Drought resistance evaluation in hulless barley seedlings: physiological markers for climate-resilient crop breeding

Yafei Shi

yafeishi@whu.edu.cn

Wuhan University https://orcid.org/0000-0003-0247-2098

Liping Niu
La Bo
Shuaihao Chen
Zhongmengyi Qin
Dawa Dondup
Lhundrup Namgyal
Xiruo Quzong
Zhuo Ga
Yanming Zhang
Xin Hou

Wuhan University https://orcid.org/0000-0002-9126-636X

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Liping Niu[1,2,3], La Bo[1,2,3], Shuaihao Chen[1,2,3], Zhongmengyi Qin [1,2,3], Dawa Dondup[2], Lhundrup Namgyal[2], Xiruo Quzong[2], Zhuo Ga[2], Yanming Zhang[1,3], Yafei Shi[3,4,5*], Xin Hou[1,2,3*]

[1] School of Ecology and Environment, Tibet University, Lhasa 850000, China

[2] State Key Laboratory of Hulless Barley and Yak Germplasm Resources and Genetic Improvement, Lhasa 850002, China;

[3] College of Life Sciences, Wuhan University, Wuhan 430072, China

[4] College of Life Sciences, Xinyang Normal University, Xinyang 464000, China.

[5] Wuhan University Shenzhen Research Institute, Shenzhen 518057, China.

*Corresponding author:

Xin Hou, Email: xinhou@whu.edu.cn; Yafei Shi, Email: yafeishi@whu.edu.cn.

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Abstract

Global climate change intensifies extreme weather-induced crop losses, necessitating drought-resilient crops. Qingke (*Hordeum vulgare var. nudum*), the staple barley of Tibet's climate-vulnerable plateau, offers genetic insights into stress adaptation. We established a seedling-stage drought evaluation system identifying four biomarkers: fresh weight, chlorophyll fluorescence (F_v/F_m, NPQ, R_{FD}), photosynthetic parameters (E and gsw), and reactive oxygen species (ROS) accumulation. Systematic screening of the physiological traits revealed these parameters as optimal predictors of drought tolerance, enabling rapid germplasm classification. Application to three uncharacterized cultivars (ZY673, ZY1403, KL14) demonstrated weak drought resistance across all lines, with ZY1403 showing extreme sensitivity. This standardized protocol for hulless barley integrates photosynthetic efficiency and oxidative stress metrics, providing breeders with actionable thresholds for climate-resilient crop development in montane agroecosystems.

Keywords: Drought tolerance; Photosynthetic efficiency; Oxidative stress; Hulless barley; Seedling-stage evaluation

Introduction

Rapid climate change and the growing scarcity of water present substantial challenges to global food security, challenges that are further intensified by the relentless need to feed a continuously growing global population[1]. Estimates indicate that, at current levels of global agricultural production, an increase of 60-110% may be required to meet the escalating demand for food security[2]. However, high-frequency and severe droughts have emerged as critical constraints on agricultural production, posing a grave threat to global food security. Drought is a significant abiotic stress factor that negatively affects plant growth, development, and ultimately crop yields[3]. In response to drought stress, plants have developed a variety of adaptive strategies, including changes in morphology, physiology, biochemistry, and gene expression patterns[4]. Plant drought resistance is a complex trait that arises from multiple mechanisms: escape (where the plant advances its reproductive phase to occur before the onset of stress), avoidance (where the plant enhances cellular water retention and prevents tissue damage), and tolerance (where the plant maintains normal growth despite having low cellular water content during drought conditions)[5]. Therefore, a deep understanding of the molecular and physiological mechanisms that govern plant responses to drought stress can offer theoretical insights and technical frameworks for improving crop drought resistance and developing drought-tolerant crop varieties. This holds immense importance for promoting efficient use of agricultural water resources, responding to the ongoing increase in food demand, and securing sustainable agricultural development[6]. Hulless barley is one of the most important plateau cereal crops on the Qinghai-Tibet Plateau, which is the highest altitude plateau in the world as well as the largest in China[7,8]. However, the stress effect of chromium on hulless barley plants has seldom been studied.

Under simulated drought stress conditions using polyethylene glycol (PEG), both Tibetan semi-wild and cultivated barley showed reduced root length and size compared to the control group, along with a corresponding decrease in root length and density[9]. By utilizing root image scanning, it was observed that under drought stress, as soil moisture levels decreased, the root development of soybeans and adzuki beans slowed

and ultimately deteriorated[10]. Several studies have documented that drought conditions lead to a decrease in chlorophyll content, a reduction in net photosynthetic rate, a lower maximum quantum yield (F_v/F_m), decreased stomatal conductance, and suppressed transpiration, ultimately negatively affecting plant metabolism and yield. Research has validated that chlorophyll content, chlorophyll fluorescence parameters, and photosynthetic parameters are strongly correlated with drought stress. Additionally, chlorophyll fluorescence parameters are commonly used in the screening for drought resistance[4,11-15]. Furthermore, drought stress increases the accumulation of reactive oxygen species (ROS) within plants. To counter this stress, plants must scavenge the excess ROS and maintain a stable ROS level. Plant cells employ a sophisticated antioxidant system to eliminate the excessively accumulated ROS, thereby mitigating detrimental effects. This system comprises enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and other enzymatic components[16].

Recently, researchers have initiated studies into exploring drought tolerance and its identification markers throughout the various growth stages of hulless barley [7,11-20]. However, research on the seedling stage is still limited, especially with regard to morphological and physiological-biochemical traits, the expression of drought-responsive genes, and the development of comprehensive identification criteria under drought stress. To address this gap, the current study aims to establish a rapid, precise, and high-throughput system for identifying drought tolerance in highland barley, facilitating a thorough and systematic assessment of drought tolerance within highland barley germplasm. The results are expected to provide valuable genetic resources for the breeding of drought-tolerant hulless barley and to offer theoretical guidance for its cultivation techniques and water management approaches.

