The *GmXTH1* Gene Improves Drought Stress Resistance of Soybean Seedlings

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Abstract

To study the role of *GmXTH1* gene in alleviating drought stress. T4 transgenic soybean seeds with *GmXTH1* gene were treated with PEG6000 at 0%, 5%, 10% and 15%, respectively. Germination potential, germination rate and germination index were measured. The results showed that the germination potential, germination rate and germination index of OEA1 and OEA2 strains overexpressed in T4 generation were significantly higher than that of control material M18. After 0d, 7d and 15d drought stress, the analysis of seedling phenotypes and root-shoot of different T4 generation transgenic soybean lines showed that under stress conditions, the growth of *GmXTH1* overexpression material was generally better than that of control material M18, and the growth of *GmXTH1* interference expression material was generally worse than that of control material M18, with significant differences in plant phenotypes.The root system of *GmXTH1* overexpressed material was significantly developed compared with that of control material M18. The analysis of physiological and biochemical indexes showed that the relative water content and the activity of antioxidant enzymes (superoxide dismutase and peroxidase) of *GmXTH1* transgenic soybean material were significantly higher than that of control material M18, and the accumulation of malondialdehyde was lower under the same stress conditions at seedling stage. Fluorescence quantitative PCR assay showed that the relative expression of *GmXTH1* gene in transgenic soybean was significantly increased after drought stress.The results showed that the overexpression of *GmXTH1* could increase the total root length, surface area, total projection area, root volume, average diameter, total cross number and total root tip number, thereby increasing the water intake and reducing the transpiration of water content in leaves, thus reducing the accumulation of MDA and producing more protective enzymes in a more effective and prompt way.Reducing cell membrane damage to improve drought resistance of soybean.

Introduction

In the face of severe climate change and the intensification of abiotic stresses such as salinization, drought, extreme temperature and waterlogging(Raza et al.,2019),plant organ morphology, physiology and biochemical levels also undergo a series of changes, including mechanical damage to protoplasts and cell walls, stomatal closure of leaves, decreased photosynthetic efficiency and cell dehydration.In addition, the biofilm system is damaged and the membrane permeability is changed, leading to metabolic disorders(Osmond et al., 1995).In response to drought stress, plants avoid drought through developmental plasticity and shortener life cycle, increase water intake and reduce water loss to avoid drought, and increase osmotic regulation, antioxidant capacity and dehydration tolerance to enhance stress tolerance(Zhang et al., 2007).Drought stress can lead to dehydration of plant cells and directly affect cell turgor pressure and then the extension of cell wall.Cells maintain cell turgor pressure by adjusting cell size and cell wall extensibility, thus contributing to plant water loss adaptation(Mohammadi et al., 2012], minimizing water loss and cell dehydration, which is crucial for drought tolerance and salt tolerance of plants and recovery of growth.

Studies have shown that xyloglucantransglycosidase/hydrolase, XTH by catalytic plant cell walls of xylan chain cutting and reconnect to modify and restructuring of cellulose, xylan skeleton, so as to change and adjust the ductility of cell wall,This in turn affects plant growth and development and stress response(Fry et al., 1992;Nishitani et al., 1992; Rose et al.,2002; Vissenberg., 2005; Miedes et al., 2011) .

According to its sequence characteristics, XTHs are divided into□,□and□(Rose et al., 2002;Campbell et al., 1999) ,III into IIIA and IIIB subclasses (Baumannet al., 2007). Among them, class IIIA showed XEH activity and specifically hydrolyzed the β-1,4 glycosidic bond of xyloglucan.Class IIIB, Class I, and Class II XTHs can internally cut xyloglucan molecules to produce a reducing terminal, which is linked to another xyloglucan chain and has significant XET activity (Baumann et al., 2007;Saladie et al., 2006;Kallas et al.,2005). The characteristic sequence of XTH enzyme is DEIDFEFLG, which contains amino acid residues that can mediate catalytic activity.The threonine or serine residues near this catalytic site are modified by N-glycosylation (Kallas et al.,2005;Vanet al.,2006),which is significantly associated with enzyme activity.N-glycation sites conserved have not been found in subclass IIIa(Baumannet al., 2007), but have been found in class I/II of XTHs proteins. The C-terminal of XTHs protein can form disulfide bonds that stabilize the protein structure because it usually contains highly conserved cysteine.Proteomic studies have shown that certain enzymes related to cell wall polysaccharide synthesis/hydrolysis, lignin biosynthesis and cell wall porosition are involved in the response to drought stress(Aranjuelo et al., 2011;Lee et al., 2007;Amor et al., 1995; Dong et al., 2011; Ashoub et al.,2013; Raorane et al., 2015; Hu et al., 2015) .

In addition, XTHs plays an extremely important role in various growth and differentiation processes, participating in the regulation of primary root elongation, hypocotyl growth, vascular differentiation, flowering, fruit ripening, petal shedding, and woody formation(Osato et al., 2006;Wu et al., 2005; Matsui et al., 2005; Harada et al., 2011;Saladie et al., 2006;Miedes et al., 2009; Singh et al.,2011; Nishikubo et al., 2011). *AtXTH* is very sensitive to metal aluminum ion stress, and Arabidopsis xth15, xth17, and xth31 mutants have improved $A1^{3+}$ stress tolerance compared with wild-type plants (Zhu et al.,2012;Zhu et al.,2013;Zhu et al.,2014). Excessive expression of *CaXTH3* in Arabidopsis thaliana and tomato can improve drought and salt tolerance of transgenic plants (Cho et al.,2006;Choiet al.,2011).Overexpression of *BcXTH1* promotes the growth of flowering shoots and thus increases plant height (Shin et al.,2006).*Atxth15, 19, 16, 17* can promote petiole elongation by regulating cell wall ductility (Sasidharanet al.,2010).*PC-XET1* may be involved in cell wall degradation during the ripening and softening process of pear (Hiwasa et al.,2003).Overexpression of *PeXTH* gene in tobacco can improve drought resistance of tobacco (Han Yansha et al., 2016).

Drought affects more than 10% of arable land and reduces the average yield of major crops by 50% (Bray et al., 2000).Soybean (Glycine Max) is an important grain and oil crop, which occupies an extremely important position in the world agricultural production.However, due to high transpiration coefficient, large water demand and relatively weak drought resistance of soybean, drought has a great impact on its growth and development, yield and quality (Farooq et al., 2017).The growth and development of soybean requires the unity of leaf photosynthesis and underground root group absorption, and the developed root

system can absorb more water to promote photosynthesis of the above-ground leaves(Hallmark et al.,1987).With the intensification of global climate change, the drought problem will be more prominent, which puts forward new requirements for the drought resistance of soybean varieties (Louren et al.,2011).It is an effective way to improve the drought resistance of soybean by discovering high-quality drought resistance genes.

The gene *GmXTH1* used in this study was a Xyloglucan transferase/hydrolase (XTH) gene isolated from soybean by RACE technology. Agrobacteria-mediated method was used to obtain OEA1 and OEA2 transgenic strains with overexpression of *GmXTH1* gene and IEA1 and IEA2 transgenic strains with interference of *GmXTH1* gene. Under drought stress stress, the transgenic strain plants germinated in response to non-growth stress, and the phenotypes at seedling stage, physiological and biochemical indexes were measured. Under drought stress, the relative expression level of *GmXTH1* gene in different transgenic lines was compared with that of endogenous E3 link-enzyme gene *GMPLR-2*(GenBank:EU362626.1)and WRKY transcription factor gene *GmWRKY35*(GenBank:KM587699.1) and the relative expression levels of transcription factor *JCVI-FLGM-14H24* (GenBank: BT095106.1) were analyzed.

Materials And Methods

Experimental materials

In the plant materials provided by the Plant Biotechnology Center of Jilin Agricultural University, the transgenic soybean lines OEA1 and OEA2 with *GmXTH1* gene overexpressed in high generation, and the transgenic soybean lines IEA1 and IEA2 with *GmXTH1* gene interfered with the expression, as well as control material M18.The reference material M18 was derived from JN18 mutants with developed roots obtained under drought stress. The sequencing company is Jilin Kumei Biotechnology Co., Ltd.

