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*Article*

# Physiological Properties and Molecular Regulation in Different Edamame Cultivars under Drought Stress

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**Abstract:** Edamame (*Glycine max* (L.) Merr.) is an important economic crop worldwide and is a good source of protein and nutrients. Kaohsiung No. 9 (KH9), Kaohsiung No. 11 (KH11), and Xiang-Ji (XJ) are three major edamame cultivars planted in Taiwan. Edamame has high water requirements in all development stages. Insufficient irrigation causes aborted blossoms, small pods, and shriveled beans, thus greatly reducing the yield. We examined the three aforementioned cultivars in drought conditions during the blooming period. The results revealed that drought stress decreased the yield in all three cultivars; however, XJ and KH11 showed better drought resistant ability than KH9 did. The reduction of the qualification rate and the dry weight of qualified pods by drought stress in XJ, KH9 and KH11 was 23%, 33%, 21% and 32%, 62%, and 44%, respectively. The quantitative reverse-transcription polymerase chain reaction results indicated that genes involved in the abscisic acid (ABA) biosynthesis, ABA-dependent, and ABA-independent pathways were upregulated by drought stress in KH11, which may explain why KH11 produced higher yields than KH9 after drought treatment. We determined that drought-related signaling transduction differed among these edamame cultivars, resulting in different drought tolerance.

**Keywords:** edamame; *Glycine max*; drought stress; yield; ABA signaling

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## 1. Introduction

Edamame, also called vegetable soybean, is rich in plant-based protein, minerals, vitamins, and dietary fiber. The protein in edamame is of high quality, comparable to the protein in meat and eggs, and is easily absorbed and used by the body. It is the only plant food that contains complete protein [1]. Edamame is also a good source of fatty acids, such as the essential linoleic and linolenic acids [2], which can improve fat metabolism and help reduce triglycerides in the body. Edamame has a relatively high $\gamma$-aminobutyric acid (GABA) content compared with other common bean species [3]. It also contains lecithin, which is an indispensable nutrient for brain development and helps improve memory and intelligence. Edamame is also rich in dietary fiber, which not only improves constipation but also helps lower blood pressure and cholesterol. Additionally, it has high potassium content and is often eaten in summer to help compensate for the loss of potassium caused by excessive sweating and the consequent loss of appetite, thereby alleviating fatigue [4]. The iron in edamame is easily absorbed. Edamame can be used as a food supplement in children. Due to its high nutritional value, edamame has been a highly competitive agricultural export product in Taiwan. In 2020, Taiwan's edamame planting area had reached 7700 hectares, with a total output of approximately 70,000 metric tons, earning more than NTD950 million profit for Taiwan farmers. Edamame is currently the largest agricultural export product in Taiwan.

Due to climate change, the risks of crops facing various abiotic stresses have also increased [5]. Crops may encounter various stresses during their growth, including drought, which can cause severe damage to plants [6]. Drought can affect the morphological appearance of plants [7]: wilting of leaves decreases the leaf expansion area, and closure of stomata to reduce water loss reduces $CO_2$ exchange, which reduces photosynthesis efficiency and growth rate, resulting in decreased plant height [8–11]. Insufficient water also has a severe impact on yield. Drought stress reduces rice yield and yield components [12,13]. Water deficit also reduces seed yield by 16.3% in sunflower, 12.8% in sesame, and 9.4% in safflower [14]. The severity of the impact of drought stress on plants varies depending on the timing of drought—drought occurring during reproductive development has a much severer yield loss [15–17].

To cope with drought stress, plants increase the biosynthesis of the phytohormone abscisic acid (ABA) to regulate related signals and improve drought tolerance [18]. ABA can maintain the development of the root system under water deficit conditions to preserve the ability to absorb water [19]. Increased ABA concentration in plant leaves induces the closure of leaf stomata and reduces leaf expansion [20], which reduces water loss from plant leaves and improves the survival rate of plants under drought stress. During the reproductive stage, ABA decreases the cell division of the developing embryo, which causes the ovaries to develop abnormally [21]. The closure of stomata reduces photosynthesis efficiency, which affects the supply of carbohydrates. ABA can also disrupt the activity of carbohydrate metabolism enzymes in the ovary, resulting in the loss of yield [22]. As the primary regulatory phytohormone in drought conditions, ABA induces the expression of many downstream genes through the cis-regulatory element ABRE (ABA-responsive element) [23,24]. Under drought conditions, the protein kinase SnRK2 is activated to promote ABA responses [25]. The activated SnRK2 phosphorylates downstream targets to transmit ABA-related physiological and molecular signals, including signals related to germination, stomata closure, and root development [26].

The average air temperature of 30 °C is considered ideal for soybean germination and seedling emergence [27]. In Taiwan, edamame is mostly planted in the southern regions because of this temperature requirement. The more frequent occurrence of high temperatures and insufficient rainfall caused by climate change has increased the possibility of edamame experiencing drought stress. In order to compare the molecular regulation under drought stress of three major edamame cultivars planted in Taiwan, we not only compared the morphological characteristics of XJ, KH9 and KH11 cultivar, but also investigated their drought tolerance and mechanisms of regulating drought-related molecular signals. Our findings can benefit researchers intending to undertake further research and farmers interested in optimizing planting.