Results

PEG treatment simulates drought stress in hulless barley varieties

Cultivating drought-resistant varieties of hulless barley is the most effective and economical strategy to alleviate the adverse impacts of drought stress on hulless barley

production[19]. However, the genetic diversity of hulless barley has significantly decreased over the course of long-term domestication, particularly in the context of modern breeding and cultivation practices. Additionally, the current assessment of drought resistance in hulless barley germplasm resources lacks sufficient depth. Therefore, it is imperative to undertake drought resistance identification and screening of the extensive hulless barley germplasm resources. Polyethylene glycol (PEG), as osmotic regulators, are capable of effectively simulating different levels of drought stress[21]. In order to establish an efficient drought resistance evaluation system for hulless barley, we selected four varieties from hulless barley germplasm resources (Supplementary Table S1): two drought-sensitive varieties (YC85 and ZY88) and two drought-tolerant varieties (ZY1252 and ZY1100). After exposing different hulless barley genotypes to simulated drought conditions using 16% PEG for 2 days, we observed phenotypic differences between drought-tolerant genotypes and drought-sensitive genotypes (Figure 1A). We found that the shoot lengths of the four hulless barley varieties were almost the same (Figure 1B). The drought-sensitive varieties (YC85 and ZY88) showed reduced fresh and dry weights with 16% PEG treatment compared to without PEG treatment. In contrast, for the drought-tolerant varieties (ZY1252 and ZY1100), there were no significant differences in fresh and dry weights between the PEG-treated and untreated cases (Figure 1C-D). But the chlorophyll contents were remained unchanged of four hulless barley varieties (Figure 1E-G). So, it is clear that PEG had a negative effect on both drought-sensitive hulless barley varieties and their fresh weight, which can serve as a drought resistance index due to its convenience for statistical analysis.

PEG-simulated drought treatment had a significant effect on photosynthetic parameters

Photosynthesis in plants is highly sensitive to drought stress, and its impact is primarily reflected through parameters such as net photosynthetic rate, transpiration rate, and stomatal conductance[22]. We observed that in drought-sensitive varieties (YC85, YC88), the photosynthetic parameters decreased significantly under PEG treatment. However,

in drought-resistant varieties (ZY1252, ZY1100), most of these parameters did not undergo significant changes under PEG treatment (Figure 2). Therefore, we selected 'E' and 'gsw' as drought resistance indices due to their large range of variation and stability.

Previous studies have shown that photosynthetic parameters in drought-sensitive varieties are significantly affected by drought stress, with PSII parameters being the most strongly impacted[23]. Consequently, we measured chlorophyll fluorescence parameters in various hulless barley varieties to further evaluate the differences among them. The results indicated that, in drought-tolerant varieties (ZY1100 and ZY1252), none of the chlorophyll fluorescence parameters underwent significant changes under PEG treatment (Fig. 3). Conversely, in drought-sensitive varieties (YC85 and YC88), several parameters including maximal quantum yield of PSII (F_v/F_m), non-photochemical quenching of fluorescence (NPQ), and fluorescence decrease ratio (R_{FD}) were decreased significantly under PEG treatment. More specifically, F_v/F_m decreased by 21% in YC85 and by 22% in YC88; NPQ decreased by 62% in YC85 and by 60% in YC88; R_{FD} decreased by 60% in YC85 and by 50% in YC88. These findings suggest that F_v/F_m, NPQ, and R_{FD} are reliable indicators of simulated drought stress in hulless barley treated with PEG (Figure 3). The above results suggest that the photosynthetic parameters, NPQ and R_{FD}, can serve as indicators for drought-resistant screening due to their large range of variation and stability.

PEG-simulated drought treatment on reactive oxygen species (ROS) levels

Previous studies have shown that ROS accumulation is a defensive response to abiotic stress [24]. We measured these parameters in drought-sensitive varieties (YC85 and YC88) and drought-tolerant varieties (ZY1100 and ZY1252) of hulless barley varieties after treating them with PEG for 2 days. The level of H_2O_2 in drought-sensitive varieties (YC85 and YC88) increased dramatically under PEG treatment, whereas it remained unchanged in drought-tolerant varieties (ZY1100 and ZY1252). Interestingly, the content of O_2^- did not exhibit significant changes under PEG treatment in any of the four varieties. These results indicate that H_2O_2 content is a more reliable indicator of

simulated drought stress than O_2^- content in hulless barley (Figure 4). Similarly, the staining intensity of DAB can serve as indicators for drought-resistant screening due to the huge differences between drought-sensitive and drought-tolerant varieties.

Verification of drought resistance evaluation system

To verify the system for evaluating and identifying drought resistance in hulless barley, we measured fresh weight, 'E', 'gsw', NPQ, R_{FD} and DAB staining levels in hulless barley seedlings. We selected ZY97, a relatively drought-tolerant variety, YC83, a relatively drought-sensitive variety, and the unknown sensitive varieties ZY673, ZY1403, and KL14 treated with or without PEG. Compared with the drought-sensitive and drought-tolerant control, we found that all of them significantly declined under drought stress, with varying degrees of decrease among different varieties. Specifically, the fresh weight was 39%, 30%, and 34% declines of the control varieties (CK), respectively (Figure 5A); the transpiration rates were 89%, 99%, and 50% declines of the CK varieties, respectively (Figure 5B) and the stomatal conductance to water vapor was 95%, 97%, and 53% of the CK (Figure 5C). Additionally, the decrease rates of NPQ were 54%, 75%, and 35% (Figure 5D); and the decrease rates of R_{FD} were 52%, 68%, and 36% (Figure 5E). Among them, ZY1403 was the most affected by drought, followed by ZY673 and KL14. Reactive oxygen species (ROS) detection results (Figure 5F) revealed that after drought treatment, the content of H_2O_2 stained with DAB increased in all three varieties, with ZY1403 showing a significant increase compared to the control group. Based on the above results, the drought resistance evaluation system we established is reliable. Therefore, based on the drought resistance evaluation system, all three varieties can be classified as having weaker drought resistance, with ZY1403 being the most sensitive.

