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EDITED BY

Praveen Guleria,
DAV University, India

REVIEWED BY

Syed Srfraz Shah,
Forman Christian College, Pakistan
Kourosh Vahdati,
University of Tehran, Iran

*CORRESPONDENCE

Shoukun Dong
dskskd2020@163.com

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Screening and identification of drought tolerance of spring soybean at seedling stage under climate change

Xin Wang¹, Xiaomei Li² and Shoukun Dong^{1*}

¹Agronomy College, Northeast Agricultural University, Harbin, China, ²Branch of Horticulture and Landscape Architecture, Harbin Academy of Agricultural Science, Harbin, China

Drought is one of the major abiotic stress factors limiting soybean growth and yield, and it frequently occur globally. Therefore, exploring resistant varieties from soybean germplasm is important under climate change. To screen drought resistant spring soybean varieties at seedling stage, pot experiment was used to detect the Survival percentage after drought stress of 60 soybean varieties at seedling stage, twice drought rehydration treatments on seedlings, to evaluate the drought tolerance of spring soybean. The results showed that at the seedling stage, seven varieties were considered drought tolerant, 17 varieties were considered drought sensitive, and 36 varieties were considered to be moderately drought tolerant. Based on this experiment, number 44 (heinong37), 48 (heinong44), 49 (heinong45), 52 (heinong48) is considered the best drought resistant, and number 3 (dongnong48), 4 (dongnong52), 27 (suinong25), 60 (heinong65) is the most sensitive. These varieties provide a reference for further study on drought tolerance and stress resistance gene screening of soybean at the molecular level. The selected soybean varieties can be planted in areas with suitable climates and frequent drought to meet the local soybean demand. In other regions, although cannot be directly grown, they can still be used as parents of selected varieties or as materials for gene screening and extraction, to assist crop breeding at the molecular level in response to increasingly severe drought stress problems under the current climate trends.

KEYWORDS

spring soybean, seedling stage, drought tolerance, identification, genetic resources

Introduction

Soybean originated in China and has been cultivated for more than 4,000 years. After years of domestication and cultivation, now soybeans are important oil and cash crops. Providing rich protein and fat for people's daily diet (Muhammad et al., 2009; Chinakwe et al., 2019), as well as having an irreplaceable role in animal husbandry and industry (Tyczewska et al., 2016). However, the current soybean production is still unable to meet people's needs, especially during the growth and development of soybeans are extremely vulnerable to various abiotic stresses, resulting in a significant decline in yield (Feng et al., 2020).

Drought is the most common abiotic stress that affects plant growth globally (Ahanger et al., 2021), and as climate change becomes more and more complex, the problem of reduced food production caused by drought is becoming increasingly serious, posing a serious challenge to world food security and food sustainability. Moreover, the effects of drought on plants vary due to the uncertainty of the occurrence time (Shen et al., 2022). Therefore, previous studies have been conducted on the various stages of plant growth subjected to drought stress (Lotfi et al., 2019; Teixido and Valladares, 2019). The purpose is to analyze the impact mechanism of drought and explore ways to cope with it.

For soybeans, adequate moisture is necessary, and drought stress at all periods of soybean growth and development can affect growth or lead to yield reduction. The developmental status of soybean in the seedling stage determines the quality of individual and population development (Hua et al., 2018). In production, only by mastering the laws of water metabolism during the soybean seedling stage can we effectively avoid drought damage and lay a solid foundation for high-yielding cultivation (Liu et al., 2017). Soybean yield formation is closely related to moisture, previous studies have explored the relationship between water content and yield (Grassini et al., 2015; Prince et al., 2016; Anda et al., 2020). Therefore, drought resistance is an important indicator in the selection and planting of soybean varieties.

Breeders have bred a large number of varieties to cope with the crises that may be encountered during plant growth. In breeding efforts, germplasm resources with excellent traits such as high yield (Fukushima et al., 2011), resistance to specific diseases or insects (Mir et al., 2022), and tolerance to abiotic adversity are crucial (Vahdati et al., 2009). Studying the excellent traits possessed by these varieties, analyzing their physiological and molecular mechanisms, mining the key genes, and breeding or transforming them so that the excellent traits are stably inherited to future generations are often the keys to breakthroughs in breeding efforts (Timerbaev et al., 2019; Mou and Zhao, 2022).

