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### **Evaluation of Drought-Tolerant Legume Varieties Under Rainfed Conditions**

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Abstract In the context of rain-fed agriculture, this study systematically evaluated the field performance of various drought-tolerant leguminous crop varieties. Firstly, the impact of drought stress on legume production and the characteristics of the target environment were analyzed, and the research objects and scope were clarified. In terms of the theoretical framework, the physiological and ecological mechanisms of drought tolerance in legume crops were summarized, key evaluation indicators and index systems were proposed, and the interaction between genotypes and the environment as well as the theory of adaptability were discussed. Subsequently, a field trial plan was designed, including the selection of test sites, the design of random blocks and the setting of control groups. Consistent cultivation and management measures were adopted to compare the drought tolerance of multiple legume varieties. By measuring yield composition, physiological and root system and other phenotypic trait indicators, as well as obtaining phenotypic data through high-throughput remote sensing methods, the performance of each variety under drought conditions was comprehensively collected. In-depth analysis of the data was conducted by using statistical methods such as yield stability analysis, drought resistance index calculation, trait correlation analysis, water use efficiency estimation and crop model simulation. The results indicated that there were significant differences in drought resistance among different varieties, and the genotype × environment interaction affected the yield stability. Some key traits (such as root systems, physiological indicators, etc.) make significant contributions to yield. By comparing the results of this study with existing literature and breeding goals, it was found that the drought-tolerant varieties selected reached or exceeded the existing levels in terms of yield and stability. Finally, variety recommendation schemes and field management optimization measures for different dryland ecological zones were proposed, and the strategies and technological development directions of drought-tolerant breeding in the future were prospected. The research provides a scientific basis for increasing the yield of leguminous crops under rain-fed conditions.

Keywords Legume crops; Drought stress; Output stability; Water use efficiency; Genotype-environment interaction

#### 1 Introduction

Rain-fed agriculture actually refers to being dependent on the weather, mainly relying on natural precipitation rather than artificial irrigation. This approach is particularly common in semi-arid and arid regions. The problem is also obvious. Droughts occur almost every year, whether seasonal or long-term, and have a significant impact on crop yields. Leguminous crops are no exception. A common description in research is that lack of water can prevent legume plants from growing, causing their leaves to shrink and their branches and leaves to wilt. The situation gets even worse when they start flowering or podding, often resulting in unstable flowers and failure to form pods. Eventually, the yield drops significantly (especially during the critical growth stage, the reduction in yield caused by insufficient water is often irreversible) (Farooq et al., 2017). However, the performance of different species is not consistent. Even within the same species, there are significant differences in drought resistance among different varieties (Daryanto et al., 2015). For instance, in years of extreme drought, the output of some soybean varieties in saline-alkali land in China may be less than half of that in normal years. Interestingly, drought not only reduces the current season's yield but also disrupts the nitrogen fixation in the soil, affecting soil fertility and even causing damage to the yield of subsequent crops (Irar et al., 2014). Therefore, for rain-fed agriculture to develop in the long term, how to deal with drought and cultivate drought-resistant and high-yield varieties has almost become an unavoidable key task.

This study did not randomly select a few crops, but focused on several leguminous food crops that are particularly representative in semi-arid regions, such as soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), peas (*Pisum* 

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sativum), and mung beans (Vigna radiata). Their respective distributions also have some differences: soybeans and peanuts are more commonly grown in rain-grown areas in northern China, while peas and mung beans are more frequently found in dryland systems in Northwest and North China. When evaluating drought tolerance, it is not just about looking at one aspect, but taking into account the entire growth process from seed germination to flowering and podding to observe the overall impact of drought on phenotypes and yields (Ali et al., 2018). As for the experimental environment, all the selected ones were typical rain-fed farmlands, which have distinct characteristics-little rainfall, uneven rainfall, light soil and high evaporation. For instance, in the Horqin Sandy Land of Inner Mongolia, the climate is a typical temperate continental monsoon: summers are short and hot with scarce rainfall, and spring and autumn are dry and windy. Such an environment is precisely the touchstone to test whether beans can withstand drought (Zhang et al., 2012). The research adopts multi-point experiments. In other words, it covers different ecological conditions to clearly see whether the drought tolerance of each variety is stable and adaptable under the interaction of genotype and environment (Yan et al., 2015).

In rain-fed environments, the differences in drought resistance among legume varieties can be seen at a glance. Some can still maintain their output during water shortages and perform quite steadily, but there are also many varieties that fail as soon as there is a drought. Generally speaking, drought-tolerant plants often have more advantages in water retention, photosynthetic efficiency or antioxidant reactions, but this is not an absolute rule. In the research, some quantitative indicators were attempted to make judgments, such as drought resistance index and yield stability parameters, etc. However, after comparison, it was found that the results of several methods did not always match (sometimes the differences were quite significant). One more point that is easily overlooked is that the interaction between the environment and genotypes is sometimes more prominent than the main effect of the variety itself. This means that when promoting, merely focusing on the variety name is unreliable; different ecological regions require a different approach. Overall, these findings, on the one hand, explain the physiological and ecological reasons behind the differences, and on the other hand, provide some practical references for drought-resistant breeding and the management of arid areas.