## 2. Materials and Methods

### 2.1. Plant Materials and Growth Condition

Edamame cultivars Xiang-Ji (XJ), Kaohsiung No. 9 (KH9), and Kaohsiung No. 11 (KH11) (*Glycine max* (L.) Merr.) were used in this study. Edamame was planted in fields and pots for different experimental analyses. For the field experiments, each cultivar was planted in multiple replicates in the spring of 2017 and 2018, and plant heights and yield component data were collected ($N \geq 3$, $n = 16$). Some plants were planted in 6-inch pots, and photos were taken at the same time point in each growth stage of XJ cultivar to compare differences growth with KH9 and KH11 cultivars. The data of the number of seeds per pod of each cultivar were also collected in the plant grown in 6-inch pots put in the field ($N \geq 3$, $n = 5$).

### 2.2. Drought Treatment

Drought treatments were conducted from the autumn of 2019 to the spring of 2020. Seedlings of each cultivars were separated into control group and drought treatment group to conduct the experiment for at least three replicates ($N \geq 3$, $n = 5$). All plants were grown in 6-inch pots in a greenhouse with cultivatable soil (Jiffy, Zwijndrecht, The Netherlands), and the water was precisely controlled in the drought experiment group. Daily irrigation was equal in the control and drought groups in the vegetative stages. Once the plant entered the reproductive stage (R1), with the first flower on the node, irrigation of the drought treatment group was held for 6 days. At the end of this period, one of the complete trifoliate leaves of each individual in the control and experiment groups was immediately frozen in liquid nitrogen and stored at $-80^{\circ}$ for further analysis. The morphological phenotypes of edamame under normal condition and drought stress and soil moisture were also recorded. After the drought treatment, the normal irrigation schedule was resumed in the experiment group. The yield data of the cultivars were further compared after the drought treatment.

### *2.3. Trait Investigation*

The plant height was directly measured using a ruler. The total number of pods was calculated as the number of all pods on a single plant. A qualified pod was defined as a fully filled green pod without shape abnormalities. The qualification rate was calculated as the number of total pods divided by the number of qualified pods. Fresh and dry weights of the pods of each cultivar were measured after harvesting. The shelling percentage was obtained by dividing the total pod weight by the total seed weight on a single plant.

### *2.4. Quantitative Reverse-Transcription Polymerase Chain Reaction*

The frozen leaf material was ground and homogenized with liquid nitrogen at a low temperature. The total RNA was extracted with Tri-reagent (Invitrogen, Waltham, MA, USA), per the manufacturer's instructions. The quality and concentration of extracted RNA were analyzed with a Nanodrop Lite Spectrophotometer. DNase digestion was performed with 1 μL of TURBO$^{\mathrm{TM}}$ DNase (2 U/μL; Invitrogen, USA) and 30 μL of 1× DNase buffer (diluted from 10× buffer with DEPC water) per sample at 37 °C for 30 min after the total RNA extraction. The extracted total RNA was then reverse transcribed using the M-MLV first-strand synthesis kit (Invitrogen, Waltham, MA, USA) to obtain cDNA. Sequences and the primer set of genes of interest were obtained from the NCBI database. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was conducted using the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) with KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, Wilmington, MA, USA). Each reaction was run in a 10 μL mixture of 3 μL of cDNA equal to 600 ng, 5 μL of 2× power SYBR mix, 0.4 μL of each primer diluted to a final concentration of 400 nM, and 1.2 μL of $ddH_2O$. Thermal cycle used for all qRT-PCR reactions was 3 min at 95 °C followed by 45 cycles of 95 °C for 3 s and 53 °C for 20 s. The GmFBOX gene was used as the housekeeping gene. Bio-Rad software was used to calculate the transcript levels with the $2^{-\Delta\Delta ct}$ method; the average values of at least three independent replicates were obtained. Primer sequences used in this experiment are listed in Table S1.

### *2.5. Statistical Analysis*

All statistical analyses were conducted using SAS. The least significant difference (LSD) post hoc test was used to compare the groups. Values represent mean ± standard deviation of at least three biologically independent experiments. Different letters indicate significant differences between cultivars ($p < 0.05$).

## **3. Results**

### *3.1. Phenotypes and Growth Rates of the Edamame Cultivars at Different Developmental Stages*

To investigate the phenotype differences between the three edamame cultivars, we planted seeds of XJ, KH9, and KH11 in plastid chambers at the same time and observed the phenotypes and growth rates of these cultivars at different growth stages (Figure 1). These cultivars exhibited different growth development speed. XJ had the fastest growth stage development among the cultivars. At day 40 of planting, XJ entered the R2 stage with obvious fruit pods, whereas KH9 and KH11 were still in the R1 flowering stage. On day 80, XJ reached the R6 harvest period, with the leaves beginning to wither and the pods being fully filled, whereas KH9 and KH11 were in the R5 stage. On day 95, XJ entered the R8 maturity stage; the entire plant was dry and withered, and the dehydrated pods turned dark brown. By contrast, KH9 and KH11 were still in the R6 harvest stage. KH9 had the longest growing period of the three cultivars. When XJ and KH11 entered the R4 stage of developing pods, KH9 was still in the R1 flowering stage. At the R8 stage, XJ had dried up and withered, most of the leaves of KH11 had also yellowed and fallen, but only a few leaves of KH9 had begun to wither.

