 had higher soil water conservation by limiting the transpiration rate and decreasing stomatal conductance (King et al. 2009). Our result was coincidence with this, in which the wilting index was strongly negatively correlated with transpiration rate and stomatal conductance. Our results found the slow-wilting was negatively correlated with a dry weight of the above part, and this was following slow-wilting germplasms resulting in yield increases in many locations in 70% or more (Sinclair et al. 2010).

Drought tolerance is a very complex trait, which involves the morphological, physiological, and biochemical characteristics of above-ground and below-ground parts. So far, there is no valid, reasonable protocol and criteria for screening drought tolerance germplasms. Root system architecture, including root morphology and development, had a significant influence on plant survival and productivity, especially in water deficit stress or excess water (Manavalan et al. 2010; Jogaiah et al. 2013). Battisti and Sentelhas (2017)

Table 3 The list of root morphological traits in soybean germplasms

Germplasm	Root length reduction %	Number of lateral root reduction %	Root diameter reduction %	Root surface area reduction %	Root volume reduction %	Wilting index
Tiefeng 31	-52.47	-169.79	15.62	-30.94	-12.42	2.75
Pengshan Huangkezi 3	14.94	13.30	14.06	26.53	36.87	3.25
Bozhi dou	-23.65	-47.49	6.60	-15.48	-7.87	3.50
Chihuang dou 2	3.16	-24.96	3.79	7.18	11.11	3.50
Fengshou 19	-17.58	-85.64	7.66	-8.24	-0.37	3.50
Jihe1 2	6.52	-25.32	-8.24	-1.79	-10.74	3.50
Jihe1 3	7.84	-11.84	-1.46	6.03	4.40	3.50
Jilin 33	-12.96	-72.80	17.65	7.01	23.43	3.50
Jiyu 403	48.09	54.03	-1.65	47.20	46.44	3.50
Qionglai Huangmaozi	15.26	-15.74	-15.96	1.41	-14.72	3.50
Qiyuehuang	19.47	14.61	-2.22	17.65	15.83	3.50
Yudou 20	5.42	8.35	9.65	15.38	24.09	3.50
Zheng 92116	32.93	22.18	2.45	34.97	36.99	3.50
Zhonghuang 63	-35.59	-103.76	19.62	-8.70	12.75	3.50
Dongnong 43	-16.30	-83.67	2.55	-12.99	-9.76	4.25
Erjizaodou-2	20.56	-17.53	-6.11	15.57	9.96	4.25
Heihe 19	36.65	19.29	0.20	37.41	37.97	4.25
Jidou 21	37.89	40.45	-0.82	37.52	37.11	4.25
Jinda Zaohuang 2	-0.70	-33.66	1.14	0.49	1.10	4.25
Liaodou 14	48.62	50.10	8.57	53.78	58.27	4.25
Longchuan Huangnumao	51.78	56.69	9.80	56.43	60.62	4.25
Yudou 15	51.82	54.12	-4.50	50.11	48.22	4.25
Zhonghuang 71	25.41	9.40	0.79	25.87	26.24	4.25
2,340,322	39.68	39.27	-0.17	39.83	39.90	5.00
Baimaozao Douzi	-2.13	-19.32	6.75	5.23	12.16	5.00
Baomuji	41.70	39.08	-13.06	34.25	25.86	5.00
Changnong 26	25.31	22.10	15.27	36.19	45.39	5.00
Daimi dou	28.15	9.91	0.51	28.79	29.34	5.00
Jimidou 2	11.79	-2.70	8.19	18.30	24.27	5.00
Jinong 27	27.30	8.84	3.20	29.77	32.04	5.00
Kenjiandou 4	17.42	-18.84	5.91	22.24	26.73	5.00
Lianjiangpo Huangdou	26.85	-12.97	2.88	28.76	30.53	5.00
Qiyuehuang 1	-3.30	-25.99	7.11	4.09	10.84	5.00

Table 3 (continued)