Experimental design

This experiment was carried out in the Plant Biotechnology Center Laboratory of Jilin Agricultural University. The test materials were strictly selected. After removing impurities, the healthy and plump soybean seeds were selected in order.

Germination test: M18 and OEA1, OEA2, IEA1 and IEA2 were soaked in 75% alcohol and 5%NaClO for 120 seconds respectively, washed with distilled water for 3 times, and then placed in 9cm petri dishes. Two layers of sterile filter paper were placed on each side and repeated for 3 times, with a total of 60 pans.Based on previous research results, PEG-6000 solutions with different mass concentrations were set to simulate different water potential as follows: 0%(CK), 5%, 10%, 15%, corresponding water potential of 0(CK), -0.1, -0.2, -0.4MPa(Shuangquan Dong et al.,2014).Each concentration was added with 20mL to simulate drought stress treatment, and the same amount of distilled water was added to the control group. The seeds were germinated in an incubator with artificial climate. The germination was accelerated at a constant temperature of 25℃ and relative humidity of 70%.The germinating standard

was that the radicle broke through the seed coat by 1 mm, and the germ was half the length of the seed(Sun Z D et al.,2001). The number of germinated seeds was recorded on a regular basis day by day, and the germinating test was ended 6 days later.After the experiment, five representative seedlings were selected from each dish to measure root length and seedling height.

Seedling test: the selected seeds were sown in a plastic basin with a height of 20cm, a width of 20cm and a length of 50cm, and each basin was filled with 10kg of sand soil. The experiment adopted a completely random design, and two factors of different strains and drought treatment were set. The watering conditions were set at three levels, which were normal, dry for 7 days and dry for 15 days respectively.Five lines, OEA1 and OEA2 were transgenic soybean lines with overexpression of *GmXTH1* gene.The transgenic *GmXTH1* gene interfered with the expression of soybean lines IEA1, IEA2 and control material M18. Each line and each treatment were repeated three times, with a total of 27 basins, and 5 seedlings were left in each basin.Potted matrix for sand, sand wash three times before use, put the good experimental material in artificial climate chamber, before the first three ternate fully expanded, normal irrigation, soil moist basic state, after the first three ternate fully expanded soybean dry processing, and then determined under normal circumstances, drought seven days, the physiological and biochemical indexes of drought for 15 days.

Determination of germination stage indexes of different soybean lines

The germination rate (GR), germination potential (GE), germination index (GI) and vigor index (VI) were calculated according to the number of germination seeds. The germination drought tolerance index and stress index were determined by referring to Wang Zan (Wang Z et al.,2008)and An Yongping(An Y P et al.,2006), and the formula was as follow:

GR(%) = total number of germinations on the 6th day ×100/ number of tested seeds

Ge (%) = number of germinations in the first 3 days ×100/ number of tested seeds

Gi =$\sum$(DG/DT),DG is the number of germinating days per day, DT is the number of germinating days of corresponding DG

VI = GI×S(S is seedling weight)

Germination drought tolerance index (GDRI) = germination index under osmotic stress/germination index of control

Germination stress index (GSI) = germination index of treated seeds/germination index of control seeds

Among them, the germination index =(1.00) Nd2 +(0.75) Nd4 +(0.50) Nd6, where Nd2, Nd4 and Nd6 are the germination rate of seeds on the second, fourth and sixth days respectively, and 1.00, 0.75 and 0.50 are the drought tolerance coefficient given by the corresponding germination days respectively.

Phenotypic identification and physiological and biochemical index determination of transgenic *GmXTH1* soybean at seedling stage

The root scanner scans the total root length, surface area, total root projected area, root volume, average root diameter, total root crossing number, and total root tip number.

Cut off all the leaves of each treated plant, quickly put them into an aluminum box with known weight, and weigh fresh weight (WF). Then take out the samples and immerse them in distilled water for 6 ~ 8 h. After that, take out the samples and wipe the water on the surface of the samples with absorbent paper.The saturated fresh weight (Wt) of the sample was obtained until the saturated weight of the sample was approximately obtained. Then, the sample was put into an aluminum box with known weight, put into a 105 ℃ oven for 15 minutes after drying, and then turned to 80 ℃ for constant weight. Then the dry weight (WD) was weighed.RWC=(WF-WD)/(WT-WD), and relative water content (RWC) refers to the percentage of water content of plant leaves in saturated water content.

Superoxide dismutase (SOD) and peroxidase (POD) were determined by Zhang Xianzheng(Zhang Zongheng et al.,1900)NBT photochemical reduction method and guaiacol method, respectively, while malondialdehyde (MDA) was determined by Liu Youliang et al.(Liu Youliang et al.,1992; Li Hesheng et al.,2000).

Expression of drought stress response gene in transgenic *GmXTH1* soybean at seedling stage

When soybean seedlings reached their first triple leaf, they were dry for 7 days and irrigated normally in the control group.β-actin was used as reference gene. .The relative expression levels of *GmXTH1* gene in different transgenic lines were analyzed by qRT-PCR with the endogenous gene E3 ligase gene *GmPLR-2*, WRKY transcription factor gene *GmWRKY35*, and transcription factor *JCVI-FLGM-14H24* in soybean.Primers are shown in Table 1.

The PCR reaction system was as follows: 2x All-in-OneTMqPCR Mix 10μL, upstream and downstream primers (10 μmol/L) 2μL each, cDNA (< 100 ng) 2μL, and sterilized ddH2O 4μL.The relative expression of the target gene was calculated by formula $2^{-\Delta\Delta CT}$.

Data processing method

DPS V17.5 and Excel 2010 were used for all statistical analyses, and Dunnet control method was used for single factor test to compare and analyze the significance of the difference between control material M18 and transgenic lines (*P < 0.05, **P < 0.01).Data are expressed as mean ± standard deviation for three replicates.

Results

Identification of drought tolerance of transgenic *GmXTH1* soybean at germination stage

As can be seen from Table 2, germination potential, germination rate and germination index of soybean seeds were significantly decreased with the increase of PEG concentration, and decreased with the increase of PEG concentration.Under the condition of clear water (CK), the germination potential and germination rate of M18, OEA1, OEA2, IEA1 and IEA2 seeds were the largest and no significant difference, indicating that all strains germinated well under normal conditions.With the increase of PEG concentration, the germinating state of OEA1 and OEA2 transgenic materials with *GmXTH1* gene overexpression was significantly stronger than that of control group M18, and the germinating state of materials with *GmXTH1* gene interference expression was significantly weaker than that of control group M18.After 5%PEG-6000 treatment, the relative germination potential of OEA1 and OEA2 were significantly higher than that of control group M18, and IEA1 and IEA2 were significantly lower than that of control group M18. Compared with water treatment, the relative germination rate of M18, OEA1 and IEA2 had no significant changes.OEA2 and IEA1 were slightly decreased. The germination index and vigor index of the *GmXTH1* overexpressed material OEA1 and OEA2 were extremely significantly higher than that of the control material M18, and the *GmXTH1* interfered expression material IEA1 and IEA2 were extremely significantly lower than that of the control material M18.After 10% and 15%PEG-6000 treatment, the relative germination potential, relative germination rate, germination index and vigor index of *GmXTH1* gene overexpression material OEA1 and OEA2 were extremely significantly higher than that of control material M18, and the *GmXTH1* gene interference expression material IEA1 and IEA2 were extremely significantly lower than that of control material M18.The results showed that the overexpression of *GmXTH1* gene could significantly increase the germination potential, germination rate and germination index of soybean seeds under drought stress.

As can be seen from Fig. 1, after 6 days, under the condition of clear water (CK), the root lengths of M18, OEA1, OEA2, IEA1 and IEA2 seeds showed no significant differences, but the number of lateral roots of OEA1 and OEA2 transgenic materials with overexpression of *GmXTH1* gene was significantly more than that of IEA1 and IEA2 transgenic materials with interfering expression of *GmXTH1* gene.After 5%PEG-6000 treatment, the root length of OEA1 and OEA2 was significantly longer than that of M18, the root length of IEA1 and IEA2 was significantly shorter than that of M18, the root length of OEA1 and OEA2 was 1.7 times of that of IEA1 and IEA2, and the number of lateral roots was also significantly more than that of IEA1 and IEA2.After 10%PEG-6000 treatment, the root length of OEA1 and OEA2 was significantly longer than that of M18, and the root length of IEA1 and IEA2 was significantly shorter than that of M18. The root length of OEA1 and OEA2 was 2.1 times of that of IEA1 and IEA2.After 15%PEG-6000 treatment, the root length of OEA1 and OEA2 was significantly longer than that of M18, and the root length of IEA1 and IEA2 was significantly shorter than that of M18. The root length of OEA1 and OEA2 was 3.1 times of that of IEA1 and IEA2.It can be seen from the phenotype that the overexpression of gene is beneficial to the generation of tested roots and the elongation of taproot during seed germination.