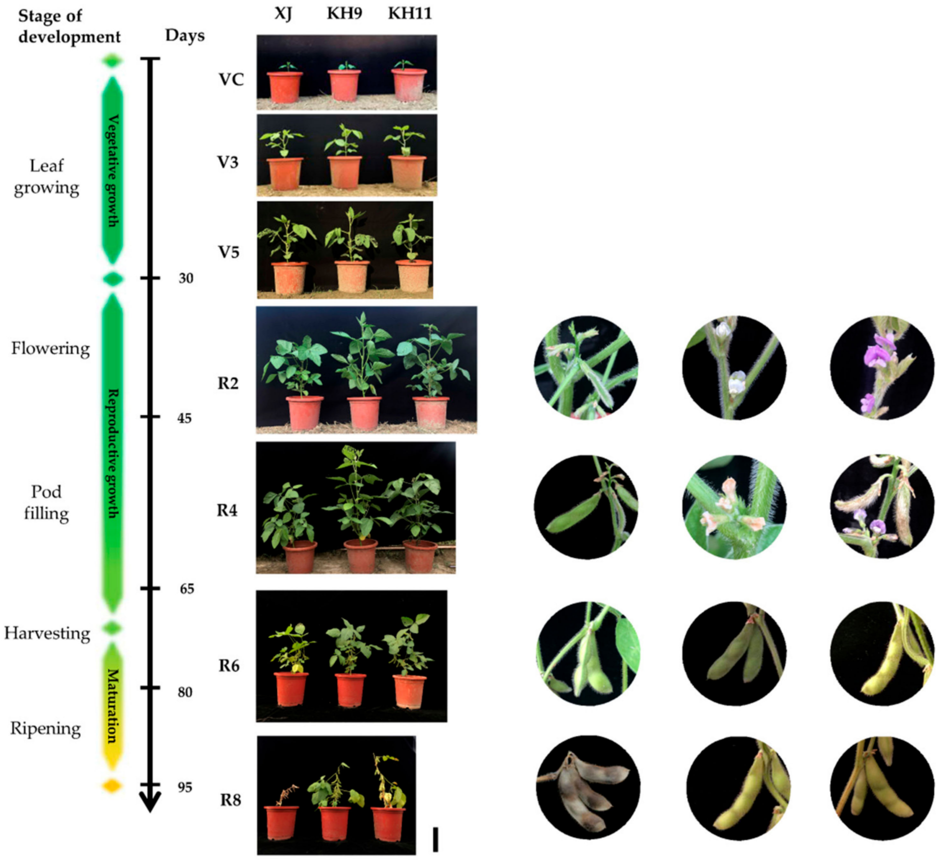

**Figure 1.** Phenotypes of XJ, KH9, and KH11 edamame cultivars during the VC to R8 growth stages. Photos of flowers and pods were taken when the plants entered the reproductive growth stage. Growth rate can be compared between cultivars according to the stage of development on a given planting day. Bar = 10 cm. XJ, Xiang-Ji. KH9, Kaohsiung No. 9. KH11, Kaohsiung No. 11. VC, vegetative cotyledon stage. V3, V5, vegetative stages 3, 5. R2–R8, reproductive stages 2–8.

We also measured the plant height of the edamame cultivars in the field for 2 years. XJ was the shortest and KH11 was the tallest. XJ showed the shortest plant height among three cultivars in almost every growth stage in 2017 and 2018. The plant height of KH11 was higher than that of KH9 in almost all of the growth stages (Figure 2).

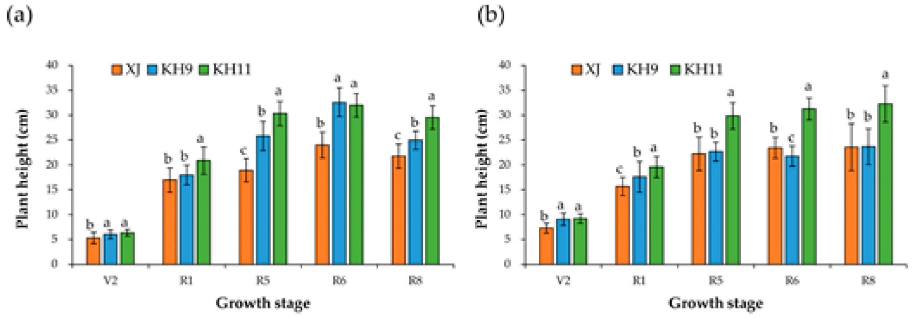

**Figure 2.** Plant heights of edamame cultivars at different growth stages in 2017 (**a**) and 2018 (**b**). Values represent the mean ± SD of at least three biologically independent experiments (N $\geq$ 3, $n$ = 16). Different letters indicate significant differences between cultivars assessed using the LSD post hoc test ($p < 0.05$). XJ, Xiang-Ji. KH9, Kaohsiung No. 9. KH11, Kaohsiung No. 11. V2, vegetative stage 2. R1–R8, reproductive stages 1–8.