Northeast China is a major soybean producing region, but frequent and severe drought in spring has a seriously impact on soybean growth and development, so it is especially important to identify and screen spring soybeans for drought tolerance at the seedling stage. In this study, we compared the drought tolerance of 60 soybean varieties through twice drought rehydration treatments at the seedling stage and observed plant survival to screen stable drought-tolerant and drought-sensitive materials, select a number of excellent soybean varieties for the soybean production area in northeastern China, and tap new germplasm resources for drought-tolerant soybean breeding so as to breed new

varieties to cope with the increasingly complex future of climate change.

Materials and methods

Test material

A total of 60 materials were collected from Soybean Research Institute of Northeast Agricultural University, Heilongjiang Bayi Agricultural Reclamation University, Heilongjiang Academy of Agricultural Reclamation Sciences, Suihua Branch of Heilongjiang Academy of Agricultural Sciences, Jiamusi Branch of Heilongjiang Academy of Agricultural Sciences, and Soybean Research Institute of Heilongjiang Academy of Agricultural Sciences (Table 1).

Test method

In the experiment, screen drought resistance at seedling stage by twice consecutive drought rehydration methods. Sow full seeds to plastic pots after disinfected (upper mouth diameter 10 cm, bottom diameter 8 cm, high 8 cm). The total amount of soil is 1 kg, sowing three holes at an equal distance in each pot, sowing two seeds in each hole, ensure three seedlings of similar growth per pot. Repeat three groups, five pots in each group. Set up the control group with the same number of pots as the drought treatment. As shown in Figure 1, treatment group irrigate normally before the first drought stress treatment, and pay attention to rain protection during the whole experiment. The water supply was stopped when the seedlings grew to the first triple compound leaf, until soil moisture content decreases to 15–20% of field capacity, then water supply was restored, so that the soil moisture reached $(85 \pm 5)\%$ of the field capacity. The number of survival seedlings was investigated 72 h after rehydration, and consider all leaves to remain green as surviving seedlings. In the second drought stress-rehydration treatment, the water supply was stopped after the first statistics of seedling survival rate, same as the first time. Drought persisted until soil moisture content decreased to 15–20% of field capacity. Then rehydration, the soil moisture reached $(85 \pm 5)\%$ of the field capacity. The number of survival seedlings was investigated after 72 h. The control group maintained $(85 \pm 5)\%$ of field capacity during the whole experiment. The number of surviving seedlings in the drought treatment group compared to the number in the control group, which is the survival rate. The mean of the three replicates was calculated as the final survival rate of the drought treatment. Real-time monitoring of soil moisture by Soil moisture meter ECH2O-TE/EC-TM (EM-50, Decagon, Washington DC, USA). Ensures the accuracy of drought or rehydration treatment.

TABLE 1 Cultivars tested.

No	Cultivars	Sources
I	Dongnong42(1), Dongnong47(2), Dongnong48(3), Dongnong52(4), Dongnong53(5), Dongnong54(6)	Soybean Research Institute of Northeast Agricultural University
II	Kennong4(7), Kennong20(8), Kennong29(9), Kennong30(10), Kennong31(11)	Heilongjiang Bayi Agricultural Reclamation University
III	Kenfeng6(12), Kenfeng7(13), Kenfeng9(14), Kenfeng12(15), Kenfeng14(16), Kenfeng17(17), Kenfeng19(18), Kenfeng20(19)	Heilongjiang Academy of Land Reclamation Sciences
IV	Suinong4(20), Suinong11(21), Suinong15(22), Suinong17(23), Suinong22(24), Suinong23(25), Suinong24(26), Suinong25(27), Suinong26(28), Suinong27(29), Suinong28(30), Suinong30(31), Suinong31(32)	Suihua Branch of Heilongjiang Academy of Agricultural Sciences
V	Hefeng26(33), Hefeng34(34), Hefeng38(35), Hefeng39(36), Hefeng41(37), Hefeng42(38), Hefeng49(39), Hefeng50(40), Hefeng51(41), Hefeng52(42), Hefeng56(43)	Jiamusi Branch of Heilongjiang Academy of Agricultural Sciences
VI	Heinong37(44), Heinong38(45), Heinong40(46), Heinong42(47), Heinong44(48), Heinong45(49), Heinong46(50), Heinong47(51), Heinong48(52), Heinong49(53), Heinong57(54), Heinong58(55), Heinong61(56), Heinong62(57), Heinong63(58), Heinong64(59), Heinong65(60)	Soybean Research Institute of Heilongjiang Academy of Agricultural Sciences

1, 2, and 3–60 represent different materials, respectively.