Germplasm	Root length reduction %	Number of lateral root reduction %	Root diameter reduction %	Root surface area reduction %	Root volume reduction %	Wilting index
Tonghua Pingdingxiang	20.07	15.86	7.00	24.56	29.00	5.00
Wuyanwo	-60.99	-139.24	5.63	-51.92	-43.39	5.00
Xiamen Tengzi	41.43	34.67	-12.67	34.30	26.35	5.00
Xihuangdou 9	-3.65	-43.08	4.74	0.88	5.23	5.00
Yannong 12	44.56	41.14	6.16	47.93	51.19	5.00
Zhonghuang 203	33.87	40.37	-2.89	32.89	29.93	5.00
Jindou 42	25.40	7.48	14.45	36.68	46.15	5.25
Liyuehuang	44.02	49.01	6.54	47.59	50.88	6.00
Shaxian green bean	11.98	-22.67	-4.57	8.05	3.88	6.00
Shaxin dou	47.80	42.67	-22.12	35.89	21.15	6.00
Suinong 10	53.47	60.96	-0.26	53.42	53.33	6.00
Yangyan dou	-8.54	-21.95	1.72	-6.35	-4.20	6.00
Lvhuangdou	15.21	21.16	7.43	21.58	27.45	7.00
Dahuangdou 2	43.05	43.97	-2.36	41.84	40.59	8.00
Hedou 13	47.64	49.37	7.48	51.42	54.90	8.00
Heihe 1	13.80	5.52	8.40	20.11	25.97	8.00
Jinong 29	50.96	54.26	-5.35	48.45	45.59	8.00
Jiyu 501	36.70	22.17	-14.67	26.96	15.73	8.00
kennong 19	3.38	6.36	-4.13	-0.64	-5.40	8.00
L-6	53.06	35.07	1.63	53.89	54.68	8.00
Madaiqingdou 2	42.44	24.01	-13.32	34.75	26.06	8.00
Tiegan 1	-5.32	-37.51	-5.31	-10.43	-16.95	8.00
Zhonghuang 66	39.37	23.84	-6.86	35.22	30.73	8.00
Zhonghuang 74	7.07	-35.96	-4.89	2.68	-1.99	8.00
Fendou 93	54.68	57.75	-4.31	53.09	51.15	9.00
Ludou 8	28.91	6.78	-14.66	18.97	7.19	9.00
Qihuang 35	53.67	49.63	-7.33	50.26	46.42	9.00
Songzi dou	16.83	-11.76	-1.44	17.10	16.83	9.00
Yudou 19	38.67	38.12	-2.70	36.95	35.18	9.00