Comparative analysis of plant types of different soybean strains under different drought stress

As can be seen from Fig. 2a, under normal water conditions, OEA1, OEA2, IEA1 and IEA2 showed good phenotypic performance and thick green stalks, which showed no significant difference compared with

the control group M18.

As can be seen from Fig. 2b, after seven days of drought treatment, OEA1 and OEA2 lines with overexpression of *GmXTH1* had slightly drooping and dark green leaves and strong and upright stalks.IEA1 was expressed by *GmXTH1* interference. The plants were moderately wilting, the leaves were moderately drooping, curled and shriveled, and the stalks were bending due to mild drought stress.IEA2 plants with moderate wilting were more serious and drooping, and the stems also showed bending phenomenon. The control material M18 had slightly wilting leaves, slightly drooping leaves, slightly yellowing, and slightly curled and wrinkled edges.After seven days of drought treatment, there were significant differences in the overall phenotypes among different strains.After rehydration for 2h, OEA1 and OEA2, the leaves gradually returned to dark green and the stalks were strong and straight.IEA1 and IEA2 after 24h, the plants gradually stood upright from wilting, and the leaves gradually recovered from drooping to rising dark green.After 12h, M18 control material returned to strong and straight stems with upturned leaves, and there was significant difference in overall recovery.The results showed that the overexpression of *GmXTH1* gene was beneficial to the improvement of drought tolerance and recovery of plants, and had a positive effect on the response of plants to drought stress.

As can be seen from Fig. 2c, after 15 days of drought OEA1, the transgenic line with *GmXTH1* overexpression, was more severely shrivelled, but a small part of the leaves extended normally and the stalks were relatively erect.OEA2 Plant leaves are seriously wrinkled, but a small part of them are normally extended, and the stalks are relatively erect.In the control group, the leaves of M18 plants were seriously wrinkled and the stems were seriously dehydrated and bent.Transforming *GmXTH1* interferes with the expression of IEA1 and IEA2. The leaves of the plants are extremely seriously wrinkled, and the stalks are also dry and short due to extremely severe dehydration.After rehydration, OEA1 and OEA2 gradually returned to the normal growth state of dark green leaves and strong and straight stalks 24h later.IEA1 and IEA2 showed no recovery after rehydration, and the plants dried up and died.The control material M18 plants did not recover after rehydration, and the plants dried up and died.The results showed that the overexpression of *GmXTH1* gene was beneficial to the improvement of drought tolerance and recovery of plants, and had a positive effect on the response of plants to drought stress.

Comparative analysis of root systems of different soybean strains under different drought stress

As can be seen from Table 3, under normal water conditions, the total root length, surface area, total root projection area, root volume, mean root diameter, total cross number of roots and total root tip number of OEA1 transplants over expressed with *GmXTH1* were significantly higher than those of control material M18.The total root length, root volume, average root diameter, total cross number and total root tip number of OEA2 transgenic line with *GmXTH1* over expression were significantly higher than those of control group M18, and its root surface area and total projected area were significantly higher than those of control group M18. The total root length and mean root diameter of IEA1 transgenic lines were significantly lower than that of control group M18, and the root volume was significantly lower than that of control group M18. The root surface area, total projection area, total cross number and total root tip

number showed no difference with that of control group M18.The total root length, total root projection area, mean root diameter, total root crossover number and total root tip number of IEA2 transgenic lines with *GmXTH1* over expression were significantly lower than those of the control material M18, and the root surface area and volume were significantly lower than those of the control material M18.

As can be seen from Table 3, in the case of 7 days of drought, the total root length, surface area, total root projection area, root volume, total cross number and total root tip number of OEA1 transgenic lines over expressed with *GmXTH1* were significantly higher than those of control group M18, and the average root diameter showed no difference with that of control group M18.The total length, surface area, total projection area, root volume and total cross number of roots of OEA2 transgenic lines over expressed with *GmXTH1* were significantly higher than those of control group M18, and there was no difference between the total projection area, average diameter and total number of root tips of OEA2 transgenic lines overexpressed with *GmXTH1* and control group M18.The total root length, surface area, total root projection area, root volume, total cross number and total root tip number of IEA1 transgenic lines were significantly lower than the control material M18, and the mean root diameter was significantly lower than the control material M18.The total root length, surface area, total root projection area, root volume, total root crossover number and total root tip number of IEA2 transgenic lines were significantly lower than the control material M18, and the average root diameter was significantly lower than the control material M18.

As can be seen from Table 3, the total root length, surface area, total root projection area, root volume, mean root diameter, total cross number and total root tip number of OEA1 transgenic lines over expressed by *GmXTH1* were significantly higher than those of control group M18 under the condition of drought for 15 days.The total root length and total root tip number of OEA2 transgenic lines over expressed with *GmXTH1* were significantly higher than those of the control material M18, and the root surface area was significantly higher than that of the control material M18. There were no differences in the total root projection area, root volume, average root diameter, and total cross number of roots of the control material M18.The total root length, total cross number and total root tip number of IEA1 transgenic lines were significantly lower than those of the control material M18, and the average diameter surface area, total root projection area and root volume of IEA1 transgenic lines were not different from those of the control material M18.The total root length, surface area, total root projection area, root volume, total cross number and total root tip number of IEA2 transgenic lines were significantly lower than those of control group M18, and the mean root diameter had no difference with that of control group M18.

As can be seen from Table 3, the total root length, surface area, total root projection area, root volume, mean root diameter, total cross number and total root tip number of all strains increased significantly after 7 days of drought compared with normal conditions, but OEA1 and OEA2 strains over expressed by *GmXTH1* were more significant.The total root length, surface area, total root projection area, root volume, mean diameter, total cross number and total root tip number of each strain decreased significantly after 15 days of drought compared with 7 days of drought, but the decrease amplitude of OEA1 and OEA2 in *GmXTH1* over expression lines was small.

The above results indicated that the overexpression of *GmXTH1* gene could significantly increase the total root length, surface area, total projection area, root volume, mean root diameter, total cross number and total root tip number of the plant root system, which promoted the more developed root system and was more conducive to the absorption of water and minerals.

Physiological and biochemical analysis of *GmXTH1* transgenic soybean at seedling stage

As can be seen from Fig. 3a, without drought treatment, RWC of leaves of different soybean strains had significant differences as a whole.After 7 days of drought treatment, the RWC of OEA1 and OEA2 leaves was significantly higher than that of M18 control group 79.14%, 80.56% and 83.97%, respectively, and the RWC of IEA1 and IEA2 leaves was significantly lower than that of M18 control group 79.14%, 75.68% and 76.25%, respectively.There were significant differences in RWC among leaves after 7 days of drought.After 15 days of drought treatment, the RWC of OEA2 and OEA1 leaves was significantly higher than that of M18 in the control group 66.93%, 79.24% and 78.54%, respectively, and the RWC of IEA2 and IEA1 leaves was significantly lower than that of M18 in the control group 66.93%, 60.35% and 61.77%, respectively.The RWC of leaves was significantly different after 15 days of drought.The results indicated that the overexpression of *GmXTH1* gene could significantly delay the decrease of RWC and reduce transpiration of water in leaves.