#### 2 Theoretical and Conceptual Framework

#### 2.1 Drought tolerance mechanism and physiological and ecological basis

The drought tolerance of leguminous crops is not the result of a single link, but rather the accumulation of multiple levels of action: the adjustment of morphological structure, the regulation of physiological state, and the coordination of molecular and biochemical mechanisms. First, let's look at the shape. Drought-tolerant varieties usually have more developed root systems that can penetrate deep into the soil to absorb water. Their leaves are often smaller, with thicker cuticle layers, and thus less transpiration. Studies have found that the root cap of drought-resistant types is generally higher than that of sensitive varieties, and their root systems are more capable of exploring downward during droughts (Vadez et al., 2008). Looking at the physiological responses, the common ones are stomata and osmotic regulation. During droughts, some varieties will partially close their stomata earlier to control water loss while still maintaining a relatively high water use efficiency (WUE). Some will adopt a "throttling" strategy when the atmosphere is particularly dry. Stomata are more sensitive to vapor pressure loss. By moderately reducing the rate of light formation, soil moisture can be saved in exchange for future use. Meanwhile, osmotic regulation is also at work. Small molecules such as proline and soluble sugars accumulate, reducing the water potential of cells and helping to maintain a relatively high water content (Zhang and Shi, 2018). Finally, at the biochemical level, the efficiency of the antioxidant enzyme system is often higher in drought-tolerant varieties, such as superoxide dismutase (SOD) and peroxidase (POD), which can eliminate excessive reactive oxygen species (ROS) produced under drought conditions, thereby reducing the peroxidation damage of membrane lipids (Ahmad et al., 2022).

#### 2.2 Key evaluation indicators and index system

Evaluating the drought tolerance of legume varieties cannot rely on a single indicator; usually, a relatively comprehensive system needs to be established. Common practices can be divided into two categories: yield type and physiological and ecological type. Output types include relative output, reduction rate, drought resistance coefficient, etc. Among them, the drought resistance coefficient measures the degree of reduction by the ratio of



output under drought conditions to that under normal conditions. The physiological and ecological category includes leaf water content, chlorophyll, photosynthetic rate, as well as proline and soluble sugar content, which are used to reflect the state of plants under drought conditions. A single indicator is often difficult to be comprehensive. Therefore, in recent years, comprehensive evaluation methods have been mostly adopted. For instance, principal component analysis is used to extract key traits, and then combined with the membership function method to calculate the comprehensive score and rank the drought resistance of varieties (Figure 1) (Bao et al., 2023). This type of method has been verified on crops such as rice, soybeans, mung beans and wheat. Research on peanuts shows that total biomass, relative moisture content, peroxidase activity and soluble sugar are important indicators for distinguishing drought-resistant varieties from sensitive ones. Research on soybeans has found that chlorophyll content, single plant grain weight and the number of root nodules contribute the most (Yan et al., 2020). Meanwhile, yield-based indicators such as the Drought Sensitivity Index (DSI), Drought Tolerance Index (TOL), Average Productivity (MP), and Geometric Mean Productivity (GMP) are also commonly used, but they are easily affected by yield levels and thus are more suitable for combination with multiple indicators (Chen et al., 2012). This study will adopt methods such as relative yield, drought resistance coefficient and the comprehensive value of membership function for cross-validation to more comprehensively characterize the drought resistance capacity of varieties.

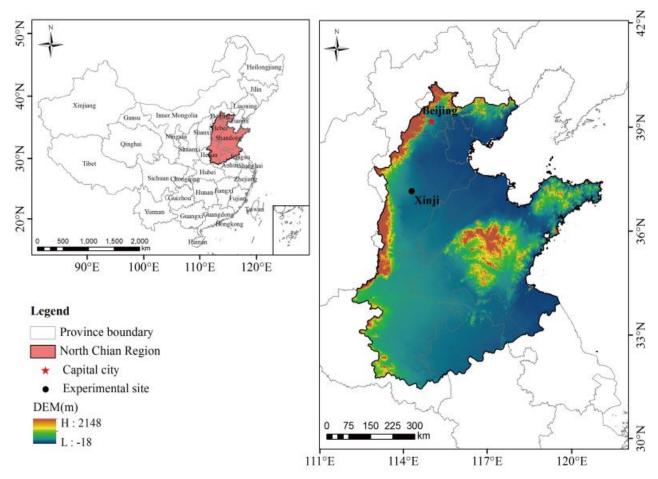


Figure 1 The geographic overview of the North China Plain and the experimental site (Adopted from Bao et al., 2023)