### *3.2. Pod Seed Numbers and Yields Were Different in the Three Cultivars*

Comparison of the appearance of the pods indicated that XJ had the shortest pods followed by KH11 and KH9. XJ and KH11 pods were linear, whereas KH9 pods were slightly curved (Figure 3a). Generally, a pod of edamame has 1–3 seeds. The proportion of two-seed pods was the same among these cultivars—approximately 68% of all pods. In XJ, the proportion of one-seed pods (19%) was significantly higher than that of three-seed pods (12%), whereas in KH9, one-seed pods accounted for 12% and three-seed pods accounted for 19% of pods. The proportion of one-seed pods and three-seed pods had no significant difference in KH11 (Figure 3b).

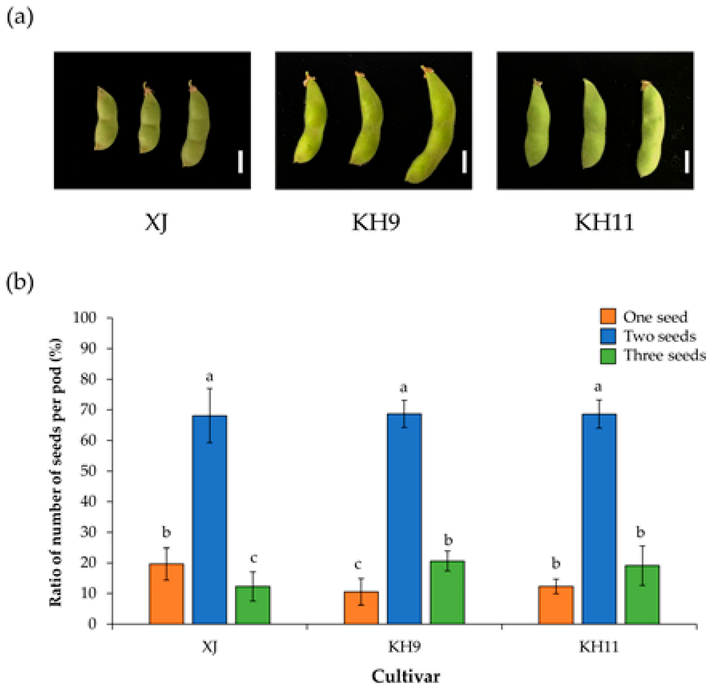

**Figure 3.** Pod phenotypes and the ratio of the number of seeds per pod of XJ, KH9, and KH11. (**a**) Phenotypes of pods of XJ, KH9, and KH11. (**b**) Number of seeds per pod of XJ, KH9, and KH11. Bar = 1 cm. Values represent the mean ± SD of three biologically independent experiments. Different letters indicate significant differences between cultivars assessed using the LSD post hoc test ($p < 0.05$). XJ, Xiang-Ji. KH9, Kaohsiung No. 9. KH11, Kaohsiung No. 11.

We collected the yield data of XJ, KH9, and KH11 grown in the field between 2017 and 2018 and compared the differences in the yield composition of these three cultivars

(Table 1). The results revealed that KH11 had the highest total number of pods. The qualified rate was defined as the number of qualified pods divided by the total number of pods. No difference in the qualified rate of these three cultivars was noted in 2017, but the qualified rate of XJ was significantly lower than that of the other two cultivars in 2018. The shelling percentage was defined as the seed weight per plant divided by the pod weight per plant; no significant difference was observed in the shelling percentage among the three cultivars. The dry weights of all pods and of qualified pods were higher in KH9 and KH11 than in XJ. These results indicated that the yields of edamame varied among these cultivars.

**Table 1.** Yield component data of XJ, KH9, and KH11 in the field.

| Year | Cultivar | Total Number of Pods | Qualified Rate (%) | Shelling Percentage (%) | Total Pods Dry Weight (g/plant) | Qualified Pod Dry Weight (g/plant) |
|---|---|---|---|---|---|---|
| **2017** | XJ | 26.20 ± 3.97 $^{b}$ | 59.69 ± 9.61 $^{a}$ | 56.15 ± 8.28 $^{a}$ | 16.70 ± 4.95 $^{b}$ | 12.20 ± 1.86 $^{b}$ |
| | KH9 | 26.06 ± 4.97 $^{b}$ | 53.63 ± 12.64 $^{a}$ | 54.37 ± 5.41 $^{a}$ | 25.16 ± 3.71 $^{a}$ | 14.66 ± 4.67 $^{a}$ |
| | KH11 | 32.06 ± 5.04 $^{a}$ | 50.14 ± 10.08 $^{a}$ | 58.87 ± 4.79 $^{a}$ | 22.62 ± 5.53 $^{a}$ | 16.14 ± 3.85 $^{a}$ |
| **2018** | XJ | 31.06 ± 6.26 $^{a}$ | 49.87 ± 8.27 $^{b}$ | 49.42 ± 7.34 $^{a}$ | 18.21 ± 2.07 $^{b}$ | 10.32 ± 2.87 $^{b}$ |
| | KH9 | 25.88 ± 6.82 $^{b}$ | 60.10 ± 11.57 $^{a}$ | 48.73 ± 12.29 $^{a}$ | 24.76 ± 4.88 $^{a}$ | 14.77 ± 4.43 $^{a}$ |
| | KH11 | 32.13 ± 5.37 $^{a}$ | 58.61 ± 8.50 $^{a}$ | 50.19 ± 11.59 $^{a}$ | 23.94 ± 4.07 $^{a}$ | 15.18 ± 3.44 $^{a}$ |

Values represent the mean ± SD of at least three biologically independent experiments ($N \geq 3$, $n = 16$). Different letters indicate significant differences between cultivars within one year assessed using the LSD post hoc test ($p < 0.05$). XJ, Xiang-Ji. KH9, Kaohsiung No. 9. KH11, Kaohsiung No. 11.