Determination index and method

At the seedling stage, the survival percentage after the first drought stress, the survival percentage after the second drought stress, the average survival rate after the first drought stress, the number of fixed seedlings before the first drought treatment, the average number of survival after the first rehydration and the average number of survival after the second rehydration. The calculation formula is as follows:

$$DS = \frac{DS_1 + DS_2}{2} = \left(\frac{X_{DS1}}{X_{TT}} \times 100 + \frac{X_{DS2}}{X_{TT}} \times 100 \right) / 2 \quad (1)$$

In the formula:

DS: Average survival rate of drought stress, %

DS₁: Survival percentage after first drought stress, %

DS₂: Survival percentage after second drought stress, %

X_{TT}: Number of surviving seedlings in the control group

X_{DS1}: Survival numbers after first drought stress

X_{DS2}: Survival numbers after second drought stress

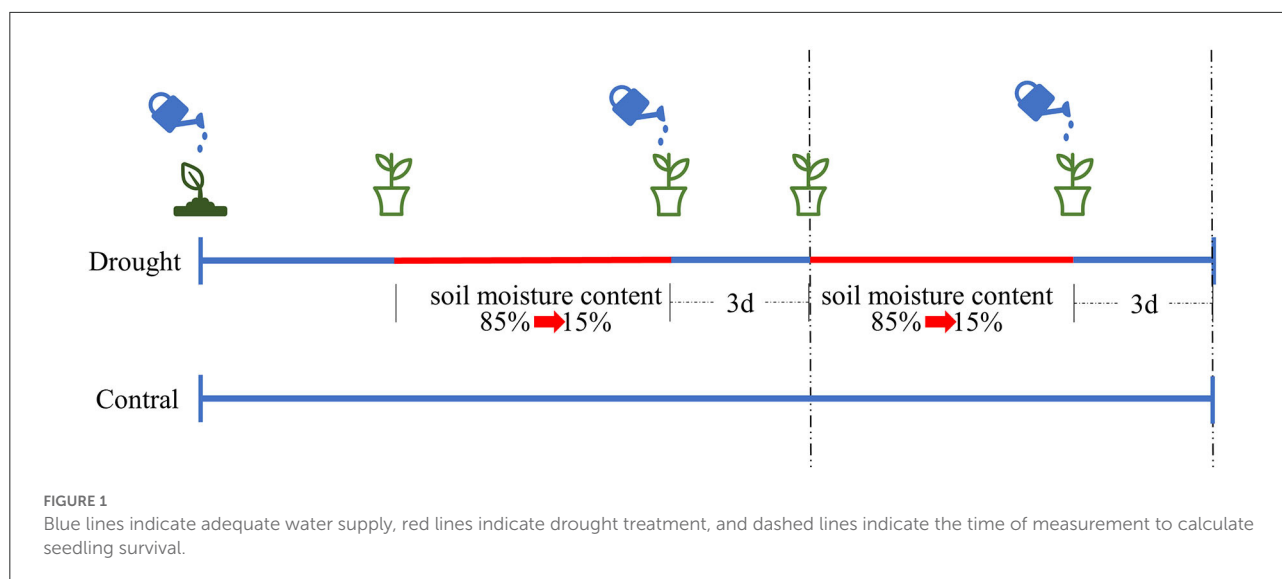
Test data processing and analysis method

All data were processed with Microsoft Office Excel 2021, statistical analysis of the data was performed using IBM SPSS software (version 23.0: IBM Corporation, Armonk, NY, USA).