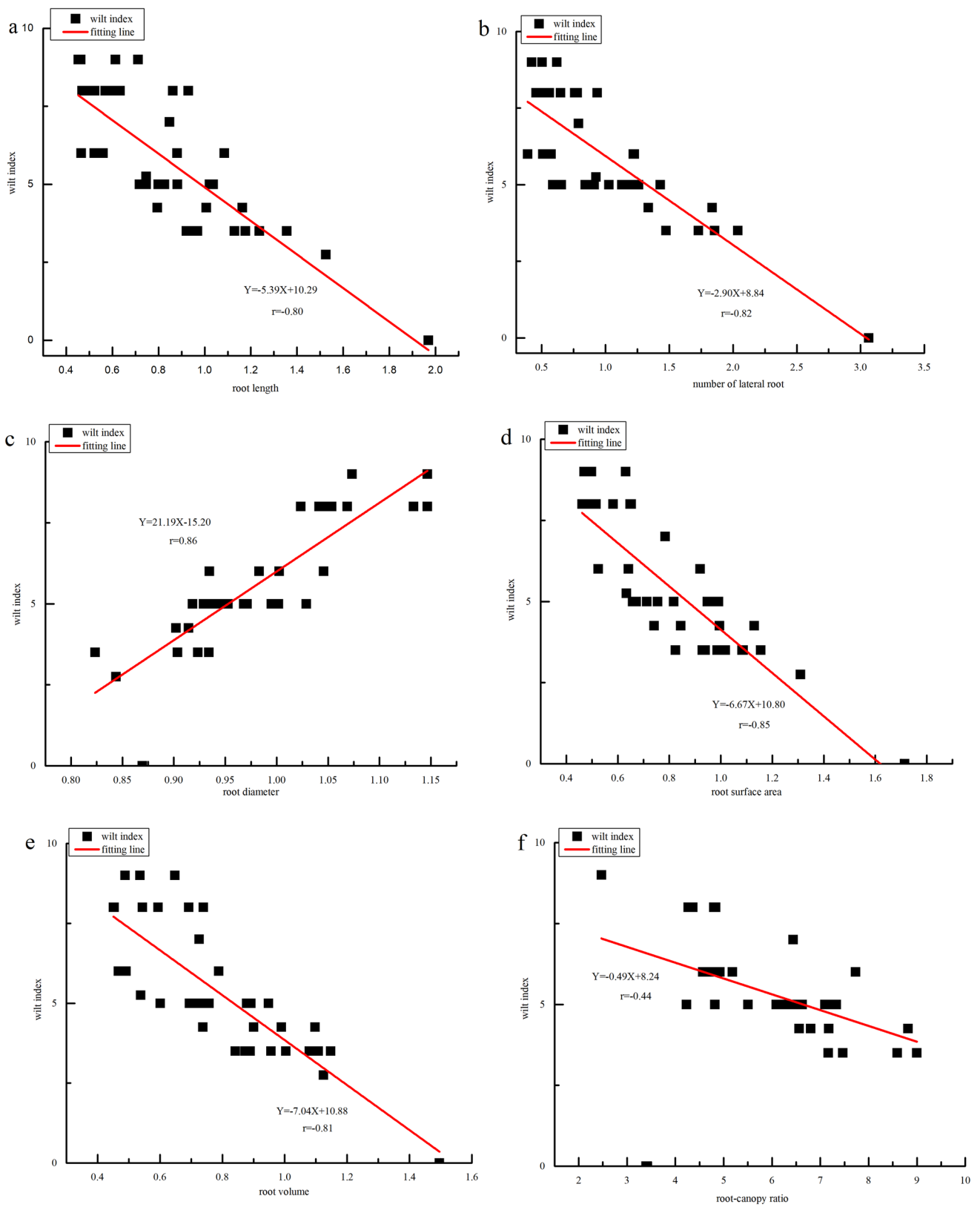


Fig. 6 Correlation analysis between the wilting index and root length (a), number of lateral roots (b), root mean diameter (c), root surface area (d), root volume (e), root-canopy ratio (f) in different soybean

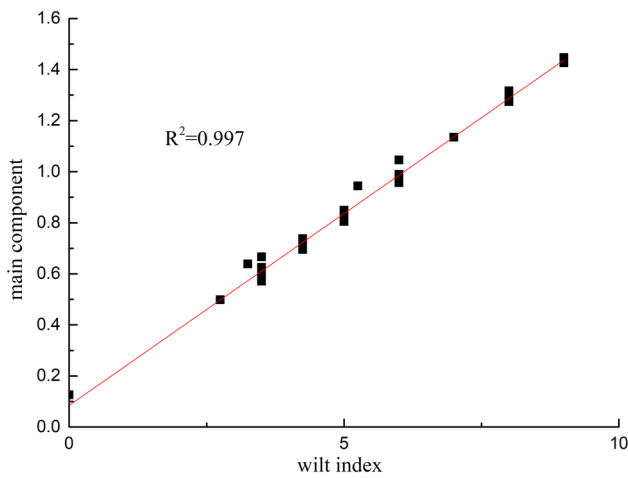


Fig. 7 Correlation analysis between the wilting index and main component composite score

found the deeper root depth was an important characteristic to make soybean crops more elastic to droughts, and that was an important strategy to be considered for adapting the crop to climate change. The root is the first organ that could experience drought stress, and soybean taproots are the first roots that formed. Therefore, screening germplasms with long taproots under non-stress conditions by a cone system to evaluate the root system architecture in soybean germplasms might determine the profound rooting ability, which is helpful to drought tolerance (Manavalan et al. 2010). Our results showed that root length, lateral root number, root surface area, root volume, and root-canopy ratio increased, and the root diameter decreased with the PEG-simulated drought stress. The wilting index was strongly negatively

correlated with root traits such as root length, the number of lateral roots, root surface area, and root volume, strongly positively correlated with root mean diameter and moderate negatively correlated with root-canopy ratio. Soybean plants that experienced drought stress before flowering had a higher yield than those stressed after flowering since the root system architecture had been developed well before flowering (Hirasawa et al. 1994). Liu et al. (2005) were also found significant correlations between drought resistance and various root traits such as dry weight, total length, root volume, and the number of lateral roots in soybean.

Recently, Prince et al. (2019) attempted to identify genes linked to root system architecture using GWAS in soybean landraces and elite cultivars and find four loci were detected in landraces for lateral root number, no major was detected for lateral root number in elite germplasm. The discovery that evaluated for drought tolerance germplasms to integrate wilting index and root system architecture could consider being used in soybean breeding programs in the future.