Discussion

Drought stress is deleterious to the growth and yield of plants. The assessment of drought tolerance in plant species can be predicated on the morphological, physiological, biochemical, and molecular alterations elicited by drought stress. Historically, the evaluation of drought tolerance has frequently hinged on the

211 determination of yield loss in response to drought conditions. Nonetheless, this
212 method is not only protracted and labor-intensive but also economically burdensome,
213 and field experiments are prone to being influenced by environmental heterogeneity.
214 Consequently, an increasing body of research is identifying drought tolerance in plants
215 by utilizing agronomic, morphological, physiological, and metabolic traits that are
216 intrinsically linked to drought tolerance[4].
217
218 Leaf wilting represents the initial external manifestation of drought stress in
219 plants. Research has demonstrated a close association between leaf wilting and plant
220 drought resistance, suggesting its utility as an indicator for assessing drought
221 resistance in plants[25,26]. In the present study, we observed phenotypic differences
222 between drought-tolerant and drought-sensitive varieties following a 2-day drought
223 treatment. Results depicted in Fig 1 indicate that the growth of the aboveground
224 portion of sensitive varieties was markedly suppressed, with leaves exhibiting
225 pronounced wilting and chlorosis. In contrast, drought-tolerant varieties displayed
226 milder symptoms. In many plant species, parameters such as plant height,
227 aboveground fresh and dry weight, and relative water content are commonly used as
228 indices of drought stress[15]. Previous studies have reported that drought stress reduces
229 fresh weight, pigment content, and relative water content in crops such as wheat[27,28],
230 barley[29,30,31], rice[32,33], and maize[34,35]. In our investigation, we compared the shoot
231 length, fresh weight, and dry weight between drought-tolerant and sensitive varieties
232 under PEG-simulated drought stress. In sensitive varieties, both plant height, fresh
233 weight and dry weight significantly declined in comparison to the control. These
234 findings suggest that fresh weight is a more appropriate indicator for assessing drought
235 resistance at the seedling stage.
236
237 Photosynthesis is highly sensitive to water stress, manifesting primarily in
238 reductions of the photosynthetic rate, transpiration rate, stomatal conductance, and
239 water use efficiency. Previous studies have established a strong correlation between
240 chlorophyll fluorescence parameters, photosynthetic parameters, and drought stress,

leading to the widespread application of chlorophyll fluorescence parameters in drought resistance screening[11,12]. In this investigation, we quantified the net photosynthetic rate (A), transpiration rate (E), stomatal conductance to water vapor (gsw), total conductance to water vapor (gtw), intercellular CO_2 concentration (Ci), and stomatal conductance to CO_2 (gst) for four cultivars under drought treatment. The data presented in Fig 2 indicate that all photosynthetic parameters declined, with the decrease being more pronounced in drought-sensitive compared to drought-tolerant cultivars. Relative to the control, photosynthetic parameters in sensitive cultivars were reduced by at least 50%. The minimum E observed in drought-tolerant cultivars was approximately 10% higher than the maximum value recorded for sensitive cultivars; the minimum gsw in drought-tolerant cultivars was about 26% higher than the maximum value in sensitive cultivars; and the minimum gtw in drought-tolerant cultivars was approximately 30% higher than the maximum value in sensitive cultivars. Consequently, this study suggests that the E and gsw can serve as reliable indicators for drought tolerance screening.

Chlorophyll fluorescence parameters serve as critical indicators of plant photosynthetic physiology, capable of delineating spatial variations in plant responses to drought stress. The analysis of chlorophyll fluorescence parameter data can be utilized to predict plant mortality under drought conditions[36]. Furthermore, these parameters can be integrated with other analytical techniques to facilitate high-throughput drought research[37]. In the current study, chlorophyll fluorescence parameters including F_v/F_m, NPQ, and R_{FD} were measured using a FluorCam system. The results presented in Fig 3 indicate that F_v/F_m, NPQ, and R_{FD} significantly decreased in drought-sensitive cultivars under water deficit conditions. Conversely, in drought-tolerant cultivars, these chlorophyll fluorescence parameters exhibited only a modest decline in response to drought stress. This finding aligns with the observations of the chlorophyll fluorescence parameters in hulless barley are adversely affected by drought stress, with drought-tolerant cultivars exhibiting a significantly smaller reduction compared to drought-sensitive cultivars[38]. Parameters such as NPQ and R_{FD}

are particularly adept at discerning the differences between drought-tolerant and drought-sensitive cultivars in terms of drought sensitivity, the rate of decline, and the speed of response.