Results and analysis

Survival number of seedlings after drought stress

It can be seen from Table 2 that the variation of seedling survival number is larger after the first drought stress and the



second drought stress, and the second drought stress is greater than the first drought stress, indicating that drought stress has a greater impact on seedling survival, that is, the seedling survival number has a large range of changes after the first drought stress and the second drought stress. The average survival number of seedlings after the first drought stress was significantly higher than that under the second drought stress, in which some varieties survived completely after the first drought stress, and no varieties survived completely after the second drought stress. However, some varieties died after the second drought stress, and no varieties died after the first drought stress.

Average survival rate of seedlings after drought stress

As can be seen from Table 3 and Figure 2, the seedling survival rate of each variety is different after the first drought stress, and most of the materials can resume growth after rehydration, the seedling survival rate is 87.22%. The results showed that there was a great difference in seedling survival rate among varieties after the first drought stress. The survival rate of 19 materials, such as Heinong44, Suinong4 and Hefeng42, reached 100% after the first drought stress, and the survival rate of all tested materials reached more than 60% after the first drought stress.

It can be seen from Table 3 and Figure 3 that after two consecutive drought stresses, the seedling survival rate of each variety decreased significantly, and the decreasing range of different varieties was different, which reflected that the drought resistance of each variety was different. The results showed that the drought tolerance of each variety after the second drought stress was more different than that of the first

drought stress. After the second drought stress, the average survival rate of seedlings decreased from 87.22% after the first drought stress to 21.44%, of which 14 varieties had a survival rate of 0, indicating that most of the tested materials were not tolerant to continuous repeated drought stress. There were only five varieties with survival rate of more than 60% (including 60%), which were Suinong30, Heinong44, Heinong37, Heinong45, and Heinong48, respectively, indicating that these five varieties showed good tolerance to continuous and repeated drought stress.

Figure 4 show that the average survival rate of the seedlings after two consecutive drought stresses, reflecting the ability of seedlings to survive after two consecutive drought stresses. The higher the average survival rate of the tested seedlings under drought stress, the stronger their survival ability under drought stress, that is, the stronger their drought tolerance. After two consecutive drought stresses, the average survival rate of Heinong44 under drought stress was 86.67%, and there were only four varieties with an average survival rate of more than 80% (including 80%) under drought stress. They were Heinong37, Heinong44, Heinong45, and Heinong48. In addition, they were the varieties with a survival rate of more than 60% under the second drought stress, indicating that these four varieties had good survival ability under continuous and repeated drought stress.

Comprehensive evaluation of drought tolerance in soybean seedlings

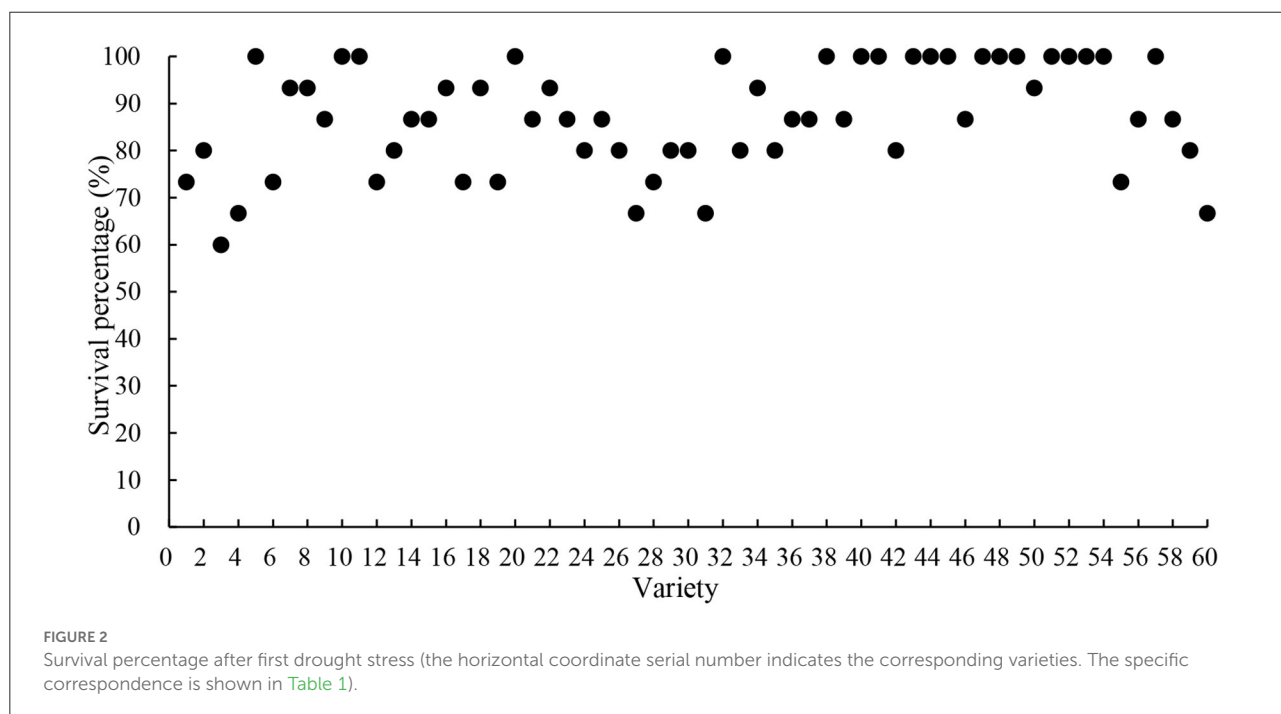
To group the studied soybean varieties according to their dissimilarity; Based on the survival rate under drought treatments, all the soybean varieties were clustered into four