Conclusion

In summary, this study provided both above- and below-ground characteristics of sixty-two soybean germplasms during PEG-simulated drought stress in the seedling stage and evaluated the drought tolerance germplasm by PCA. The results showed the wilting index was positively correlated with plant height, electrolyte leakage and root mean diameter, but negatively correlated with shoot dry weight, stomatal conductance, transpiration rate, root-canopy ratio, root length, number of lateral roots, root surface area and root volume. Six drought-tolerance evaluation indexes were

Table 4 Principal component analysis results of different drought resistance traits

Trait	Component 1	Component 2	Component 3
Root length (cm)	0.968	0.054	- 0.067
Number of lateral root	0.948	0.057	- 0.030
Root mean diameter (cm)	- 0.321	- 0.494	0.337
Root surface area (cm ²)	0.976	- 0.089	0.016
Root volume (cm ³)	0.906	- 0.250	0.116
Root and crown ratio	0.313	- 0.445	0.542
Plant height (cm)	0.550	0.159	- 0.214
Aboveground growth (g/box)	0.756	- 0.280	- 0.234
Electrolyte leakage (%)	- 0.093	0.277	- 0.698
Stomatal conductance (mol H ₂ O m ⁻² s ⁻¹)	0.181	0.871	0.361
Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)	0.218	0.824	0.353
Variance contribution rate	0.434	0.194	0.115
Cumulative contribution rate	0.434	0.628	0.743

The bold data in the table indicates a large contribution rate

obtained by wilting index, correlation analysis, and PCA, which contain stomatal conductance, transpiration rate, root length, lateral root number, root surface area, and root volume. Furthermore, by comparison, the wilting index and the main component overall score, the drought-tolerant cultivar Tiefeng 31, and the drought-sensitive cultivar Fendou 93 were evaluated. Therefore, the selected drought-tolerant and drought-sensitive soybean cultivars could be used to understand further the mechanism of drought tolerance and breed soybean drought-tolerant cultivars in the future.

Acknowledgements This work was supported by the National Major Special Project for Transgenic Organisms, Ministry of Agriculture in China (No. 2016ZX08004-004-005).