Generally, drought stress diminishes chlorophyll content due to the drought-induced elevation in ROS accumulation, which triggers lipid peroxidation and ultimately leads to chlorophyll degradation[39,40]. Contrarily, in the present investigation, the levels of chlorophyll a and b in the genotypes remained unaltered (Fig 1E-G). This discrepancy may be attributed to the substantial reduction in leaf area and the concomitant increase in leaf thickness of hulless barley under drought stress[41], which could potentially lead to an elevated chlorophyll content. However, previous studies have documented that the chlorophyll content in plants either exhibits a slight increase or remains stable under drought stress across various plant species[42,43]. Additionally, previous reported shown that the chlorophyll content in peanuts initially increases under mild to moderate drought stress, yet significantly declines under severe water deficit conditions[44]. Consequently, the extant literature suggests that there is no consistent correlation between chlorophyll content and drought stress. In summary, we found that the fresh weight, 'E', 'gsw', NPQ, RFD, and DAB staining levels of hulless barley seedlings, which serve as indicators in our drought resistance evaluation system, are reliable in this study.

Methods

Plant material

Drawing upon local cultivation experience in the Qinghai-Tibet Plateau, we selected drought-tolerant varieties such as ZY1100, ZY1252, and ZY97, as well as drought-sensitive varieties including YC85, YC88, and YC83. Furthermore, due to their heightened susceptibility to environmental influences, YC85 and YC88 demonstrate a higher requirement for water and nutrient inputs. Additionally, we included varieties with unknown drought resistance grades, namely ZY673, ZY1403, and KL14, based on established ideal indexes for drought resistance assessment at the seedling stage.

Plant Growth and Drought Treatments

Hulless barley seeds were imbibed for 2 days and subsequently germinated on moist filter paper placed within 100-mm petri dishes for an 8-day period. Following germination, the seedlings were transferred to a hydroponic system containing Yoshida nutrient solution for a 4-day growth period. Drought stress was induced by exposing the seedlings to a 16% (w/v) PEG solution (mixed in Yoshida nutrient solution) for 13 days. The growth conditions consisted of a temperature of 22°C, a light intensity of 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a photoperiod of 16 hours of light followed by 8 hours of darkness.

Morphological and biomass measurement

Phenotypic diversity was observed among various genotypes following a 2-day PEG treatment. After 7 days post-treatment, the following traits were quantified for each genotype: seedling height, aboveground fresh biomass, and dry biomass (after desiccation at 65°C for 48 hours). A representative subset of 6 seedlings per genotype was sampled. The experimental protocol was conducted in triplicate to ensure reproducibility.

Assessment of photosynthetic parameters

Photosynthetic parameters were assessed using a Li 6800 (LI-COR Inc., Lincoln, NE, USA) photosynthesis system. Following a 2-day exposure to a 16% PEG-induced drought stress, the net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gsw), total leaf conductance to water vapor (gtw), and additional photosynthetic parameters were measured in the fully expanded first leaves of various plant genotypes, which were randomly sampled for analysis.

Measurement of Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters were quantified utilizing a FluorCam 800-C (Photon System Instruments, Brno, Czech Republic) chlorophyll fluorescence imaging system. Following a 16% PEG-induced drought treatment for 2 days, chlorophyll fluorescence parameters were assessed in four fully expanded first leaves from various

plant genotypes, which were randomly selected and measured in the dark for a duration of 15 minutes. Each individual seedling representing a specific genotype treatment was considered a biological replicate, with the experimental protocol being replicated four times across three independent experimental runs.

Measurement of Chlorophyll Content

The leaves exposed to PEG and the control leaves were harvested and weighed, then soaked in 80% acetone containing 1 mg/ml of acetone and incubated in an oven for 16 hours at 37°C. The absorption values at 663 nm and 645 nm were measured using a NanoDrop 2000C (Thermo Scientific, USA) spectrophotometer. The concentrations of chlorophyll a (Chla) and chlorophyll b (Chlb) were calculated using the following formulas: Chlorophyll a (mg/g FW) = (12.7 * A663) - (2.69 * A645) Chlorophyll b (mg/g FW) = (22.9 * A645) - (4.68 * A663) Total chlorophyll content (mg/g FW) = chlorophyll a + chlorophyll b.

Detection of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) were detected using diaminobenzidine (DAB) and tetrazolium blue (NBT) staining methods. Solutions of 1 mg/mL DAB and 1 mg/mL NBT were prepared for the staining process. The first fully expanded leaf from hulless barley seedlings that had been treated with 16% PEG for 3 days was immersed in the DAB and NBT staining solutions. Subsequently, the leaf was placed in a vacuum oven for 20-30 minutes, kept in the dark at room temperature for 2 days, and then bleached with 80% ethanol at 85°C in a water bath prior to observation and photography. The staining intensity of the leaves was quantified using ImageJ (https://imagej.net/ij/).

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Authors contributions

Liping Niu: Data curation, Formal analysis. **Yafei Shi:** Formal analysis, Conceptualization, Validation, Writing – original draft. **La Bo:** Data curation, Formal analysis. **Shuaihao Chen:** Data curation. **Zhongmengyi Qin:** Data curation. **Dawa Dondup:** Provide hulless barley. **Lhundrup Namgyal:** Provide hulless barley. **Xiruo Quzong:** Data collection, **Zhuo Ga:** Data collection. **Yanming Zhang:** Data collection. **Xin Hou:** Data curation, Formal analysis, Project administration, Resources, Supervision, Writing – review & editing.

Competing interests

The authors declare that they have no conflict of interests.