TABLE 2 Survival numbers under drought stress.

Survival numbers after first drought stress				Survival number after second drought stress			
Min	Max	Variation	Average	Min	Max	Variation	Average
9.00	15.00	6.00	13.08	0.00	11.00	11.00	3.22

TABLE 3 Survival percentage under drought stress.

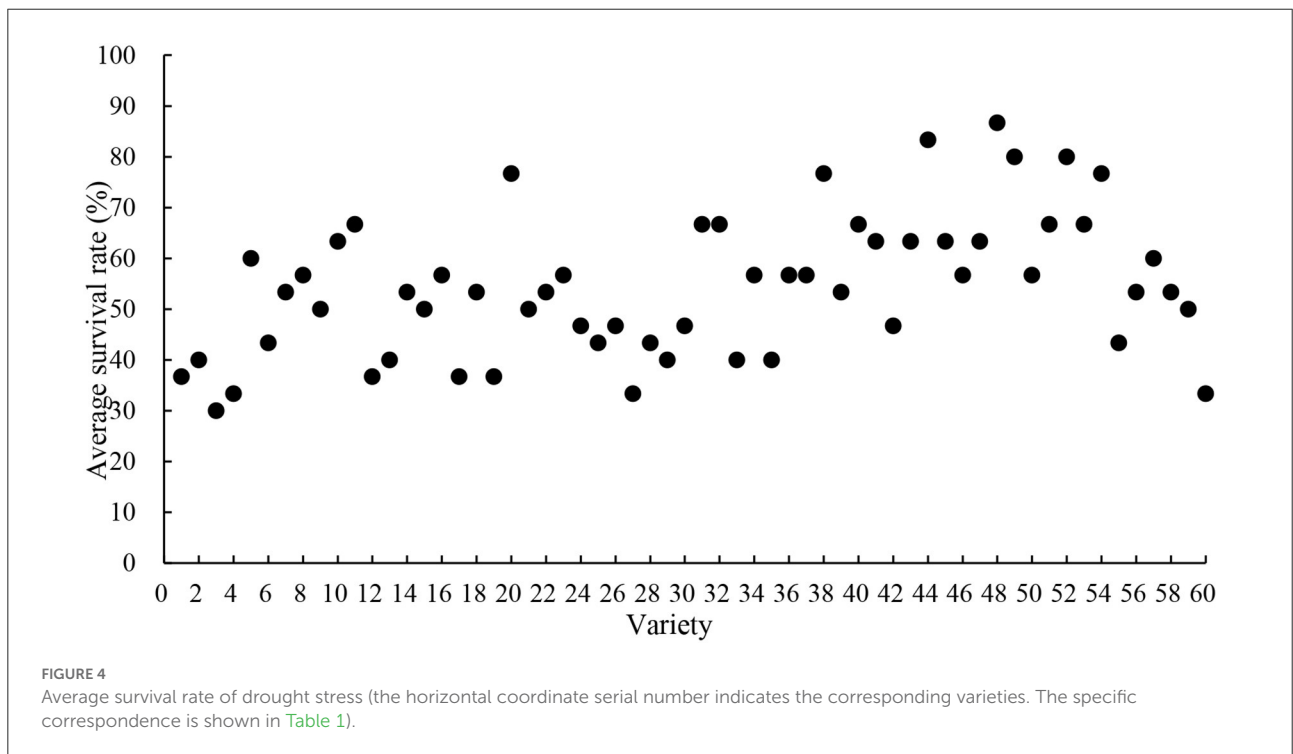
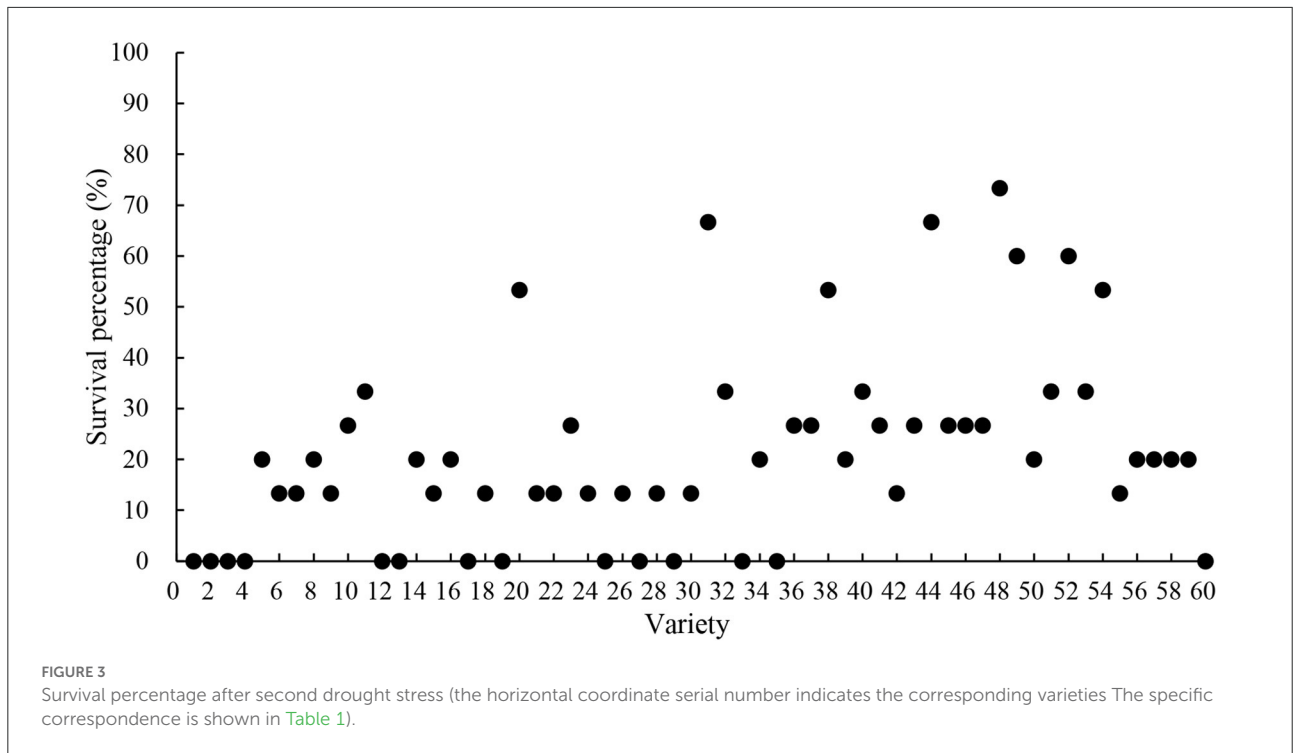
Survival percentage after first drought stress				Survival percentage after second drought stress			
Min	Max	Variation	Average	Min	Max	Variation	Average
60.00	100.00	40.00	87.22	0.00	73.33	73.33	21.44



groups, all drought tolerant varieties were grouped in cluster IV and sensitive varieties in cluster I. Cluster II and cluster III are considered moderately tolerant genotypes (Figure 5). Comparison in terms of survival rate, the order of drought tolerance from strong to weak is cluster IV, cluster II, cluster III, and cluster I. There were seven varieties were classified in Cluster IV, accounting for 11.67% of the total materials tested, they were Heinong37, Heinong44, Heinong45, Heinong48, Heinong57, Suinong4, and Hefeng42. In the cluster I, there were 17 varieties were considered drought sensitive, accounting for 28.33% of the total materials tested.