### 3.3. Drought Stress Decreased the Yield in Edamame

To investigate whether drought stress tolerance varied among cultivars, we planted XJ, KH9, and KH11 in growing pots at the same time. When the edamame reached the R1 stage, the plants were subjected to no irrigation for 6 consecutive days. The phenotypes of the control and experiment groups were recorded before and after the drought stress treatment (Figure 4a). The results revealed that all three cultivars exhibited severe wilting after 6 days of drought treatment, indicating that each cultivar was under severe drought stress, with no significant difference between cultivars. The soil water content and plant water content data of the control and drought groups indicate that the stress intensity was the same in each cultivar (Supplementary Figure S1).

To further investigate the effects of drought stress on the yield of different edamame cultivars, daily irrigation was resumed after 6 days. No significant difference was observed between the control and drought treatment groups in the total number of pods, indicating that drought did not have a significant effect on the total number of pods (Figure 4b). No significant difference was observed in the qualification rate and the total pod dry weight of these cultivars in the control group. Nevertheless, the qualification rate and the total pod dry weight were significantly lower in the drought-treated group than in the control group (Figure 4c,d), indicating that the yield of these three cultivars would be reduced by drought stress. The qualification rates of XJ, KH9, and KH11 in the control group were 71%, 60%, and 63%, respectively, and in the drought treatment group, the rates were 48%, 27%, and 42%, respectively. The qualified pod dry weight of XJ, KH9, and KH11 was 7.3, 10.1, and 8.1 g, respectively, in CK and 5, 3.9, and 4.5 g in the drought group, respectively. According to these results, KH9 was the cultivar most affected by drought stress among these three cultivars.