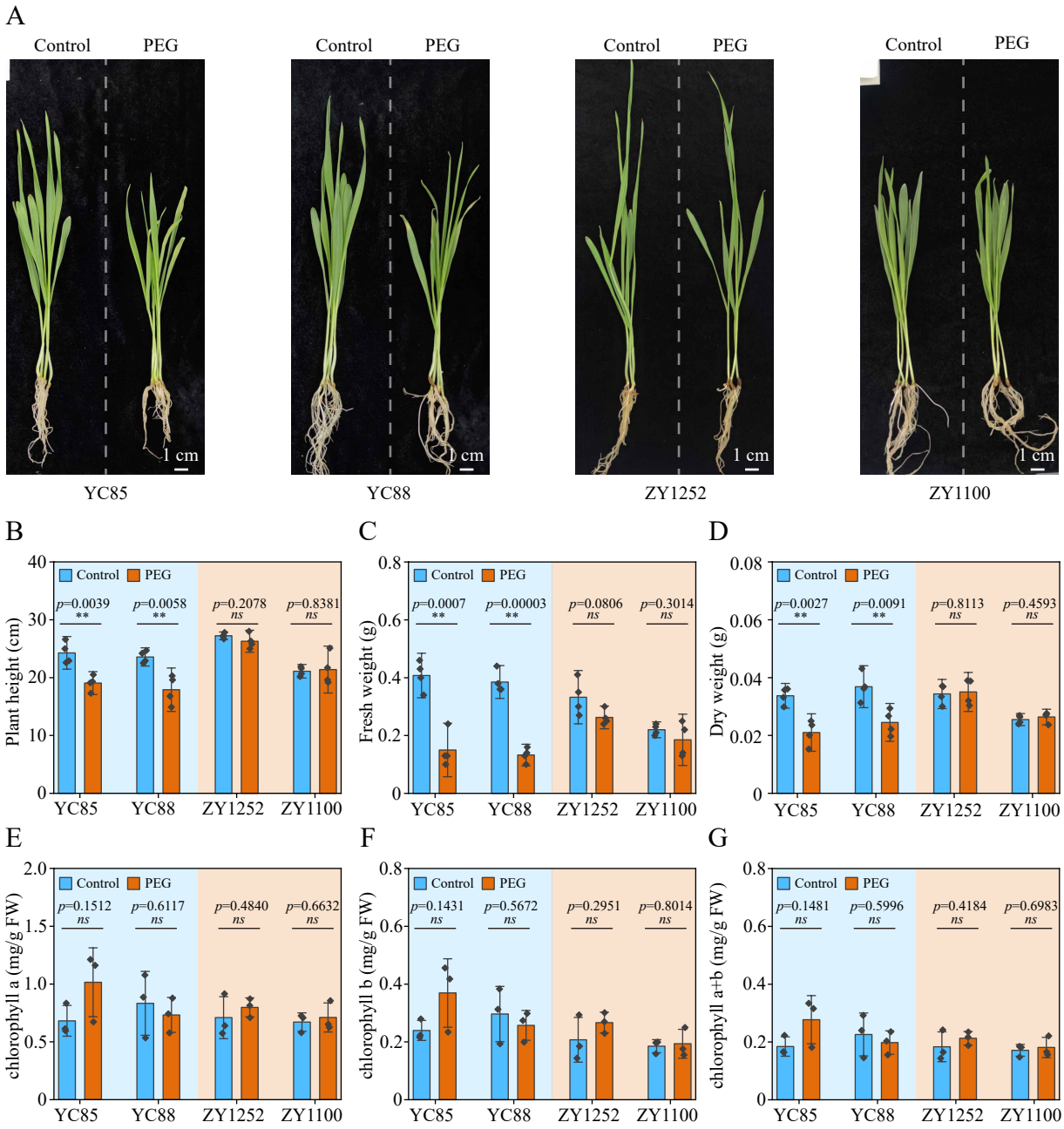

Fig. 1. Effects of drought stress simulated by polyethylene glycol (PEG) on different varieties of hulless barley plants. The drought-sensitive (YC85 and YC88) and drought-tolerant hulless barley (ZY1252 and ZY1100) were selected for the experiment. **A,** Phenotypes of highland barley plants treated with or without PEG. 13-day-old plants grown under Yoshida (Control) were treated with Yoshida or 16% PEG for another 7 days. Bar=1 cm. **B to D,** Effect of PEG on shoot length (B), shoot fresh weight (C) and shoot dry weight (D) of hulless barley plants. 13-day-old plants grown under Yoshida (Control) were treated with Yoshida or 16% PEG (Drought) for another 7 days. **E to G,** Effect of PEG on chlorophyll a (E), chlorophyll b (F), and chlorophyll a+b (G) of hulless barley plants. 13-day-old plants grown under Yoshida (Control) were treated with Yoshida or 16% PEG for another 3 days. The values indicate the means ± SDs (n=4). **P < 0.01 and *P < 0.05, Student's *t* test.