Correlation among drought tolerance indexes at seedling stage

Table 4 showed that the correlation coefficients between the average survival rate of drought stress and the survival rate of the first drought stress and the second drought stress were 0.875 and 0.943, respectively, which reached a very significant level. The correlation coefficient between the survival rate of the second drought stress and the survival rate of the first drought stress is higher than that of the first drought stress.



Discussion

There have been many studies focusing on the physiological responses of plants under drought stress, including the

production of reactive oxygen species (Zhanassova et al., 2021), changes in enzyme activity (Moloi et al., 2016), accumulation of osmoregulatory substances (Lotfi et al., 2010; Lu et al., 2019). changes in pigment content (Saha et al., 2020) and

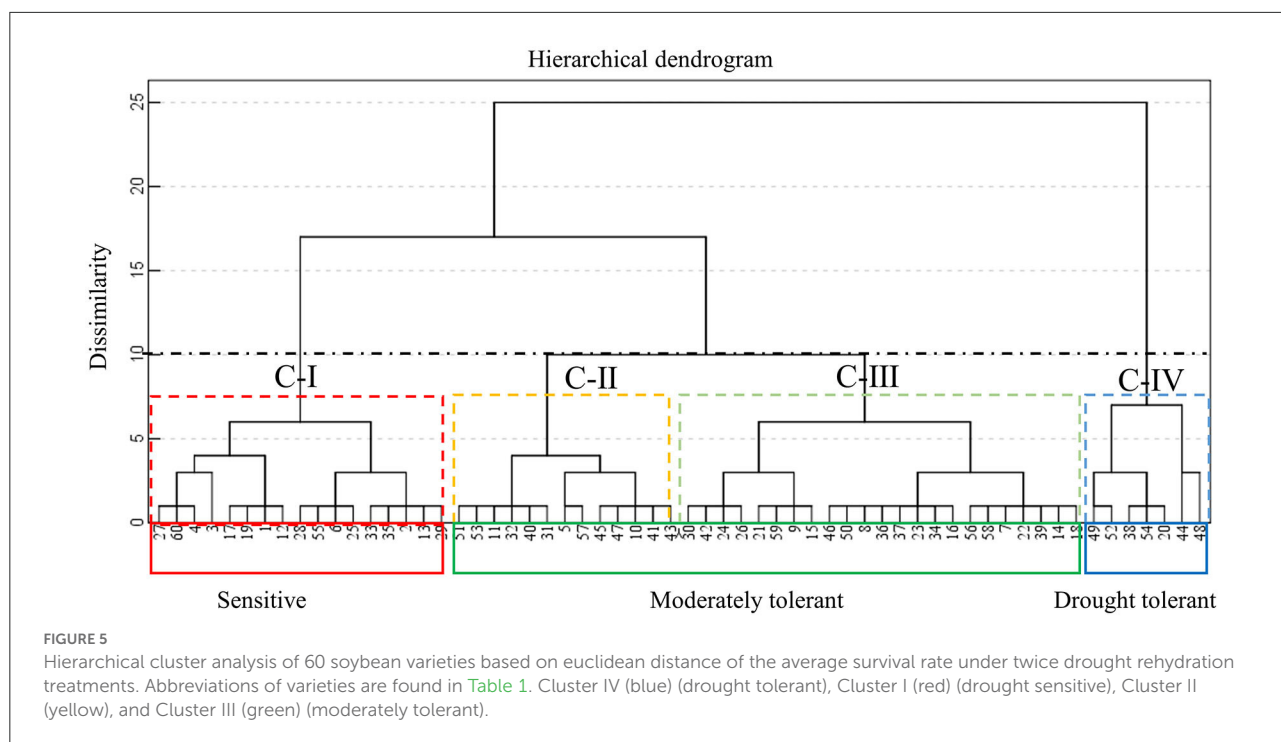


TABLE 4 Correlation analysis for parameters of drought screening at seedling stage.