References

- Bagherzadi L, Sinclair TR, Zwieniecki M, Secchi F, Hoffmann W, Carter TE, Ruffy TW (2017) Assessing water-related plant traits to explain slow-wilting in soybean PI 471938. *J Crop Improv* 31(3):400–417
- Battisti R, Sentelhas PC (2017) Improvement of soybean resilience to drought through deep root system in Brazil. *Agron J* 109:1612–1622
- Bengough AG, McKenzie B, Hallett P, Valentine T (2011) Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *J Exp Bot* 62:59–68
- Bouslama M, Schapaugh WT (1984) Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci* 24(5):933–937
- Charlson DV, Bhatnagar S, King CA, Ray JD, Sneller CH, Carter TE, Purcell LC (2009) Polygenic inheritance of canopy wilting in soybean [*Glycine Max* (L.) Merr.]. *Theor Appl Genet* 119:587–594
- Dogan E, Kirnak H, Copur O (2007) Deficit irrigations during soybean reproductive stages and CROPGRO-soybean simulations under semi-arid climatic conditions. *Field Crop Res* 103:154–159
- Douglas I, Alam K, Maghenda M, McDonnell Y, McLean L, Campbell J (2008) Unjust waters: climate change, flooding and the urban poor in Africa. *Environ Urban* 20:187–205
- Fenta BA, Beebe SE, Kunert KJ, Burr ridge JD, Barlow KM, Lynch JP, Foyer CH (2014) Field phenotyping of soybean roots for drought stress tolerance. *Agronomy* 4:418–435
- Fletcher AL, Sinclair TR, Allen LH Jr (2007) Transpiration responses to vapor pressure deficit in well-watered and ‘slow wilting’ and commercial soybean. *Environ Exp Bot* 61:145–151
- Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, Connell C, Ray DK, West PC, Balzer C, Bennett EM, Carpenter SR, Hill J, Monfreda C, Polasky S, Rockström J, Sheehan J, Siebert S, Tilman D, Zaks DPM (2011) Solutions for a cultivated planet. *Nature* 478:337–342. <https://doi.org/10.1038/nature10452>
- Gilbert ME, Zwieniecki MA, Holbrook NM (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *J Exp Bot* 62(8):2875–2887
- Hasanah Y, Nisa T, Armidin H, Hanum H (2015) Isoflavone content of soybean (*Glycine max* (L.) merr.) cultivars with different nitrogen sources and growing season under dry-and conditions. *J Agric Environ Int Dev (JAEID)* 109(1):5–17
- Hirasawa T, Tanaka K, Miyamoto D, Takei M, Ishihara K (1994) Effects of pre-flowering moisture deficits on dry matter production and ecophysiological characteristics in soybean plants under drought conditions during grain filling. *J Crop Sci* 63:721–730
- Hoagland DR, Arnon DI (1938) The water-culture method for growing plants without soil. University of California Agricultural Research Station, Davis, C.A. Circular 347:1–39
- Hudak CM, Patterson RP (1996) Root distribution and soil moisture depletion pattern of a drought-resistant soybean plant introduction. *Agro J* 88:478–485
- Jogaiah S, Govind SR, Phan Tran LS (2013) Systems biology-based approaches toward understanding drought tolerance in food crops. *Crit Rev Biotechnol* 33(1):23–39
- King CA, Purcell LC, Brye KR (2009) Differential wilting among soybean genotypes in response to water deficit. *Crop Sci* 49(1):290–298
- Liu X (1986) Discussing assessment methods of drought resistance of soybean. *Chin J Oil Crop Sci* 4(2):23–26
- Liu F, Anderson MN, Jacobson SE, Jensen CR (2005) Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. *Environ Exp Bot* 54:33–40
- Manavalan LP, Guttikonda SK, Phan Tran LS, Nguyen HT (2009) Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol* 50(7):1260–1276
- Manavalan LP, Guttikonda SK, Nguyen VT, Shannon JG, Nguyen HT (2010) Evaluation of diverse soybean germplasm for root growth and architecture. *Plant Soil* 330:503–514
- Oosterhuis DM, Walker S, Eastham J (1985) Soybean leaflet movements as an indicator of crop water stress1. *Crop Sci* 25:1101–1106
- Pantalone VR, Rebetzke GJ, Burton JW, Carter TE (1996) Phenotypic evaluation of root traits in soybean and applicability to plant breeding. *Crop Sci* 36:456–459
- Passioura JB (1983) Root and drought resistance. *Agric Water Manag* 7:265–280
- Pathan SM, Lee JD, Slepser DA, Fritschi FB, Sharp RE, Carter TE, Nelson RL, King CA, Schapaugh WT, Eilersieck MR, Nguyen HT, Shannon JG (2014) Two soybean plant introductions display slow leaf wilting and reduced yield loss under drought. *J Agron Crop Sci* 200:231–236
- Prince SJ, Valliyodan BB, Ye H, Yang M, Tai SS, Hu WS, Murphy M, Durnell LA, Song L, Joshi T, Liu Y, de Velde JV, Vandepoele K, Shannon JG, Nguyen HT (2019) Understanding genetic control of root system architecture in soybean: insights into the genetic basis of lateral root number. *Plant Cell Environ* 42:212–229
- Ries LL, Purcell LC, Carter TE, Edwards JT, King CA (2012) Physiological traits contributing to differential canopy wilting in soybean under drought. *Crop Sci* 52:272–281
- Sadok W, Gilbert ME, Raza MS, Sinclair TR (2012) Basis of slow-wilting phenotype in soybean PI 471938. *Crop Sci* 52:1261–1269
- Sloane RJ, Patterson RP, Carter TE (1990) Field drought tolerance of a soybean plant introduction. *Crop Sci* 30:118–123
- Sinclair TR, Messina CD, Beatty A, Samples M (2010) Assessment across the united states of the benefits of altered soybean drought traits. *Agron J* 102(2):475–482
- Sinclair TR, Zwieniecki MA, Holbrook NM (2008) Low leaf hydraulic conductance associated with drought tolerance in soybean. *Physiol Plant* 132:446–451
- Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. *Science* 327:818–822
- Thao NP, Tran LSP (2012) Potentials toward genetic engineering of drought-tolerant soybean. *Crit Rev Biotechnol* 32:349–362
- Ye H, Roorkiwal M, Valliyodan B, Zhou LJ, Chen PG, Varshney RK, Nguyen HT (2018) Genetic diversity of root system

architecture in response to drought stress in grain legumes. *J Exp Bot* 69:3267–3277

Yue CL, Huang YX, Yan Z (2003) *SAS system and economic statistical analysis*. China University of Science and Technology Press, Hefei (in Chinese)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.