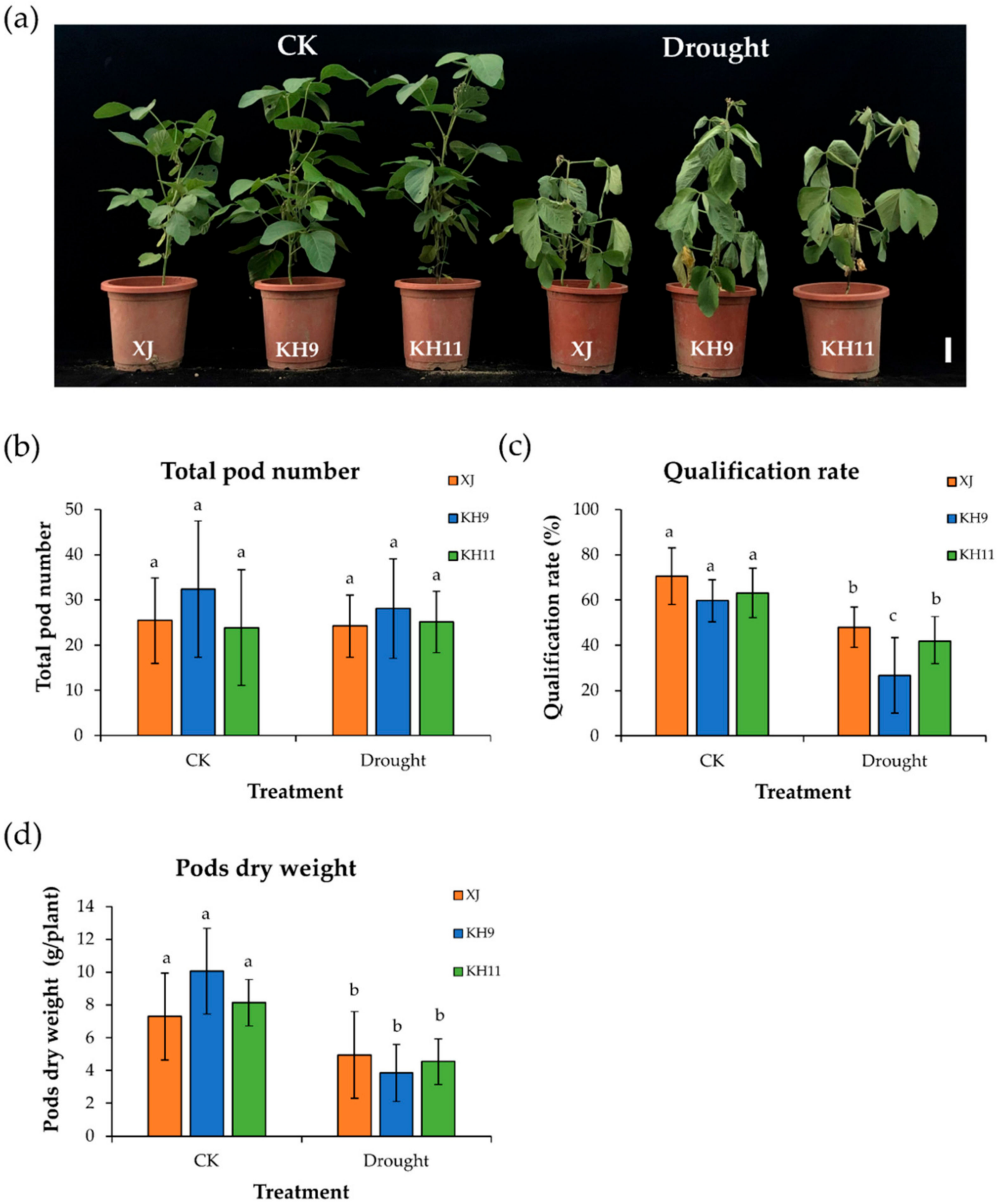

**Figure 4.** Drought affected the morphological appearances and the yields of XJ, KH9, and KH11. (**a**) Phenotypes of XJ, KH9, and KH11 under normal and drought conditions. (**b**) Total number of pods, (**c**) qualified rate, and (**d**) pods dry weight of XJ, KH9, and KH11 under normal and drought conditions. Bar = 5 cm. Values represent the mean ± SD of three biologically independent experiments. Different letters indicate significant differences between cultivars assessed using the LSD post hoc test ($p < 0.05$). XJ, Xiang-Ji. KH9, Kaohsiung No. 9. KH11, Kaohsiung No. 11. CK, control check.

### *3.4. Effect of Drought Stress on the Expression Level of Genes Involved in ABA Biosynthesis and the ABA Signaling Pathway*

When plants encounter abiotic stress, they trigger hormones biosynthesis or signaling transduction pathways, which help plants to survive the stress condition. ABA has been reported to be strongly related to plant drought stress. To investigate the regulation of drought stress and ABA-related genes in these three cultivars, we used qRT-PCR to analyze the ABA biosynthesis pathway-related genes and drought stress-related signal genes of these edamame cultivars (Figure 5). The 9-*cis*-epoxycarotenoid dioxygenase (NCED) and the abscisic aldehyde oxidase 3 (AAO3) genes encode enzymes that catalyze steps in the ABA biosynthesis pathway [28–30]. *GmNCED3* expression was significantly induced in

XJ and KH11 but not in KH9 by drought treatment, whereas *GmAAO3* expression was significantly upregulated only in KH9. *GmERD1*, *GmRD29A*, *GmRD22*, and *GmRD20A* are downstream signaling genes regulated by drought stress [31,32]. Among them, *GmRD22* and *GmRD20A* belong to the ABA-dependent pathway and *GmERD1* and *GmRD29A* belong to the ABA-independent pathway. The transcript levels of *GmRD22*, *GmRD20A*, *GmERD1*, and *GmRD29A* were significantly upregulated by drought stress in KH11 but did not significantly changed in XJ and KH9. Our results revealed that the molecular regulation of these ABA-dependent and ABA-independent genes varied in XJ, KH9, and KH11 under drought conditions.