#### 2.3 Genotype-environment interaction and adaptability theory

In multi-environment experiments, it is often encountered that a variety has a high yield in one place, but it falls behind once the environment is changed. This is actually what is called the interaction between genotype and environment (G×E). This situation is even more pronounced in drought-tolerant breeding. Some varieties perform well in arid areas, but they do not have an advantage when placed in humid conditions. The methods for analyzing the adaptability and stability of varieties are also changing. In the past, linear regression and double-label plots

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were commonly used, but nowadays, AMMI and GGE double-label plots are more popular. AMMI separates genotype, environment and interaction effects, and then uses principal component analysis to reveal the patterns among them. GGE is even more straightforward, presenting the average output and stability in a single chart (Thangavel et al., 2011; Rao et al., 2023). Take the Huang-Huai-Hai region as an example. When conducting GGE analysis on 11 summer soybean varieties, the results showed that the pilot could be divided into two "super environments", and in each environment, there were relatively better varieties. It should be noted that the performance of drought-resistant varieties cannot be judged solely by the years of drought. Some can maintain high yields under extreme drought conditions, but their advantages are not significant in bumper years. There are also some that remain relatively stable in different years and are more suitable for promotion (Sharma et al., 2022). This research will conduct G×E analysis using multi-year and multi-point data in order to screen out drought-tolerant varieties with wide adaptability and specific adaptability. Considering the trend of climate change, the "yield resilience" of varieties is becoming increasingly crucial.

### 3 Experimental Design and Material Methods

#### 3.1 Selection of test sites and description of environmental characteristics

This study selected three rain-cultured sites in northern China, but the climatic conditions varied greatly. Tongliao is located in the Horqin Sandy Land, with an annual rainfall of approximately 350 mm. The soil is sandy loam and it often suffers from spring drought and summer drought (Wang et al., 2015). Wuwei is located on the edge of the Hexi Corridor. With an annual rainfall of only 200mm, it is accompanied by an evaporation of over 1500mm, making it a typical dry farming area. Although Anyang has an average annual rainfall of 600 millimeters, most of the rainfall is concentrated in summer, and short-term droughts occur frequently (Shi et al., 2017). All the test sites rely on natural precipitation and no irrigation is carried out. To ensure representativeness, the experiment was conducted for two consecutive years: the rainfall in the first year was lower than the average of the years, while in the second year it was relatively higher, but there were still periods of drought. Rainfall, temperature and soil moisture content were recorded in detail at each point. The results show that drought often occurs during the flowering and podding stage: In Wuwei, there was only 20 mm of rainfall within 45 days from the beginning of flowering to the grain filling stage, with severe water deficiency. Tongliao experienced nearly 30 days without rain from the seedling stage to the initial flowering stage. Although Anyang has abundant precipitation, there is also an intermittent drought of about 20 days in July. Overall, these points are covered from semi-humid and slightly dry to typical arid areas, providing a suitable platform for drought resistance evaluation.

#### 3.2 Field design and control setup methods

The layout of this experiment is quite conventional yet a bit special. Random blocks were used in the design, with each point repeated three times, mainly to reduce the errors caused by environmental differences. The selection of the comparison was quite thoughtful: Zhonghuang 13 was used for soybeans, Longwan 3 for peas, Yinchuan Dalu for mung beans, and a variety of peanuts from the Luhua series with relatively weak drought resistance was chosen. In each district group, a control is repeatedly installed to facilitate subsequent comparisons. The division of the community is determined by the crops. For soybeans and peanuts, it is 4 rows ×5 meters, approximately 15 square meters. Peas and mung beans are 6 rows by 4 meters, approximately 10 square meters. The row spacing and density are kept consistent with the conventional practice. The blocks are randomly arranged inside, with a 1-meter isolation belt and protective rows added. One rather special point is that initially, there was no control of "adequate irrigation", and water stress was entirely formed by the differences in precipitation at different years and points. However, in the following year, at Henan Point, an additional irrigation area was added to see what level the potential output could reach. The sowing time varies from place to place according to local customs. In Henan, soybeans are sown in summer, while in Inner Mongolia, they are sown in spring. The planting density is carried out in accordance with the recommended values. Apply only one base fertilizer, no top dressing, and carry out regular pest and disease control. Throughout the entire growth period, water is basically not given. Only when it is extremely dry will "life-saving water" be applied, but this has not happened in the past few years of the experiment. In this way, when the water conditions are basically the same, the differences in drought tolerance among varieties become clearer.

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#### 3.3 Variety materials and cultivation management measures

There are a total of 20 genotypes in this material, including 6 for soybeans, 5 for peas, 4 for mung beans, and 5 for peanuts. Both conventional varieties and new strains with stronger drought resistance are included. The key points to consider when making a selection are that the regional adaptability and drought tolerance differences should be more obvious. Among soybeans, Zhonghuang 13 is the control, Heinong 44 has relatively weak drought resistance, while Sili Hong is more drought-resistant. The peas were controlled with Kewan No. 3 and also with Ningwan No. 4. There are two types of mung beans: "Green Star" from Gansu Province and "Jilu No. 2" from Jilin Province. The peanuts include HO-1, which is high in oleic acid (with good drought tolerance), and Luhua 11. Before sowing, the germination rate of all seeds was measured and they were uniformly coated.