As can be seen from Fig. 3b, MDA content of different strains showed no significant difference without drought treatment.After 7 days of drought treatment, the MDA content of OEA2 and OEA1 was significantly lower than that of M18, and the MDA content of IEA2 and IEA1 was significantly higher than that of M18, and the growth rates of MDA content of OEA2 and OEA1 were 46.57% and 37.50%, respectively, significantly lower than that of control M18 73.67%.The MDA content of IEA2 and IEA1 increased by 200.24% and 206.95%, respectively.After 15 days of drought treatment, the MDA content of OEA2 and OEA1 was significantly lower than that of M18, the MDA content of IEA2 and IEA1 was significantly lower than that of M18, and the MDA content of OEA2 and OEA1 increased by 104.30% and 82.94%, respectively, significantly lower than that of control M18 144.84%.The MDA content of IEA2 and IEA1 increased by 297.72% and 296.99%, respectively.The results indicated that the overexpression of *GmXTH1* gene could slow down the peroxidation degree of membrane lipid.

As can be seen from Fig. 3c, there was no significant difference in POD activity between different strains before drought treatment (0d).After 7 days of drought treatment, the POD activity of OEA2 and OEA1 was significantly higher than that of M18, and the POD activity of IEA2 and IEA1 was significantly lower than that of M18, and the growth rates of POD activity of OEA2 and OEA1 were 176.45% and 155.24% respectively, which were significantly higher than that of control M18 113.54%.The POD activity of IEA2 and IEA1 increased by 67.66% and 61.27%, respectively.After 15 days of drought treatment, the POD activity of OEA2 and OEA1 was significantly higher than that of M18, and the POD activity of IEA2 and IEA1 was significantly lower than that of M18, and the growth rates of POD activity of OEA2 and OEA1 were 69.12% and 65.59% respectively, which were significantly higher than that of 57.00% of control M18.The POD activity of IEA2 and IEA1 increased by 44.65% and 40.94%, respectively. The results indicated that the effective removal of harmful substances during the seedling stage of soybean

transgenic *GmXTH1* gene overexpression could produce more protective enzymes and resist the damage caused by drought.

As can be seen from Fig. 3d, without drought treatment, SOD activity of different strains showed no significant difference.After 7 days of drought treatment, the SOD activities of OEA2 and OEA1 were significantly higher than that of M18, and the SOD activities of IEA2 and IEA1 were significantly lower than that of M18, and the SOD activity growth rates of OEA2 and OEA1 were 126.89% and 156.57%, respectively, significantly higher than that of control M18 94.65%.The SOD activity of IEA2 and IEA1 increased by 84.98% and 74.63%, respectively.After 15 days of drought treatment, the SOD activities of OEA2 and OEA1 were significantly higher than that of M18, and the SOD activities of IEA2 and IEA1 were significantly lower than that of M18, and the SOD activity growth rates of OEA2 and OEA1 were 56.63% and 59.50% respectively, which were significantly higher than that of control M18 33.55%.The SOD activity of IEA2 and IEA1 increased by 21.88% and 22.88%, respectively.The results indicated that the effective removal of harmful substances during the seedling stage of soybean transgenic *GmXTH1* gene overexpression could produce more protective enzymes and resist the damage caused by drought.

Relative expression levels of target gene and other endogenous genes in transgenic *GmXTH1* soybean at seedling stage

As can be seen from Fig. 4a, under normal water conditions, the expression level of OEA1 *GmXTH1* in soybean roots and leaves was increased by 44.39% and 50.00% respectively.The expression level of OEA2 strain *GmXTH1* in soybean roots and leaves was increased by 56.37% and 42.90% respectively.The expression level of *GmXTH1* of IEA1 strain was decreased by 74.74% in soybean root and 61.31% in leaf.The expression level of *GmXTH1* of IEA1 strain was decreased by 44.71% in soybean root and 85.39% in leaf.

As can be seen from Fig. 4b, after 7 days of drought, the expression level of OEA1 *GmXTH1* in soybean roots and leaves increased by 113.61%.The expression level of OEA2 strain *GmXTH1* in soybean roots and leaves was increased by 120.38% and 171.32% respectively.The expression of IEA1 strain *GmXTH1* in soybean roots and leaves was reduced by 32.40% and 68.00% respectively.The expression level of *GmXTH1* of IEA1 strain was decreased by 60.50% in soybean roots and 34.48% in soybean leaves.

According to Fig. 4c, under normal water conditions, the expression level of OEA1 strain *JCVI-FLGM-14H24* in soybean roots and leaves was increased by 50.52% and 7.92% respectively.The expression level of OEA2 strain *JCVI-FLGM-14H24* in soybean roots and leaves was increased by 43.40% and 14.87% respectively.The expression level of IEA1 strain *JCVI-FLGM-14H24* was decreased by 33.10% in soybean root and 61.31% in soybean leaf.The expression level of IEA1 strain *JCVI-FLGM-14H24* was decreased by 49.65% in soybean roots and 74.30% in soybean leaves.The above results indicated that the overexpression of *GmXTH1* promoted the expression of *JCVI-FLGM-14H24* under normal water conditions, while the interference of *GmXTH1* inhibited the expression of *JCVI-FLGM-14H24.*

As can be seen from Fig. 4d, after 7 days of drought, the expression level of OEA1 strain *JCVI-FLGM-14H24* in soybean roots and leaves increased by 80.25% and 12.51%.The expression level of OEA2 strain *JCVI-FLGM-14H24* was increased by 48.45% in soybean roots and 10.96% in leaves.The expression level of IEA1 strain *JCVI-FLGM-14H24* was decreased by 19.34% in soybean roots and 12.95% in soybean leaves.The expression level of IEA1 strain *JCVI-FLGM-14H24* was decreased by 25.26% in soybean roots and 13.55% in soybean leaves.The above results indicated that the overexpression of *GmXTH1* promoted the expression of *JCVI-FLGM-14H24*, while the interference expression of *GmXTH1* inhibited the expression of *JCVI-FLGM-14H24* under drought for 7 days.

As can be seen from Fig. 4e, under normal water conditions, the expression level of OEA1 strain *GmWRKY35 i*n soybean roots and leaves increased by 80.25% and 536.43%.The expression level of OEA2 strain *GmWRKY35* was decreased by 78.61% in soybean roots and increased by 748.55% in leaves.The expression level of IEA1 strain *GmWRKY35* in soybean roots and leaves was increased by 471.60% and 1608.90% respectively.The expression level of IEA2 strain *GmWRKY35* in soybean roots and leaves was increased by 56.37% and 1656.95% respectively.The above results indicated that the low expression level of *GmXTH1* under normal water condition was conducive to the expression of *GmWRKY35*.

As can be seen from Fig. 4f, after 7 days of drought, the expression of OEA1 strain *GmWRKY35* in soybean roots decreased by 49.48% and increased by 44.89% in leaves.The expression level of OEA2 strain *GmWRKY35* was decreased by 30.27% in soybean roots and increased by 39.47% in leaves.The expression level of IEA1 strain *GmWRKY35* was decreased by 56.02% in soybean roots and increased by 25.70% in soybean leaves.The expression level of IEA1 strain *GmWRKY35* was decreased by 69.75% in soybean roots and 11.12% in soybean leaves.The above results indicated that the overexpression of the target gene *GmXTH1* was beneficial to the expression of *GmWRKY35* in soybean leaves under drought for 7 days, while the expression of the target gene *GmXTH1* inhibited the expression of *GmWRKY35* in soybean roots, and the higher the expression level, the weaker the inhibition.

As can be seen from Fig. 4g, under normal water conditions, the expression level of OEA1 strain *GmPLR-2* in soybean roots and leaves was increased by 217.11% and 8171.06% respectively.The expression level of OEA2 strain *GmPLR-2* was decreased by 95.00% in soybean roots and increased by 3512.69% in leaves.The expression level of *GmPLR-2* of IEA1 strain was decreased by 44.91% in soybean root and increased by 6344.52% in soybean leaf.The expression level of *GmPLR-2* of IEA1 strain was decreased by 85.39% in soybean roots and increased by 6711.97% in soybean leaves.The results showed that the expression of target gene *GmXTH1* inhibited the expression of *GmPLR-2* in soybean roots and promoted the expression of *GmPLR-2* in soybean leaves under normal water conditions.

According to Fig. 4h, after 7 days of drought, the expression level of OEA1 strain GmPLR-2 in soybean roots and leaves decreased by 25.26% and 19.34%.The expression level of OEA2 strain *GmPLR-2* in soybean roots was increased by 5.70% and decreased by 15.03% in leaves.The expression level of *GmPLR-2* of IEA1 strain was decreased by 22.62% in soybean roots and increased by 227.16% in soybean leaves.The expression of IEA1 strain *GmPLR-2* was decreased by 60.09% in soybean roots and

increased by 244.62% in soybean leaves.The above results indicated that the target gene *GmXTH1* was overexpressed and inhibited the expression of *GmPLR-2* in soybean leaves under drought for 7 days, while the target gene *GmXTH1* interfered with the expression and promoted the expression of *GmPLR-2* in soybean leaves.