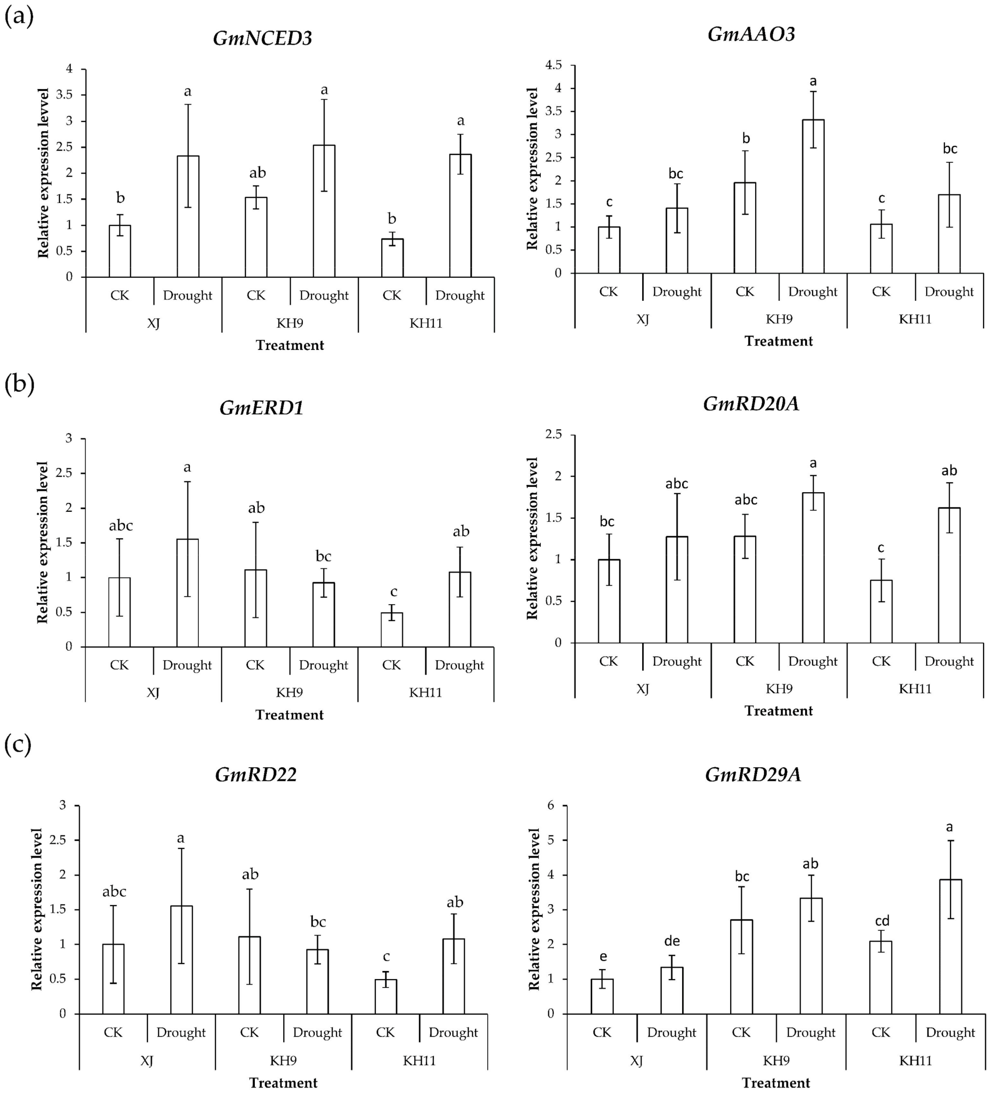

**Figure 5.** Transcript levels of drought-related genes in XJ, KH9, and KH11 under normal and drought stress conditions. Expression levels of genes in the (**a**) ABA biosynthesis pathway, (**b**) ABA-dependent pathways, and (**c**) ABA-independent pathways under drought stress. Values represent the mean ± SD of three biologically independent experiments. Different letters indicate significant differences between cultivars assessed using the LSD post hoc test ($p < 0.05$). XJ, Xiang-Ji. KH9, Kaohsiung No. 9. KH11, Kaohsiung No. 11. CK, control check. AAO, abscisic aldehyde oxidase. ERD, early responsive to dehydration. NCED, 9′-*cis*-epoxycarotenoid dioxygenase. RD, responsive to dehydration.

## 4. Discussion

XJ, KH9, and KH11 are the most popular edamame cultivars in Taiwan. KH9 is bred by Taiwan's Kaohsiung Agricultural Research and Extension Station. It is currently the main edamame cultivar exported from Taiwan [33]. KH9 is also widely planted, with high yield and good taste. XJ originated from Japan. XJ pods have a unique taro aroma and a short growing period. KH11 is also bred by the Kaohsiung Agricultural Research and Extension Station in Taiwan by using the XJ cultivar and local cultivar KVS1329 as parent plants. KH11 is a cultivar with the same taro aroma of XJ and good characteristics such as high yield and suitability for machine harvesting [34]. For the cultivar phenotypes at different growth stages (Figure 1), their growth rates were different. XJ had the shortest growth period; it took approximately 65 days to be harvestable, whereas KH9 and KH11 took roughly 71 and 75 days, respectively. However, XJ had the lowest total pod dry weight and qualified pod dry weight among the cultivars in our 2-year yield components data (Table 1), suggesting that the yields may be positively correlated with the growth duration [35]. XJ was also the shortest cultivar among the three cultivars at every growth stage, which meant that it might have better lodging resistance than the other two cultivars.

Sufficient water supply is critical for the growth and development of edamame, especially in the reproductive stage [36]. Numerous studies have shown that drought stress would significantly decrease the yield of soybean [37–39]. According to our results in drought treatment, the phenotypes of these three cultivars were all obviously affected by the drought stress, causing severe leaf wilting and withering. The decreases on the qualified rate and the qualified pod dry weight rather than on the total number of pods in these three cultivars by drought stress may imply that drought stress reduced the pod filling efficiency rather than changed the number of pods in these cultivars (Figure 4). Drought condition during pod filling would reduce seed size but had no significant effect on pod set, which is consistent with our result [40]. Besides drought stress, cultivar is also significant regarding plant height, fertile pod, seeds per plant, seeds per pod and the protein and oil content of the seeds [41]. The three cultivars in our experiment showed different severity on the yield affected by drought. When comparing control group and drought treatment, the reduction in the qualification rate and the qualified pod dry weight by drought stress in XJ, KH9 and KH11 was 23%, 33%, 21% and 32%, 62%, and 44%, respectively (Figure 4). Compared with other cultivars, the yield of KH9 was significantly reduced due to drought stress, indicating that its drought tolerance may be the lowest.

ABA is synthesized from β-carotene through several enzymatic steps. 9′-*cis*-epoxycarotenoid dioxygenase (NCED) catalyzes the oxidative cleavage of 9′-*cis*-neoxanthin to xanthoxin in the ABA biosynthesis pathway, and abscisic aldehyde oxidase (AAO) is responsible for the conversion of ABA-aldehyde to ABA. The expression level of *GmNCED3* was significantly upregulated by drought in XJ and KH11, whereas the expression of *GmAAO3* was significantly upregulated by drought only in KH9 (Figure 5a), indicating that different cultivars had varying regulatory mechanisms in the regulation of drought-stimulated ABA biosynthesis. Drought-induced decrease in photosynthetic rate and the increase in ABA content is significant in inducing pod abortion [42]. In our result, it can be inferred that KH9 with higher *GmAAO3* expression may have higher ABA content, which can disrupt the carbohydrate metabolism and reduce the formation of pod sets. This might explain why KH9 yield decreased significantly after drought.