Cultivation and management should be kept as consistent as possible, but there are still some differences in details among various regions. For instance, in Inner Mongolia and Gansu, soil moisture retention is achieved through compaction, while in Henan, no tillage and stubble retention are adopted. Sowing should be uniformly carried out using precision seeders, with the depth controlled between 3 and 5 centimeters. The seeding rate should be based on the basic number of seedlings, approximately 10 kilograms per mu for soybeans and 15 kilograms per mu for peas. Standardize the application of fertilizers, applying 20 kilograms of N, 60 kilograms of P<sub>2</sub>O<sub>5</sub> and 30 kilograms of K<sub>2</sub>O per hectare at one time. Field management was also carried out in accordance with uniform requirements, including replanting, pest and disease control, and chemical weeding. Additional supports were set up for the peas to prevent them from lodging. Throughout the entire growth period, there was basically no irrigation. It relied on natural precipitation, and no extreme cases occurred. During the experiment, phenology and weather conditions were also recorded. Protective rows were set up around the plot to reduce marginal interference, making the obtained data more reliable (Wang et al., 2020).

#### 4 Phenotypic Traits and Data Collection

#### 4.1 Yield and constituent trait indicators

Yield and its composition indicators are the key to measuring drought tolerance, but the harvest times for different crops are not the same. For instance, soybeans and mung beans should be harvested only when over 80% of the pods have turned yellow. Peas are usually harvested in the middle to late July, and peanuts need to be picked before the frost. When collecting, remove the edge rows and only calculate the weight of the effective area in the middle. In addition to the total output, we also measured the constituent factors such as the number of pods per plant, the number of grains, and the weight per hundred grains. The peanuts also recorded the number of full and unfull fruits. Randomly select 10 samples from each plot and calculate the average value to represent them. These indicators respectively record the values under drought and normal conditions, and then uniformly convert them into relative yields and reduction rates to analyze the drought resistance of varieties (El-Nabarawy et al., 2016; Yan et al., 2020). In terms of results, the differences in yield reduction among different varieties are quite significant: some pea varieties only have a 10% reduction in yield during severe drought, but others can exceed 40%. Among the soybeans, the output of Heinong 44 has decreased by more than 30%, while that of Silihong and Suinong 28 is less than 15%, remaining relatively stable. From the perspective of traits, generally speaking, varieties with better drought resistance have less reduction in the number of pods or grains, indicating that the flowering and podding period is less affected by drought. These measurements provide a basis for the subsequent quantitative evaluation of drought tolerance.

#### 4.2 Physiological characteristics and root system characteristics indicators

To understand the differences in drought tolerance mechanisms among various varieties, we measured some physiological and root system indicators during the period when drought was more obvious. For instance, in terms of leaf moisture, the relative moisture content (RWC) and leaf water potential were measured during the flowering period. Generally speaking, the RWC of drought-tolerant varieties is higher than that of sensitive ones-some drought-tolerant peas can even maintain a RWC of over 80%, but sensitive varieties are less than 70%. Photosynthesis and gas exchange were also measured by instruments. It was found that the photosynthetic rate (Pn) of drought-tolerant varieties decreased less, the stomatal opening degree (Gs) decreased appropriately, and the

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water use efficiency increased instead. For instance, in a certain mung bean variety, the Pn only dropped by 15%, while in the control variety, it decreased by over 30% (Zhang and Shi, 2018).

In terms of osmotic regulation, the proline and soluble sugar content of drought-tolerant varieties have significantly increased. For instance, the proline content of the soybean "Four Grains Yellow" has risen by more than three times, indicating that its ability to regulate osmosis is stronger. In the antioxidant system, the activities of SOD and POD in drought-tolerant varieties are relatively high, while the increase in MDA is not significant. However, for sensitive varieties like Heinong 44, the MDA content is more than twice as much as before, and the enzyme activity keeps dropping (Ahmad et al., 2022). In terms of root systems, drought-tolerant ones generally have deeper roots, more fine roots, higher root trunk weight and root-crown ratio, and some can even maintain a decent number of root nodules (Vadez et al., 2008). Overall, drought-resistant varieties do perform better in water retention, photosynthesis, osmotic regulation, antioxidation, and root water absorption. This should also be the reason why their yields are more stable.