Fig. 4. Relative expression levels of *GmXTH1* and *JCVI-FLGM-14H24* in different transgenic soybean lines under different drought conditions at seedling stage (a.Under normal circumstances, the relative expression of *GmXTH1* in OEA1,OEA2, IEA1 and IEA2 roots and leaves;b. Relative expression levels of *GmXTH1* in OEA1,OEA2, IEA1 and IEA2 roots and leaves after 7 days of drought :c.Under normal conditions, the relative expression levels of *JCVI-FLGM-14H24* in OEA1,OEA2, IEA1, and IEA2 roots and leaves: d. *JCVI-FLGM-14H24* in OEA1,OEA2, IEA1, and IEA2 roots and leaves:e.Relative expression levels of *GmWRKY35* in roots and leaves of OEA1,OEA2, IEA1 and IEA2;f. The relative expression levels of *GmWRKY35* in OEA1,OEA2, IEA1 and IEA2 roots and leaves;g. The relative expression levels of *GmPLR-2* in OEA1,OEA2, IEA1 and IEA2 roots and leaves;h. The relative expression levels of *GmPLR-2* in OEA1,OEA2, IEA1 and IEA2 roots and leaves)

Discussion

Germination and phenotypic data analysis of *GmXTH1* transgenic soybean

Seed germination stage is a relatively important stage for the study of drought resistance of plants, which can be used for early identification of drought tolerance of plants(Sun J K et al.,2006).Crop varieties with strong drought resistance have a fast water absorption rate and can sprout quickly under drought stress, with better germination indexes such as relative germination rate, relative germination potential and drought resistance coefficient of germination(Hou J H et al.,1995).The test results showed that when the water content of seeds was normal at germination stage, the root number of transgenic OEA1 and OEA2 strains with over expression of *GmXTH1* was significantly higher than that of control material M18, and the root number of transgenic IEA1 and IEA2 strains with interference expression of *GmXTH1* was significantly lower than that of control material M18, indicating that *GmXTH1* promoted lateral root meristem of soybean root. With the increase of PEG-6000 concentration, the germination potential, germination rate and germination index of OEA1 and OEA2 strains transgenic with *GmXTH1* over expression were significantly higher than those of control group M18.The germination potential, germination rate and germination index of IEA1 and IEA2 expressed by *GmXTH1* interference were significantly lower than those of control group M18.Moreover, the transgenic lines OEA1 and OEA2 with over expression of *GmXTH1* had better growth and longer soybean roots under the same conditions. It was inferred that the over expression of *GmXTH1* promoted lateral root meristem and root elongation of soybean roots, and improved drought tolerance of soybean seeds.

During seed germination, the activities of various enzymes in cotyledon are enhanced to promote the decomposition of stored substances. In addition to ensuring its own respiration, most of the hydrolysates are transferred to the flourishing parts such as radicle or embryo to promote the rapid development of

hypocotyl and radicle(Fu J R et al.,1985).Droughts stress can inhibit the germ length, taproot length, root weight and other indexes during soybean germination, and the greater the drought degree, the more obvious the inhibition effect is(Mo J G et al.,2014).At the same time, under drought stress, nutrients will give priority to radicle, promote root elongation and development, and inhibit hypocotyl growth(Li L L et al.,2007).Therefore, the developed root system and higher transport rate of storage matter are significantly associated with drought tolerance of plants positive correlation(Benjamin J G et al.,2006;Chen X Z et al.,2005).The total root length, surface area, total projection area, root volume, mean diameter, total cross number and total root tip number of *GmXTH1* transgenic material OEA1 and OEA2 were significantly higher than those of control material M18.IEA1 and IEA2 of transgenic *GmXTH1* gene interference expression materials were significantly lower than that of control material M18.The results showed that the overexpression of *GmXTH1* gene could significantly improve the root condition of the plant.There were significant differences in plant type performance of different soybean strains at seedling stage under drought stress.With the increase of drought time, the plant type performance of OEA1 and OEA2 transgenic materials with *GmXTH1* gene over expression was significantly better than that of the control group, and the plant type performance of IEA1 and IEA2 transgenic materials with *GmXTH1* gene interference expression was significantly worse than that of the control group. The results showed that the over expression of *GmXTH1* gene can significantly improve the drought resistance of plants.

Root traits of different soybean strains at seedling stage were significantly different under drought stress. The total root length, surface area, total root projection area, root volume, average root diameter, total root crossing number and total root tip number of *GmXTH1* transgenic materials OEA1 and OEA2 were significantly higher than those of control material M18.The *GmXTH1* gene interference expression materials IEA1 and IEA2 were significantly lower than the control material M18.The results showed that the over expression of *GmXTH1* gene could significantly improve the root meristem.

Analysis of physiological and biochemical indexes of *GmXTH1* transgenic soybean

Leaf RWC and other indicators are sensitive to water deficit and are usually used as an important indicator for drought resistance identification(Bai Z Y et al.,2008).There was a significant correlation between leaf water content and soil water content (Wang Fan et al.,2019), and the results of this study showed that leaf RWC decreased with the increase of drought time.The RWC of OEA1 and OEA2 transgenic materials with over expression of *GmXTH1* gene was significantly higher than that of the control material at the same period, and the RWC of IEA1 and IEA2 transgenic materials with interference expression of *GmXTH1* gene was significantly lower than that of the control material at the same period, indicating that the over expression of *GmXTH1* gene could significantly delay the water loss of leaves.

Drought stress produces antioxidant enzymes that can remove free radicals, reduce cell membrane damage and enhance drought resistance of varieties(Xie C et al.,2008).This study showed that the SOD and POD of OEA1 and OEA2 transgenic materials with over expression of *GmXTH1* gene responded to drought more quickly and had higher activity than the control variety M18.Compared with the control

variety M18, the response of SOD and POD of *GmXTH1* transgenic interference expression materials IEA1 and IEA2 to drought was slower and the activity was lower, which indicated that *GmXTH1* transgenic over expression soybean material could produce more protective enzymes timely in the effective removal of harmful substances in the seedling stage of soybean and resist the damage caused by drought.With the increase of drought time, the activities of SOD and POD basically increased first and then decreased in the three measured times, which was consistent with the results of Wang Qiming and Mo Hong et al. (Wang Qiming et al.,2006;Mo Hong et al.,2007).

MDA is the product of membrane lipid peroxidation and reflects the strength of plant response to stress conditions(Liu J et al.,2009;Lou L J et al.,2013; Jiao J et al.,2006 ;Yan M L et al.,2007) showed that MDA content increased with the extension of drought time, and similar results were also shown in this study.The increase of MDA content in the *GmXTH1* overexpression materials OEA1 and OEA2 was significantly lower than that in the control materials of the same period, and the increase of MDA content in the *GmXTH1* interference expression materials IEA1 and IEA2 was significantly higher than that in the control materials of the same period, indicating that the overexpression of *GmXTH1* can slow down the peroxidation degree of membrane lipid.