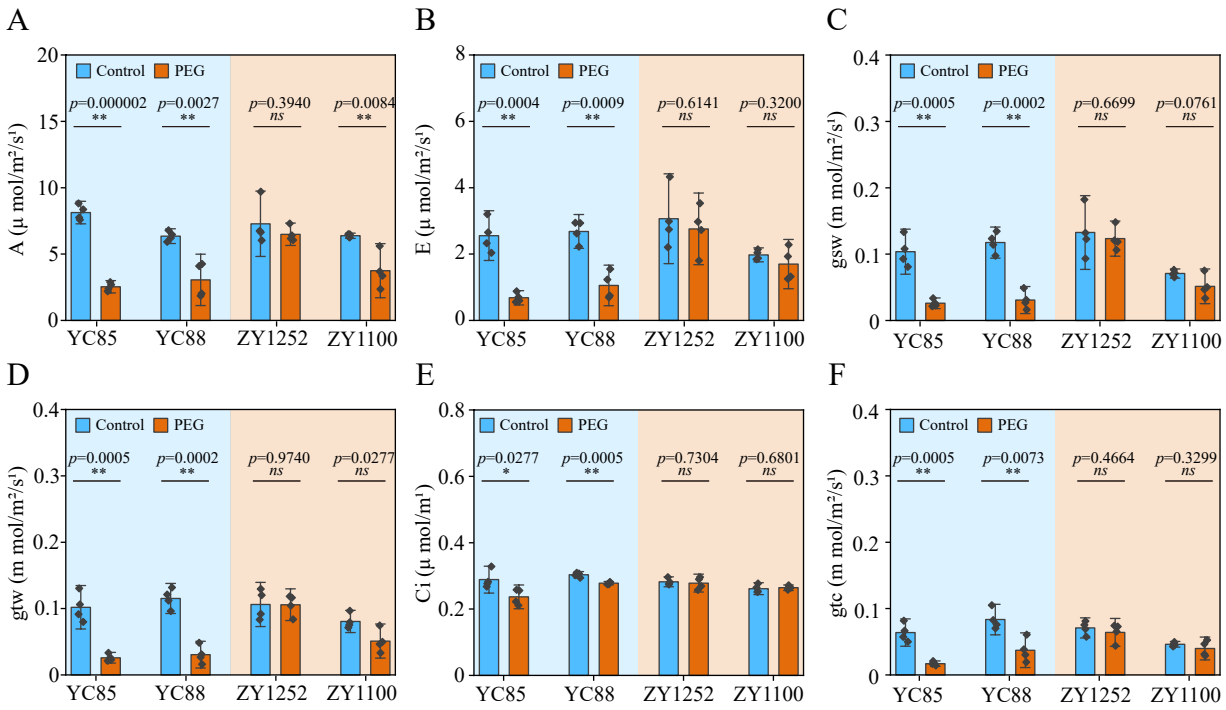

Fig. 2. Photosynthetic physiological parameters of hulless barley plants under control and PEG treatment. The photosynthetic gas exchange of YC85, YC88, ZY1252 and ZY1100 was measured with LI-6800 (LI-COR, USA) under control and PEG treatment. 13-day-old plants grown under Yoshida (Control) were treated with Yoshida or 16% PEG for another 2 days. The drought-sensitive (YC85 and YC88) and drought-tolerant hulless barley (ZY1252 and ZY1100) were selected for the experiment. **A,** Net photosynthetic rate (A); **B,** Transpiration rate (E). **C,** Stomatal conductance of water vapor (gsw); **D,** Total conductivity to water vapor (gtw); **E,** Intercellular CO2 concentration (Ci) and **F,** Stomatal conductance of CO_2 (gtc). The values indicate the means ± SDs (n=4). **P < 0.01 and *P < 0.05, Student's *t* test.