#### 4.3 High-throughput and remote sensing phenotypic data acquisition

Traditionally, although physiological data can be obtained through manual measurement, the timeliness and spatial representativeness are not very satisfactory. So this time we used some remote sensing and high-throughput methods to assist in monitoring. For instance, for soybeans and peanuts, we use drones to conduct multi-spectral flights every two weeks from emergence to grain formation, extracting NDVI and moisture index, which are then used to calculate leaf area and water content. It was found that the NDVI of drought-tolerant varieties was 10% to 20% higher than that of sensitive varieties under drought conditions, and the decline in leaf area index was also less, approximately 10%, while sensitive varieties might have a decrease of more than 30% (Balota and Oakes, 2017). In addition, infrared thermal imaging shows that the canopy temperature of drought-tolerant varieties is actually 1 to 2 degrees higher, indicating that their stomata close more promptly and save water. In Gansu Province, we also used a high-throughput platform to quickly test the leaves and root systems of some soybeans. From the perspective of fluorescence parameters, the Fv/Fm of drought-resistant varieties decreased less, and the photosystem was less damaged. Root scanning is indeed much faster than manual measurement. Overall, these remote sensing and high-throughput results are basically consistent with those measured manually, and they have a larger coverage. Preliminary analysis shows that NDVI and yield are still positively correlated (r≈0.7), indicating that it is somewhat helpful for estimating yields under drought conditions (Carvalho et al., 2015). However, we mainly rely on manual measurement, and remote sensing only plays an auxiliary verification role.

#### 5 Data Analysis and Statistical Methods

#### 5.1 Analysis methods for output stability and adaptability

To clarify whether the yields of different varieties are stable and their adaptability is strong, we conducted variance analysis and stability assessment on the yield data of multiple experimental sites over two years. In fact, the impact of the environment is even greater than that of the variety itself, accounting for more than half of the variations. For instance, in the case of soybeans, location differences account for 55.3%, while genotypes themselves make up less than 6%. However, the interaction between varieties and the environment is also quite obvious, accounting for about 38%. This indicates that although the environment is dominant, it is also very important that varieties perform differently in different places (Firew et al., 2019).

We used the AMMI model and the GGE bigraph to analyze this interaction, and the results were quite intuitive: the pilot was roughly divided into two groups, and the best-performing varieties in each group were not the same. For instance, "Xu 9416-8" had the highest yield in one group, while "Liu Dou 108" performed better in another group. In terms of stability, the main parameters examined were CV%, regression coefficient bi and  $\sigma^2$ \_d. A small CV indicates a stable yield. A bi close to 1 is considered a variety with wide adaptability. A CV less than 1 May be more resilient to adverse conditions, but it is not outstanding under high water and fertilizer conditions. A CV greater than 1 is suitable for high-yield environments, but it is prone to yield decline when encountering drought. Like "Handou 13", which has a small  $\sigma^2$ \_d and bi close to 1, it is a type that is both high-yielding and stable.

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"Shengyu 6" also approaches this ideal characteristic. The situation of other beans is similar. Two pea varieties have been quite stable for two years, with CVS both below 10%. However, there is a new variety of mung bean that has a high yield when there is good rainfall, but it drops significantly when there is a drought, with a CV exceeding 25%. These analyses help us clearly understand the adaptability types of the varieties and are very helpful for breeding varieties that are both high-yielding and stable in yield (Rao et al., 2023).

#### 5.2 Index calculation and trait association analysis

To compare the drought resistance of different varieties, we used several indices, such as DSI, TOL, MP, GMP and STI, and also conducted correlation and principal component analyses. For instance, for soybeans, a DSI lower than 1 indicates good drought resistance and less yield reduction. A small TOL is often drought-tolerant but the yield may not be high. MP, GMP and STI simultaneously reflect output and stability. The correlations among these indicators are very strong. In peas, STI is highly positively correlated with GMP (r=0.98), while negatively correlated with DSI (r≈-0.85) (Ryabukha et al., 2023). Principal component analysis revealed that the first two principal components accounted for over 90% of the variations. PC1 was mainly determined by STI, GMP and MP, while PC2 was negatively correlated with DSI (Rehman et al., 2019). The results sorted by PC1 are basically consistent with the actual yield performance in the field. Regression analysis indicated that the chlorophyll retention rate, root dry weight and proline content of soybeans could jointly explain approximately 85% of the yield changes. The survival rate of pea seedlings and the relative water content of leaves during the flowering period were significantly correlated with the yield (r approximately 0.77 and 0.72). The relative germination rate of mung beans during the germination period is also correlated with the yield reduction rate (r=0.68) (Morovati and Kordenaeej, 2021). From the perspective of coefficient of variation, the CV of yield under drought conditions is approximately 20%, while the cumulative CV of proline reaches 40%, indicating a higher degree of differentiation. Overall, varieties with good drought resistance generally have the characteristics of low DSI, high STI and strong green retention. These indicators can provide a basis for drought-resistant breeding.