Analysis of target gene and its endogenous gene expression in transgenic *GmXTH1* soybean

Transcription factor (TF), also known as trans-acting factor, is a regulation product of gene coding, which can specifically bind with cis-acting elements in gene promoter region, so as to ensure the combination of protein molecules expressed by target gene at a specific intensity and at a specific time and space(Yang Wenjie et al.,2009).In plant stress response, it has the function of signal transduction and gene expression regulation, such as CDPK, MAPK and other protein kinases that sense and transact stress signals, as well as transcription factors that regulate gene expression such as bZIP, bHLH, NAC, DREB, ERF, RAV, WRKY and MYB(Riechmann J R et al.,2000).The target gene of *GmXTH1* transgenic soybean was expressed in both roots and leaves, and there was no difference in the expression between roots and leaves. The expression level of this gene increased with the degree of drought. Under normal water conditions, the overexpression of *GmXTH1* promoted the expression of *JCVI-FLGM-14H24*, while the interference expression of *GmXTH1* inhibited the expression of *JCVI-FLGM-14H24*, and the low expression level of *GmXTH1* was conducive to the expression of *GmWRKY35*.The expression of target gene *GmXTH1* inhibits the expression of *GmPLR-2* in soybean roots, while the expression of target gene *GmXTH1* promotes the expression of *GmPLR-2* in soybean leaves. Under drought for 7 days, overexpression of *GmXTH1* promoted the expression of *JCVI-FLGM-14H24*, while interference with *GmXTH1* inhibited the expression of *JCVI-FLGM-14H24*.The overexpression of the target gene *GmXTH1* is beneficial to the expression of *GmWRKY35* in soybean leaves, while the expression of the target gene *GmXTH1* inhibits the expression of *GmWRKY35* in soybean roots. E3 ligases play an important role in the UPS (Ubiquitin-Proteasome) system, which is responsible for specific recognition, recruitment and transport of target proteins(Moon J et al.,2004), and then ubiquitination modification to regulate different physiological processes of plants.A large number of studies have shown that RING-H2-type E3 ligase is related to plant resistance to abiotic stress, especially drought stress. The higher the expression level, the

weaker the inhibition.The overexpression of target gene *GmXTH1* inhibited the expression of *GmPLR-2* in soybean leaves, while the interference of the expression of target gene *GmXTH1* promoted the expression of *GmPLR-2* in soybean leaves.

Conclusions

Illustrated by the above phenomenon, under drought stress, excess *GmXTH1* gene expression, in turn, increase root length, surface area, root total projection area, root volume, root average diameter, root total number of cross, the total number of root, increase water intake, reduce transpiration, leaf water content and decrease MDA accumulation, more effective to produce more protective enzyme in time,To low resistance to the hazards of drought.This study lays a theoretical foundation for further understanding the biological function and molecular mechanism of *GmXTH1* in soybean stress, and also provides a reference for soybean stress breeding research.

Declarations

Author contributions Conceptualization,Y.Z.,Y.S.andP.W.; methodology,Y.Z.,Y.S., H.Z., J.Y.,Y.D., J.Q. and P.W.; investigation, Y.Z.; resources, Y.S., P.W.,J.Q.; data curation, Y.Z.; writing—original draft preparation, Y.Z.,H.Z; writing—review and editing, Y.Z.,H.Z.,Y.S.; visualization, Y.Z.; supervision, Y.D.; project administration, J.Q. All authors have read and agreed to the published version of the manuscript.

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Code availability Not applicable

Supplementary Materials The following are available online at www.mdpi.com/xxx/s1, Table S1: title.

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Tables

Table 1. Primer sequences used in the PCR analysis

Targeted Gene	Primer Sequence
Actin2	Forward:5′-CTGAGGTTCTATTCCAGCCATCC-3′
	Reverse:5′-CCACCACTGAGGACAACATTACC-3′
GmXTH1	Forward:5′-CATTCCCAAAGGAGCAGCCA-3′
	Reverse:5′-GGAGGAGGCAGAGTTGGAAGTG-3′
JCVI-FLGm-14H24	Forward:5′-ATCCCATCCAAAATCATTAGGC-3′
	Reverse:5′-ATGCCTTATGTGTATTTTCCTTTGC-3′
GmPLR-2	Forward:5′-ATCGCACTTCACTGTATGGACC-3′
	Reverse:5′-CCTCAGACAATCCTGTGCTCAC-3′
GmWRKY35	Forward:5′-CTGCGGAACCCAGAGTCTATCG-3′
	Reverse:5′-CGACGACGAGCACAGTTGTTTAG-3′

Table. 2. Comparison and analysis of traits of *GmXTH1* transgenic soybeans with different PEG-6000 concentrations at germination stage

	Normal circumstances					5%PGE-6000				
	Genotype									
	M18	OEA1	OEA2	IEA1	IEA2	M18	OEA1	OEA2	IEA1	IEA2
Normal circumstances	98.33±1.67	100.00±0.00*	95.00±2.89*	98.33±1.67	98.33±1.67	65.00±2.89	86.67±1.67**	86.67±1.67**	58.33±1.67**	55.00±2.89**
Germination rate	98.33±1.67	100.00±0.00*	98.33±1.67	98.33±1.67	98.33±1.67	98.33±1.67	100.00±0.00*	95.00±2.89*	96.67±1.67*	98.33±1.67
Germination Index	9.44±0.03	9.67±0.10*	9.39±0.17*	9.47±0.10	9.28±0.10	7.47±0.07	8.56±0.10 **	8.78±0.18**	6.69±0.27**	6.53±0.10**
Vigor index	6.77±0.13	7.34±0.08*	7.07±0.24*	6.54±0.17	6.34±0.04*	4.18±0.12	5.76±0.14**	5.94±0.14**	3.55±0.18**	3.33±0.08**
GDRI						0.80±0.01	0.88±0.00**	0.93±0.02**	0.70±0.03**	0.71±0.02**
GSI						0.79±0.01	0.89±0.00**	0.93±0.01**	0.71±0.02**	0.70±0.02**
	10% PGE-6000					15%PGE-6000				
	Genotype									
	M18	OEA1	OEA2	IEA1	IEA2	M18	OEA1	OEA2	IEA1	IEA2
Normal circumstances	51.67±1.67	75.00±0.00**	81.67±1.67**	41.67±1.67**	36.67±1.67**	26.67±4.41	41.67±1.67**	40.00±2.89**	16.67±1.67**	15.00±2.89**
CGermination rate	91.67±4.41	96.67±1.6**	93.33±1.67**	90.00±2.89**	88.33±4.41**	68.33±6.01	86.67±7.26**	90.00±5.77**	71.67±4.41**	61.67±9.28**
Germination Index	6.11±0.15	7.36±0.22**	7.31±0.15**	5.64±0.07**	5.25±0.34**	3.78±0.35	5.11±0.16**	5.14±0.24**	3.69±0.07**	3.17±0.27**
Vigor index	2.63±0.09	4.19±0.12**	4.24±0.13**	2.11±0.09**	1.99±0.10**	1.43±0.12	2.28±0.17**	2.31±0.09**	1.27±0.05**	1.01±0.11**
GDRI	0.70±0.02	0.76±0.03**	0.76±0.02**	0.66±0.02**	0.64±0.05**	0.41±0.01	0.52±0.02**	0.53±0.03**	0.40±0.02	0.35±0.04**
GSI	0.65±0.02	0.76±0.03**	0.78±0.01**	0.60±0.01**	0.57±0.04**	0.40±0.03	0.53±0.03**	0.55±0.03**	0.39±0.02	0.34±0.04**

Within a row and treatment (normally or drought), values followed by asterisks are significantly different from M18

*P < 0.05; **P < 0.01

Table 3. Comparison and analysis of root traits of transgenic *GmXTH1* soybean at seedling stage under different drought conditions