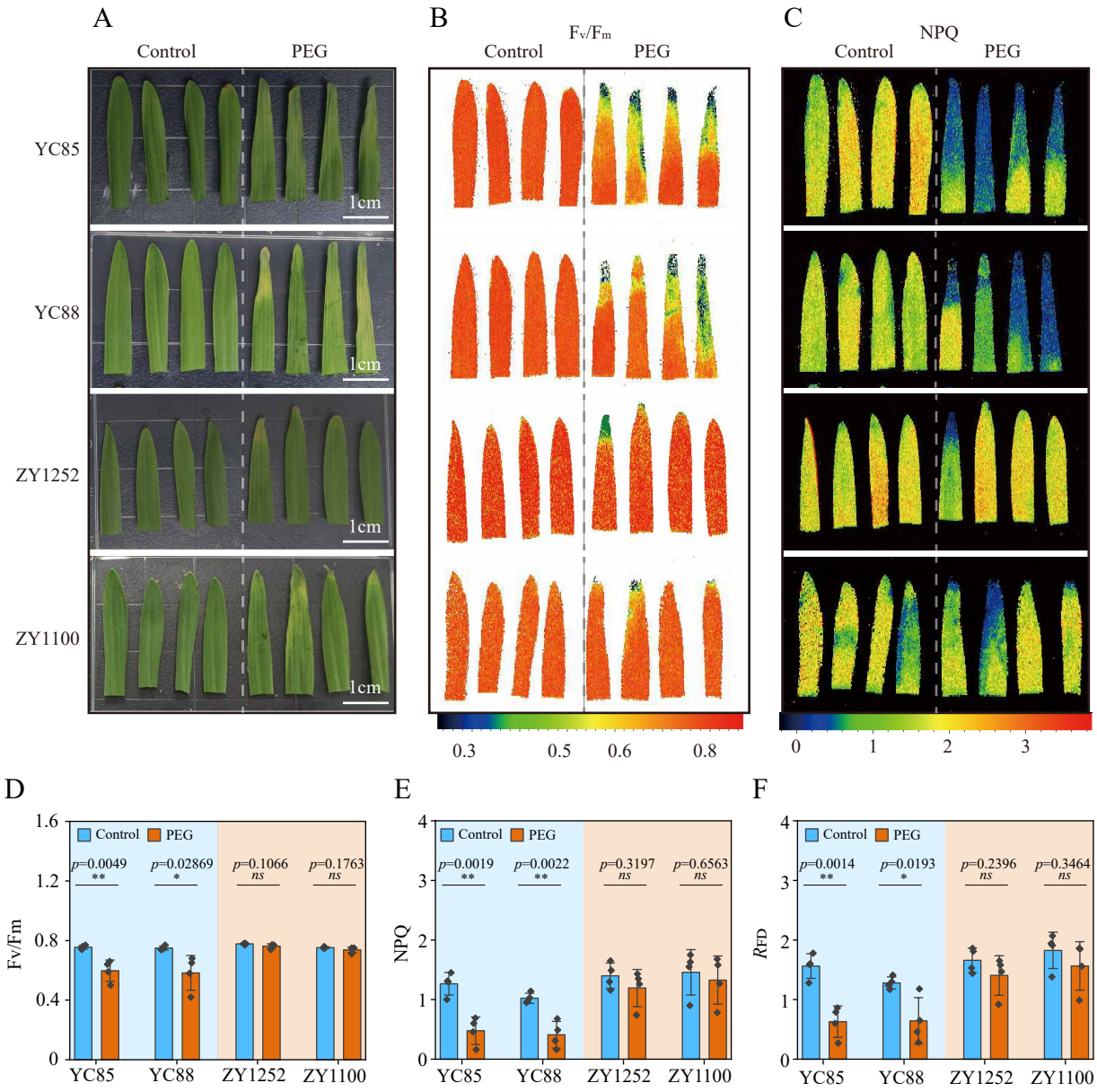

Fig. 3. Chlorophyll fluorescence parameters of hulless barley plants under control and PEG treatment. The photosynthetic gas exchange of plants was measured with FluorCam (PSI, Czech) under control and PEG treatment. 13-day-old plants grown under Yoshida (Control) were treated with Yoshida or 16% PEG for another 2 days. The drought-sensitive (YC85 and YC88) and drought-tolerant hulless barley (ZY1252 and ZY1100) were selected for the experiment. The experimental materials were obtained from two different media, and the first fully unfolded leave taken from each medium after 15 min dark adaptation for the measurement of chlorophyll fluorescence parameters. **A,** Phenotypes of hulless barley plants treated with or without PEG. Bar=1cm; **B,** Imaging of maximum quantum yield of PSII (Fv/Fm); **C,** Imaging of non-photochemical quenching (NPQ); **D,** maximum quantum yield of PSII (Fv/Fm); **E,** non-photochemical quenching (NPQ) and **F,** non-photochemical quenching (R_{FD}). The values indicate the means ± SDs (n=4). **P < 0.01 and *P < 0.05, Student's *t* test.

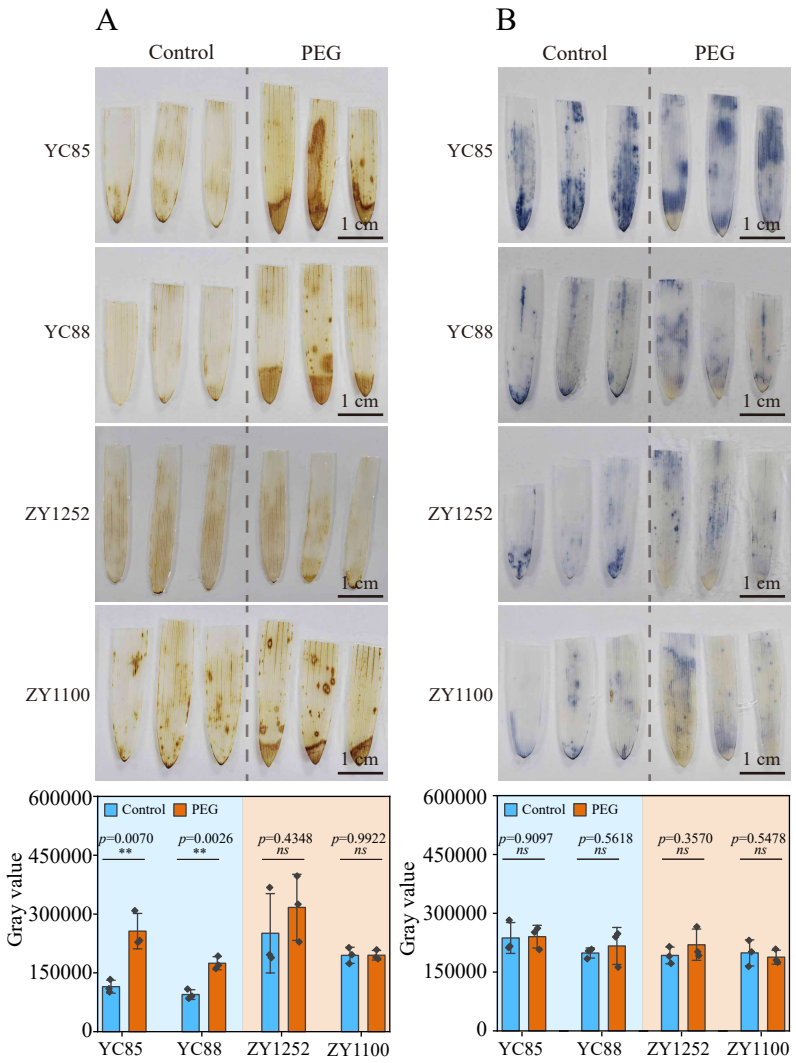

Fig. 4. ROS accumulation in hulless barley plants under control and PEG treatment. 13-day-old plants grown under Yoshida (Control) were treated with Yoshida or 16% PEG for another 3 days. The drought-sensitive (YC85 and YC88) and drought-tolerant hulless barley (ZY1252 and ZY1100) were selected for the experiment. **A,** DAB staining was used to determine the hydrogen peroxide content under control and PEG treatment, upper. The relative amount of DAB was estimated using ImageJ, with measurements taken in the above plants, lower. **B,** NBT staining was used to assess the superoxide anion under control and PEG treatment, upper. The relative amount of NBT was estimated using ImageJ, with measurements taken in the above plants, lower. The values indicate the means ± SDs (n=3). **P < 0.01 and *P < 0.05, Student's *t* test.