#### 5.3 Water use efficiency and crop model simulation

Under drought conditions, water use efficiency (WUE) often becomes the key to stable production. However, the differences among various varieties are quite obvious. First, we estimate the WUE by combining the output and actual water consumption, and then use the model to examine the long-term performance. The calculation mainly uses the Penman-Monteith model and the measured evapotranspiration data to obtain the grain WUE. The results are quite intuitive: In Gansu Province, some soybean varieties consume approximately 400 liters of water to produce 1 kilogram of seeds, while the control variety consumes more than 500 liters (Park et al., 2014). The results of gas exchange are similar. The A/T ratio of drought-tolerant varieties is generally higher, indicating that water usage during photosynthesis is more cost-effective. On the model side, after calibration with CROPGRO, 20 years of meteorological data were input to run the simulation. The results show that in particularly dry years, drought-resistant varieties may suffer a reduction in yield of approximately 15%, while the control varieties may drop by more than 30% (Bulacio et al., 2023). It is worth noting that if the climate continues to warm up, the yield reduction of those drought-tolerant varieties will be more moderate due to their earlier closure of stomata and lower evaporation. The simulation also reminds us that the upper limit of yield is mainly determined by the water that the environment can provide, but drought-tolerant varieties are more likely to approach this upper limit. For instance, when the annual rainfall is 300 mm, drought-resistant soybeans can yield 1.8 tons per hectare, which is close to 90% of the potential, while common varieties only have about 1.5 tons. From the comparison of the measured and simulated results, it can be seen that both point to one conclusion: drought-resistant varieties are more water-efficient and efficient, which can be regarded as a very practical basis in variety screening (Silva et al., 2022).

#### 6 Results and Discussion

### 6.1 Analysis of inter-species differences and genotype-environment interactions

After field trials, it was found that the yields of different legume varieties vary quite a lot under drought conditions where they rely on the weather. For instance, regarding soybeans, the average yield of the three sites over the past two years ranged from 1.3 to 2.2 tons per hectare. "Handou 13" performed the best and was the most

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stable, with an increase of about 10% compared to the control medium, Huang 13. However, "Jindou 40" is a bit inferior, only 80% of the comparison. Drought-resistant varieties like Silihong and Suinong 28 have a yield reduction of less than 15%, but sensitive types such as Jiyu 86 may experience a drop of over 30% in drought (Asfaw et al., 2009; Belay et al., 2019). Other beans are more or less the same. Among the peas, "Qinghai 13" can still have an 85% yield in a drought year, while some varieties only have a 60% yield. We also found that the interaction effect between varieties and the environment is quite obvious: some varieties grow well everywhere and belong to the widely adaptable type; some are particularly picky about the location. For instance, there is a new variety of mung bean that performs outstandingly in the arid regions of Inner Mongolia, but in the more humid areas of Henan, it is just so-so. This interaction effect can explain 30% to 40% of the variation. It can also be seen from the GGE chart that the most suitable varieties for different regions are actually not the same (Dadras et al., 2017). There is no "universal variety" that is strong in everything. Although the influence of the environment accounts for more than half, the output can still be increased through variety and regional matching. In conclusion, when breeding, it is best to select drought-resistant varieties based on different ecological regions. Of course, widely adaptable varieties may become increasingly useful in the future, after all, the weather is unpredictable.

#### 6.2 The contribution of key traits to yield performance

Based on our field observations and data analysis over the past few years, there are indeed significant differences in drought resistance among different varieties. For instance, in terms of root systems, varieties with deep roots and large root mass usually have higher yields. For example, the root dry weight of the soybean "Si Li Hong" is 25% higher than that of "Ji Yu 86", and the leaves remain green and the grains are plump as well. The water retention capacity of leaves is also very important. Under drought conditions, leaves with a higher relative water content (RWC) tend to maintain better yields. The leaf temperature of drought-resistant varieties is usually slightly higher, indicating that the stomata close earlier and save water. In terms of photosynthetic and water use efficiency (WUE), varieties with less decline in photosynthetic rate naturally have more stable yields. This type of variety generally maintains good chlorophyll and has a high WUE. In addition, drought-tolerant individuals generally accumulate a large amount of proline and soluble sugar, have high antioxidant enzyme activity, and suffer less membrane damage. Overall, deep root systems, good water retention, high WUE and strong oxidation resistance are common features of drought-resistant varieties. By using the root-crown ratio, chlorophyll retention rate and proline content for discrimination, nearly 90% of the varieties can be correctly classified. These rules are quite consistent in legumes and should have good reference value for material selection in breeding.

### 6.3 Comparison with existing research and breeding objectives

Compared with previous research and breeding goals, our results this time have both similarities and differences. For instance, the soybean variety "Handou 13" has increased production by approximately 10% compared to Zhonghuang 13, which is close to the performance of the widely applicable varieties that have been reported. The drought resistance of the pea variety "Qinghai 13" in the dry land of the plateau has also been verified. In terms of drought tolerance mechanisms, features such as deep root systems, good water retention, high water use efficiency and strong antioxidant capacity are indeed crucial, which is consistent with some previous conclusions (Islam et al., 2022; Xu et al., 2023). However, some different situations have also been found. For instance, some mung bean varieties are drought-tolerant during the seedling stage, but suffer from severe drought during the reproductive period, indicating that the entire growth period should be examined. There are also some varieties that belong to the "tolerant type", with good leaf green maintenance but not necessarily high yield. Some other "avoidant" types avoid drought by maturing early, resulting in more stable yields. These results not only verify the existing understanding but also provide some new basis for drought-resistant breeding.