	Normal irrigation				
	Genotype				
	M18	OEA1	OEA2	IEA1	IEA2
TL(cm)	130.17±0.28	176.81±2.41**	159.03±0.89**	106.32±3.01**	100.96±4.45**
SA(cm^2)	20.14±2.18	34.18±3.56**	31.37±4.15*	20.59±1.71	15.19±0.44*
PA(cm^2)	6.41±0.69	10.88±1.13**	9.99±1.32*	6.55±0.55	4.83±0.14**
Vol(cm^3)	0.25±0.05	0.53±0.10**	0.51±0.14**	0.32±0.06*	0.18±0.02*
AvgD(mm)	0.49±0.05	0.61±0.06**	0.63±0.08**	0.62±0.07**	0.48±0.03
TNT	892.33±32.20	1293.00±139.08**	1151.30±168.00**	854.67±76.22	790.00±42.06**
TNF	897.33±149.63	1564.00±242.09**	1559.00±13.80**	909.67±24.22	791.67±78.25**
TNC	60.33±9.21	127.67±6.84**	101.00±7.77**	56.00±10.69	47.67±7.42*
	Drought 7 days				
	Genotype				
	M18	OEA1	OEA2	IEA1	IEA2
TL(cm)	337.66±10.31	508.61±36.97**	392.67±17.74*	242.28±2.56**	239.18±3.10**
SA(cm^2)	91.87±12.20	134.47±5.51**	107.74±28.40*	52.08±1.69**	51.47±7.56**
PA (cm^2)	29.24±3.88	42.80±1.75**	34.30±9.04*	16.58±0.54**	16.38±2.41**
Vol(cm^3)	2.05±0.54	2.84±0.16**	2.59±1.01*	0.89±0.07**	0.92±0.25**
AvgD(mm)	0.86±0.10	0.84±0.04	0.85±0.20	0.68±0.03*	0.69±0.10*
TNT	2751.67±237.17	3608.00±87.50**	2376.33±269.22*	1637.67±156.33**	2012.67±160.32*
TNF	5871.33±259.68	9039.33±147.54**	5782.00±1231.13	2400.33±38.67**	3211.33±494.89**
TNC	434.33±23.38	671.00±36.59**	404.33±57.91	150.00±3.00**	222.00±43.11**
	Drought 15days				
	Genotype				
	M18	OEA1	OEA2	IEA1	IEA2
TL(cm)	243.86±13.06	295.09±6.74**	280.27±3.22**	206.55±10.91**	199.16±6.44**
SA(cm^2)	50.33±14.08	80.15±9.88**	58.12±5.55*	51.65±9.92	38.63±2.39**
PA (cm^2)	16.02±4.48	25.51±3.14**	18.50±1.77	15.09±2.10	12.30±0.76*
Vol(cm^3)	0.91±0.45	1.78±0.38**	0.98±0.17	0.83±0.16	0.60±0.06**
AvgD(mm)	0.64±0.14	0.87±0.09**	0.66±0.06	0.70±0.05	0.62±0.03
TNT	1847±132.22	2342.33±111.44**	1859±160.07	1292.67±99.34**	1380±17.39**
TNF	2986.67±785.96	5311.33±444.03**	3661.33±727.30**	2196.33±371.68**	2102±129.19**
TNC	238.67±20.51	379±9.07**	283.33±63.60**	136.67±31.00**	145±1.00**

Within a row and treatment (normally or drought), values followed by asterisks are significantly different from M18

*P < 0.05; **P < 0.01

Figures

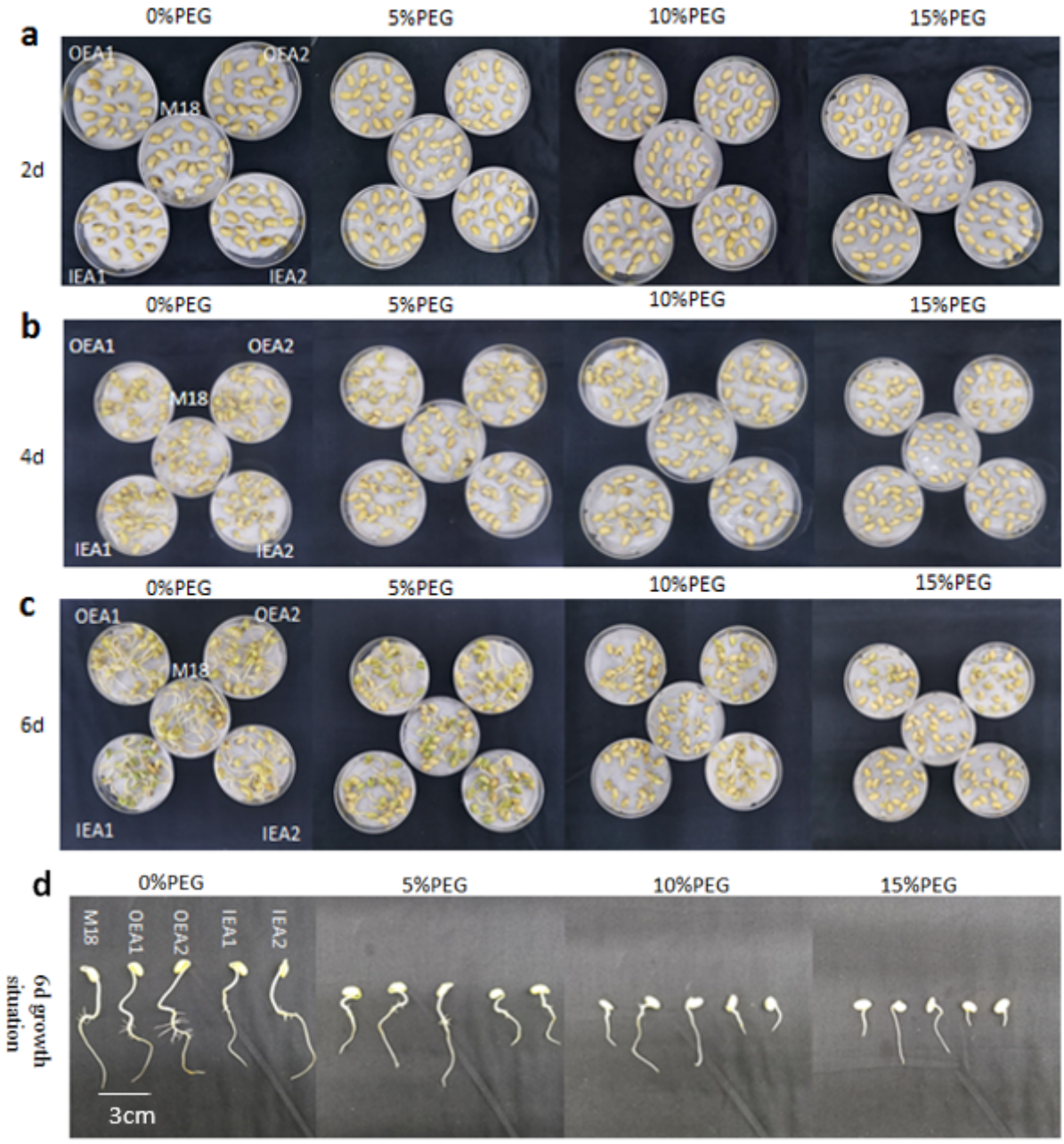

Figure 1

Phenotype analysis of germination of M18 and GmXTH1 transgenic soybean under different concentrations of PEG-6000 stress (a.The germination phenotypes of M18 and OEA1, OEA2, IEA1 and IEA2 were analyzed under 0%, 5% and 10%PEG stress at 2 days of germination.b. Phenotypic analysis of germination of M18 and OEA1, OEA2, IEA1 and IEA2 under 0%, 5% and 10%PEG stress at 4 days of germination c.The germination phenotypes of M18 and OEA1, OEA2, IEA1 and IEA2 were analyzed under 0%, 5% and 10%PEG stress at 6 days of germination.d. Phenotypes of root growth of M18 and OEA1, OEA2, IEA1 and IEA2 under 0%, 5% and 10%PEG concentration stress)

Figure 2

Seedling phenotypic analysis of M18 and GmXTH1 transgenic soybean under different drought stress (a.After 30 days of germination, the phenotypes of M18 and OEA1, OEA2, IEA1 and IEA2 at seedling stage were analyzed under 0d drought stress.b. Phenotypic analysis of M18 and OEA1, OEA2, IEA1 and IEA2 at seedling stage under drought stress for 7 days after germination for 30 days;c. Phenotypic analysis of M18 and OEA1, OEA2, IEA1 and IEA2 at seedling stage under drought stress for 15 days after germination for 30 days;)