Among the drought-related downstream signaling genes, the transcript levels of *GmRD22* and *GmRD20A* in the ABA-dependent pathway and *GmERD1* and *GmRD29A* in the ABA-independent pathway between XJ, KH9, and KH11 were compared after drought treatment. All genes were significantly upregulated by drought stress in KH11, whereas no significant regulatory patterns were observed in XJ and KH9 (Figure 5). This result implies that the signal regulation caused by drought was more sensitive in KH11 than in the other cultivars. This also explains why KH11 was less affected by drought than KH9 in terms of the qualified rate and qualified pod dry weight (Figure 4c,d). In the regulation results of *GmNCED3* and *GmAAO3*, XJ and KH11 exhibited the same regulation patterns, possibly due to the phylogenic relationship between XJ and KH11. *GmAAO3*, *GmERD1*, and *GmRD22* expression levels in KH11 were lower than those in KH9 under normal conditions (Figure 5). ABA-related drought signaling may be considerably vigorous in KH9 under normal conditions, but the expression patterns of these genes were much higher in KH11 due to drought, indicating that the signaling to drought may be more sensitive in KH11 than in other cultivars.

On the basis of these gene expression findings, we made a diagram of the regulation of drought-related signaling pathways in edamame (Figure 6). When edamame encounters drought stress, it stimulates ABA biosynthesis, increasing *GmNCED3* and *GmAAO3* expression in edamame and also increasing the transcript level of related transcription factors such as *GmRD20A* and *GmRD22* in the ABA-dependent pathway; however, drought stress also stimulates the expression of transcription factors such as *GmERD1* and *GmRD29A* in the ABA-independent pathway to improve the drought tolerance ability of edamame.

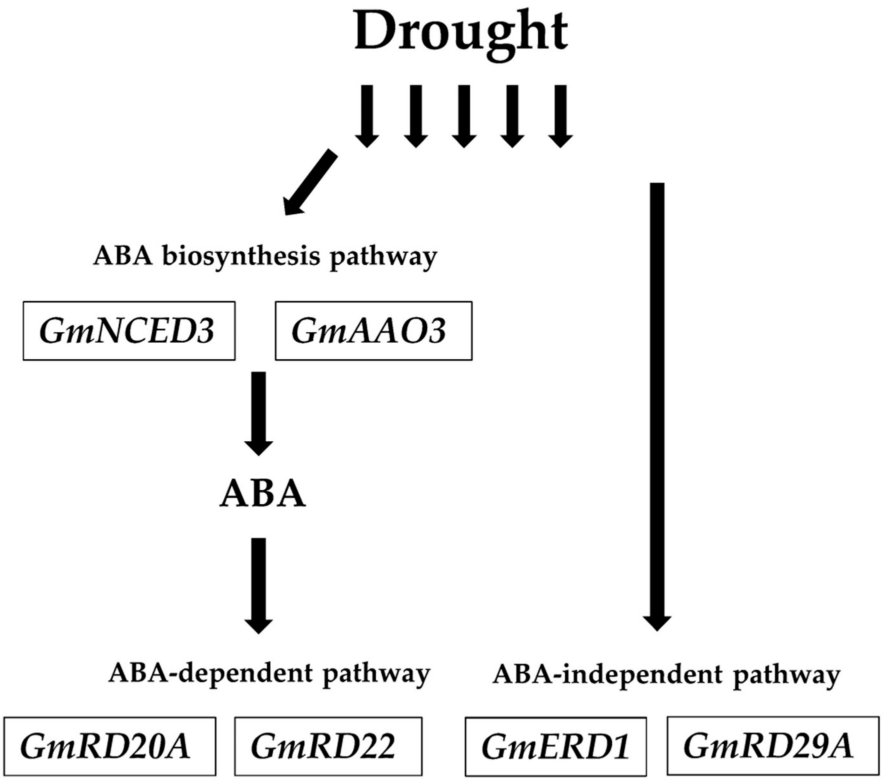

**Figure 6.** Regulation of genes in ABA-dependent and ABA-independent pathways in response to drought stress in edamame. Different cultivars exhibited different expression patterns in these drought-related genes. Genes in both ABA-dependent and ABA-independent pathways were significantly upregulated in KH11 by drought stress. AAO, abscisic aldehyde oxidase. ERD, early responsive to dehydration. NCED, 9′-*cis*-epoxycarotenoid dioxygenase. RD, responsive to dehydration.

Taken together, our results indicated that XJ and KH11 exhibited similar patterns in yield data and that they were less affected by drought stress than KH9, probably because they are close relatives. Under normal conditions, KH9 and KH11 produce higher yields than XJ; however, under drought conditions, KH11 might be a good choice for farmers. The characteristics of cultivars varied considerably. Our data can guide farmers in their planting considerations, and our results on ABA signaling and drought tolerance can also provide basic information for further research by scientists or breeders.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy11050939/s1, Figure S1: Detection of the soil water content and plant water content, Table S1: Primers used for qRT-PCR.

**Author Contributions:** C.-Y.Y. conceived and planned the experiments. C.-T.C. carried out the experiments. C.-T.C. collected the data. C.-Y.Y. and C.-T.C. wrote the manuscript. C.-T.L., J.T.C.T. and C.-Y.Y. supervised the project. All authors have read and agreed to the published version of the manuscript.

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