#### 7 Suggestions for Agronomy and breeding Applications

### 7.1 Target environment zoning and variety recommendation scheme

Based on the performance of the varieties in rain-fed environments, the dryland farming areas in the north can be roughly divided into three categories, and the corresponding varieties are recommended. For instance, in semi-arid areas like Horqin in Inner Mongolia, where the annual rainfall is 300 to 400 millimeters and spring drought is

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frequent, it is suitable to grow soybeans such as Si Li Hong and Sui Nong 28, and peas like Qinghai 13 can be chosen. In the semi-humid and slightly arid area in the northern part of the Huanghuai region, the annual rainfall is 500 to 600 millimeters, but it is prone to drought in summer and autumn. For soybeans, Handou 13 and Shengyu 6 are recommended. For peanuts, HO-1 can be chosen. For mung beans, early-maturing types such as "Jingyan 7" are suitable. In the more arid Hexi Corridor and the Northwest Plateau, the annual rainfall is less than 250 millimeters. It is recommended to plant mung beans "Longly No.1" and peas "Ningwan No.1". Peanuts carry a high risk, so it is necessary to choose small-sized early-maturing varieties such as "Huayu 28" (Zhang et al., 2011). In addition, crop rotation or intercropping (such as peas/millet) can help retain moisture and increase production. Combining 2 to 3 complementary varieties in the same area can also reduce risks. In conclusion, it is very necessary to precisely recommend varieties based on local characteristics.

#### 7.2 Optimization measures for field management

Although drought-resistant varieties are important, if they are not managed properly, their effectiveness will also be compromised. In terms of moisture retention, mulching or minimal no-tillage is often better than bare land, and the soil moisture content during the seedling stage can be 2%-5% higher. The density is not constant. In dry years, it is appropriate to reduce it by 10%-15%. For instance, if the yield of soybeans is reduced from 330 000 plants per hectare to 280 000 plants, the yield per plant can still increase. When there is more rain, the plants can be planted more densely. Fertilization depends on the situation. During drought, potassium and boron fertilizers can help maintain water balance. Spraying some potassium dihydrogen phosphate and micro-fertilizer on peas during the flowering period can increase production by approximately 8%. In some places, water is stored through rainwater collection ditches and small plots for planting. Even in extremely dry years, some water is replenished to prevent "neck drought". Intercropping is also quite interesting. For instance, when soybeans and corn are interplanted in strips, water can be utilized more fully. The sowing period can also be adjusted. If summer soybeans are sown earlier or mung beans are sown at different times, the summer drought can be avoided. Some varieties have their own "exclusive combinations". For instance, HO-1 peanuts are suitable for ridging and mulching, while Qinghai No. 13 peas have the best topdressing effect during the flowering period. Ultimately, whether drought resistance potential can be fully exploited depends not only on the variety but also on keeping up with management measures.

#### 7.3 Development of drought-tolerant breeding strategies and methodologies

This research has provided some ideas for drought-resistant breeding to some extent. Yield is of course important, but focusing solely on it is not enough. Factors such as whether the root system is deep enough, whether it can retain water, and the efficiency of water use all need to be taken into consideration. In fact, initial screening can be conducted during the seedling stage or even in greenhouse conditions, such as observing the root morphology, proline accumulation or whether the leaves remain green, and then determining the target based on different ecological zones. Molecular technology is now becoming increasingly practical. Whole-genome scanning can identify QTLS and markers, accelerating the process. Gene editing can also directly replicate known drought-resistant variations. However, traditional methods should not be abandoned either. It is more reliable to use them in combination. High-throughput methods can also be put to use. Remote sensing and thermal imaging can both quickly assess the drought resistance level of large groups. Models like CROPGRO can also simulate the performance of varieties in specific environments, which is very helpful for arranging experiments. The "old tricks" such as deep root systems and early maturity to avoid drought are still effective, but now we still have to deal with the superimposed situation of high temperatures and drought, and the requirements for compound stress resistance are even higher (Figure 2) (Wu et al., 2024). If there is truly policy support and molecular design becomes increasingly mature, the breeding cycle is expected to be shortened, and drought-resistant and high-yield varieties will have a greater chance of being truly implemented in agriculture in arid areas.