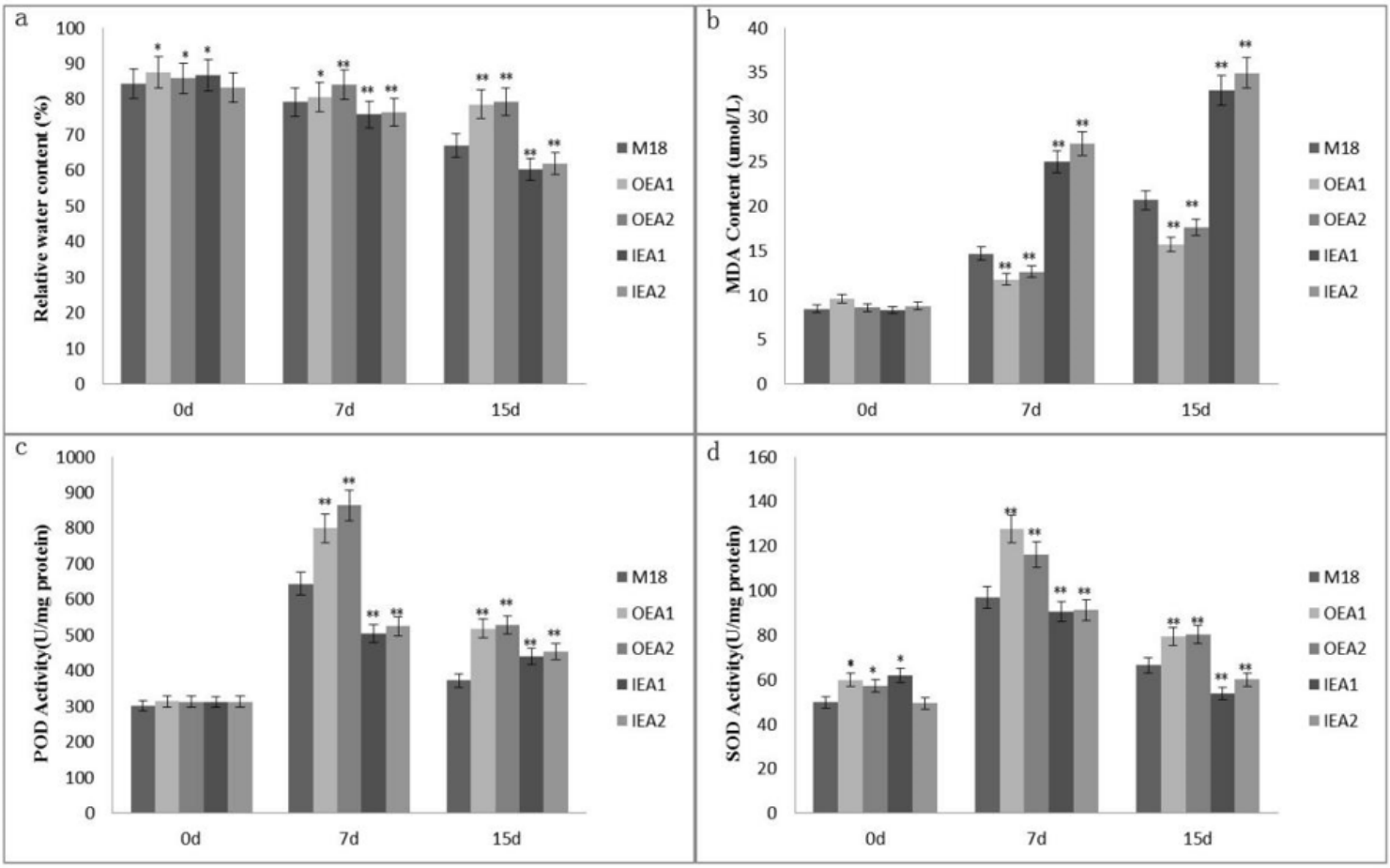

Figure 3

Physiological and biochemical indexes at seedling stage of different GmXTH1transgenic soybean lines under different drought conditions (a.Leaf relative water content;b. Malondialdehyde content: c. Peroxidase activity: d. Superoxide dismutase activity.* p < 0.05;* * P < 0.01)

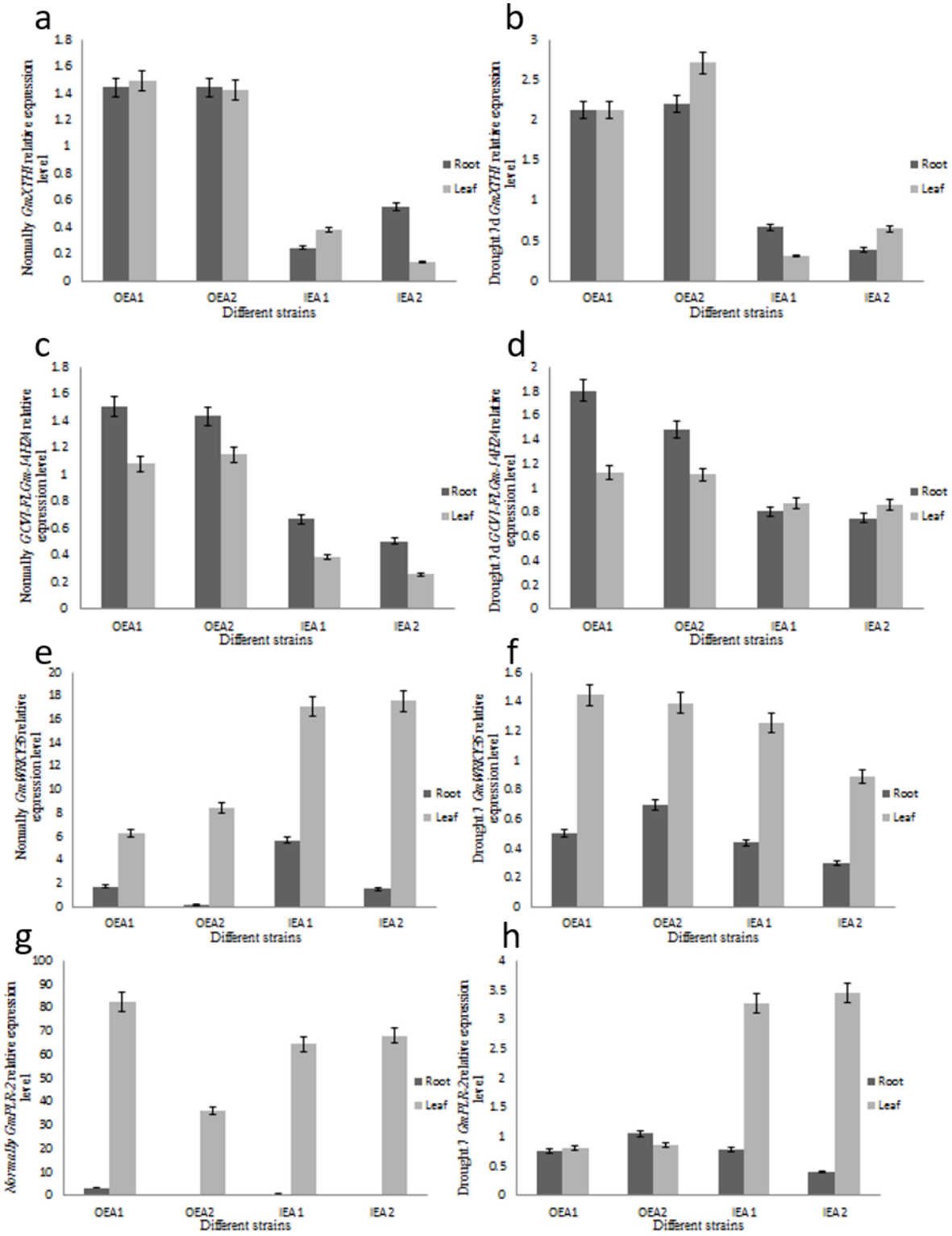

Figure 4

Relative expression levels of GmXTH1 and JCVI-FLGM-14H24 in different transgenic soybean lines under different drought conditions at seedling stage (a.Under normal circumstances, the relative expression of GmXTH1 in OEA1,OEA2, IEA1 and IEA2 roots and leaves;b. Relative expression levels of GmXTH1 in OEA1,OEA2, IEA1 and IEA2 roots and leaves after 7 days of drought :c.Under normal conditions, the relative expression levels of JCVI-FLGM-14H24 in OEA1,OEA2, IEA1, and IEA2 roots and leaves: d. JCVI-

FLGM-14H24 in OEA1,OEA2, IEA1, and IEA2 roots and leaves:e.Relative expression levels of GmWRKY35 in roots and leaves of OEA1,OEA2, IEA1 and IEA2;f. The relative expression levels of GmWRKY35 in OEA1,OEA2, IEA1 and IEA2 roots and leaves;g. The relative expression levels of GmPLR-2 in OEA1,OEA2, IEA1 and IEA2 roots and leaves;h. The relative expression levels of GmPLR-2 in OEA1,OEA2, IEA1 and IEA2 roots and leaves)