Indicators	Survival percentage after first drought stress	Survival percentage after second drought stress	Average survival percentage
Survival percentage after first drought stress	1.000**		
Survival percentage after second drought stress	0.665**	1.000**	
Average survival percentage	0.875**	0.943**	1.000**

Multiple comparison using LSD test, ** mean extremely significant difference.

related molecular processes (Wang et al., 2022), etc. Resistant and sensitive varieties may differ in several of these indexes, however, the ultimate difference is either survival or death at a certain critical moisture or a certain critical stress time. Therefore, although several different physiological indicators have been reported previously to evaluate the tolerance of germplasm resources in an integrated manner (Belay et al., 2021; Xie et al., 2021), this experiment did not measure any physiological parameter but used twice drought rehydration treatments to observe plant survival. The varieties with strong drought resistance have higher survival rate under drought stress, and *vice versa* (Denton et al., 2018). The drought resistance of varieties was evaluated by the percentage of surviving seedlings. This method is simple, economical, and reliable. Ability to screen a large amount of plant material in a short period of time. Researchers proposed to identify the drought tolerance of plants at seedling stage by repeated drought stress, and many scholars used this method to identify

the drought tolerance of sorghum and cotton, respectively (Nour et al., 1978; Longenberger et al., 2006).

In this study, all leaves remained green after drought stress and were considered as surviving seedlings. The leaf is an important organ for photosynthesis in plants (Khan et al., 2022). As long as the leaf remains green, it means that the assimilation process is still going on, i.e., the plant is still alive. And under drought stress, leaves lose water and wilt, chlorophyll content decreases and a series of other physiological changes (Song et al., 2021), which eventually lead to the loss of green and death of the leaves and hinder the normal physiological activities.

The results of seedling experiment showed that the survival rate of tested materials after the first drought stress was significantly higher than that after the second drought stress, which may be that the time of the first drought stress was short. The degree of drought stress is not enough to reach the wilting coefficient of most of the tested materials, but it also

causes varying degrees of damage to the plants, resulting in yellow leaves and death. After the second drought stress, only a few of the tested materials showed relatively high survival rate, because the degree of drought stress for a long time reached the wilting coefficient of most of the tested materials, resulting in their withering. Rehydration can not alleviate the damage caused by drought (Martinez-Fernandez et al., 2015). In addition, there is a possible reason that soybean plants had only one compound leaf at the time of the first drought stress, while after experiencing drought and rehydration, the plants had grown new leaves at the time of the second drought treatment, and the number of leaves may imply a different transpiration effect, thus leading to an apparent difference in plant survival after the first and second drought stress (Gu et al., 2019). Our results indicate that the ability of plants to tolerate the second drought stress seems to be more important, while the damage of the second drought stress to plants is significantly higher than that of the first. However, in many previous studies, recurrent drought stress led to stress memory, causing relevant physiological changes or gene expression (Li et al., 2019; Kim et al., 2020), which eventually improved tolerance to subsequent stress. The key to the difference may be the rehydration issue, this experiment was rehydrated and treated for 3 days, which is similar to the first drought time. This may lead to changes in gene expression levels caused by stress memory, resulting in heavier damage to the second drought stress. Of course, this needs further exploration.

Conclusion

According to the screening and identification of drought tolerance of spring soybean at seedling stage, each variety was evaluated comprehensively by calculating the survival rate of seedlings under repeated drought stress. According to cluster analysis, 60 varieties were divided into four groups, all drought tolerant varieties were grouped in cluster IV, including Heinong37, Heinong44, Heinong45, Heinong48, Heinong57, Suinong4, and Hefeng42. A total of 17 sensitive varieties were grouped into Group I, accounting for 28.33% of the total materials tested. The remaining 36 varieties were considered to

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be moderately drought tolerant, accounting for 60% of the total materials tested.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

XW wrote the draft of the manuscript. XW and XL contributed to data curation, analysis, and visualization. SD contributed to manuscript revision. All authors approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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