#### **8 Limitations and Future Research Directions**

This time, we evaluated a batch of drought-tolerant legume varieties under rain-fed conditions. Although some discoveries were made, there were also many limitations. For instance, the experiment only covered three pilot areas in the north. Areas like the karst regions in Southwest China or the winter and spring arid regions in the



south were not included. Therefore, caution should be exercised when extending the conclusion. The data is only for two years and cannot be considered long-term. No extreme drought years have been encountered, and the performance of the varieties may still change in the event of a severe drought. Most points also did not have a control with adequate irrigation, so the absolute yield potential and drought resistance penalty are actually not very easy to quantify. There are also errors in the field itself. Soil differences, microclimates, and measurement operations may all affect the results. Although repetition and model cross-validation were used, methods like AMMI and GGE also carry a certain subjective element. Physiological indicators such as leaf and root system measurements are easily affected by sampling time and location. The sample size of root systems is not large, and it is more about observing the trend. Breeding suggestions are still at the empirical level at present. Specific genes and hybrid combinations still need to be genetically verified. The 20 varieties tested cannot cover all the materials. Among those not tested, there might be even more drought-resistant ones. In conclusion, the research has provided some basis for drought resistance evaluation, but longer-term, multi-environmental and deeper genetic verification is still needed.

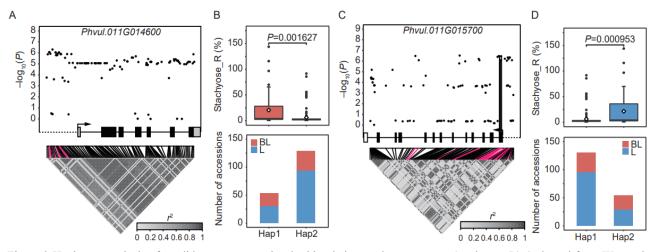


Figure 2 Haplotype analysis of candidate genes associated with relative stachyose content (stachyose\_R) (Adopted from Wu et al., 2024)

Image caption: A, local Manhattan plot (upper) around *Phvul.011G014600*, the gene structure (middle) of *Phvul.011G014600* and the linkage disequilibrium (LD) heatmap (below). B, phenotypic analysis (upper) and the number (below) of different haplotypes based on *Phvul.011G014600*. C, local Manhattan plot (upper) around Phvul.011G015700, the gene structure (middle) of *Phvul.011G015700* and the LD heatmap (below). D, phenotypic analysis (upper) and the number (below) of different haplotypes based on *Phvul.011G015700*. For each Manhattan plot, the vertical line indicates the position of the SNP resulting in a missense mutation. For each gene structure diagram, the white boxes, the black boxes, the black thick lines and the arrows indicate the UTRs, exonic regions, intronic regions and gene orientations, respectively. For each LD heatmap, red lines indicate the positions of significant SNPs. The r2 values are indicated using the color bars. For each boxplot, the middle line indicates the median, the rhombus indicates the mean, the box indicates the range of the 25th and 75th percentiles of the total data, the vertical lines indicate the interquartile range, and the outer dots represent outliers. BL, breeding line; L, landrace. Two-tailed Student's *t*-test (Adopted from Wu et al., 2024)

In fact, we have done a lot of work to enhance the reliability and repeatability of the data. The field trials employed random blocks and multiple repeat designs. The coefficient of variation of the yield data was on average controlled within 10%, and the accuracy was still acceptable. Physiological indicators such as RWC and enzyme activity are all obtained by taking the average of multiple samples and repeated measurements. The results over two years are basically consistent, and there is still some credibility. The data analysis employs relatively mature statistical models, and the appendix also includes complete trait data for easy traceability and verification. We also made some comparisons with existing literature and found that some stabley-yielding varieties and key traits were in line with those of others' research, which also increased our confidence a little. We have eliminated data with obvious anomalies, such as abnormal yields caused by local pests. Of course, there are always some uncontrollable factors in field experiments, so it should be fine to repeat the conclusion under the

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same conditions. However, if it is to be extended to other environments, further verification is still needed. It is suggested that more experiments be conducted in more places and in different years in the future, especially in breeding practice to test whether indicators such as root-crown ratio and WUE are effective. Overall, through reasonable design, standardized operation and statistical cross-validation, the quality of this batch of data is quite good and it also has certain reference value.

There are still many directions that can be expanded for future research on drought tolerance of legumes. For instance, in terms of genetic resources, more drought-tolerant genes from wild soybeans and peas can be utilized and attempted to be applied to cultivated varieties. In terms of molecular mechanisms, key genes and common pathways can be identified by using methods such as transcriptomics and metabolomics. Symbiotic microorganisms such as rhizobia and mycorrhizal fungi are also worthy of attention. Screening or inoculating superior strains may enhance drought resistance. Intelligent breeding is currently quite popular. It uses AI and big data to predict the relationship between genotypes and the environment, and gene editing may also introduce drought-resistant new varieties. In agronomy, models can be combined to study the performance of different varieties under different sowing periods and densities, and to form supporting plans. Under climate change, drought is often accompanied by high temperatures. It is necessary to design composite stress experiments to screen materials that are both drought-resistant and heat-resistant. In conclusion, multi-disciplinary collaboration is needed, from mechanisms to breeding and then to field management, to strive to cultivate smarter legume varieties and provide support for agriculture in arid areas.

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#### **Conflict of Interest Disclosure**

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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