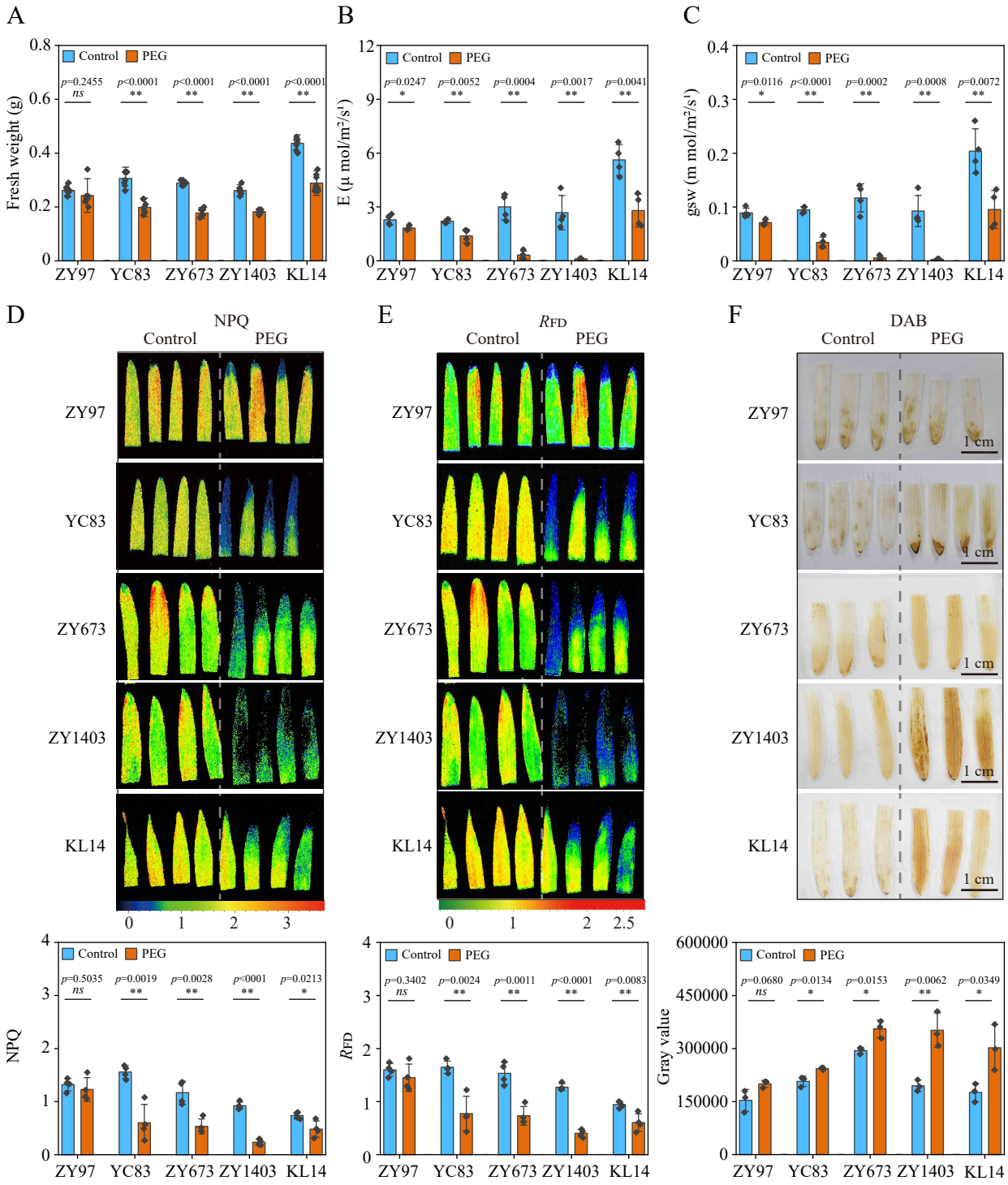

Fig.5 Verification of drought resistance evaluation. 13-days-old seedlings of ZY97, YC83, ZY673, ZY1403 and KL14 were treated with 16% PEG is drought group, and treated with Yoshida is control group. The experimental materials were obtained from two different media, and the first leave was taken from each medium for the measurement of different parameters. **A to C,** Effect of PEG simulated drought treatment on Fresh weight (A), transpiration rate, E (B), and stomatal conductance of water vapor, gsw (C). The values indicate the means ± SDs (n=8). **P < 0.01 and *P < 0.05, Student's *t* test. **D,** The photosynthetic gas exchange of plants was measured with FluorCam (PSI, Czech) under control and PEG treatment. Imaging of non-photochemical quenching (NPQ), upper. The level of NPQ, lower. E, Imaging of fluorescence decrease ratio (*R*FD), upper. The level of *R*FD, lower. The values indicate the means ± SDs (n=4). **P < 0.01 and *P < 0.05, Student's *t* test. **F,** DAB staining was used to determine the hydrogen peroxide content under control and PEG treatment, upper. The relative amount of DAB was estimated using ImageJ, with measurements taken in the above plants, lower. The values indicate the means ± SDs (n=3). **P < 0.01 and *P < 0.05, Student's *t* test.

Supplementary table S1. Names and sources of hulless barley varieties

Number	Variety name	Variety origin	Drought-resistant ability
YC85	Zangqing 320	Lhasa Tibet	no
YC88	Zangqing 2000	Lhasa Tibet	no
ZY1252	Ziqingke	Lhasa Tibet	yes
ZY1100	Guoluo	Milin Nyingchi	yes
ZY97	Duobujiu	Rikaze Tibet	yes
YC83	Zangqing 148	Lhasa Tibet	no
ZY673	Naina	Linzhou Lhasa	unknown
ZY1403	Lanqingke	Jiangda Chamdo	unknown
KL14	Kunlun14	Qinghai	unknown