A Sweetpotato Auxin Response Factor Gene (IbARF5) Is Involved in Carotenoid Biosynthesis and Salt and Drought Tolerance in Transgenic Arabidopsis

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Auxin response factors (ARFs) constitute a family of transcription factors and have been found to play major roles in the process of plant growth and development. However, their roles in plant carotenoid biosynthesis and responses to abiotic stresses are rarely known to date. In the present study, we found that the IbARF5 gene from sweetpotato (Ipomoea batatas (L.) Lam.) line HVB-3 increased the contents of carotenoids and enhanced the tolerance to salt and drought in transgenic Arabidopsis. The transgenic Arabidopsis plants exhibited the increased abscisic acid (ABA) and proline contents and superoxide dismutase (SOD) activity and the decreased H$_2$O$_2$ content. Furthermore, it was found that IbARF5 positively regulated the genes associated with carotenoid and ABA biosynthesis and abiotic stress responses. These results suggest that IbARF5 is involved in carotenoid biosynthesis and salt and drought tolerance in transgenic Arabidopsis. This study provides a novel ARF gene for improving carotenoid contents and salt and drought tolerance of sweetpotato and other plants.

Keywords: sweetpotato, IbARF5, Arabidopsis, carotenoid content, salt and drought tolerance

INTRODUCTION

In nature, more than 750 kinds of carotenoids are characterized structurally, which are widely found in bacteria, fungi, algae, and plants (Hirschberg, 2001; Takaichi, 2011). The biosynthesis pathway of carotenoids has been extensively studied in plants, and nearly all of the key genes have been isolated and characterized (Cunningham and Gantt, 1998; Fraser and Bramley, 2004; Colasuonno et al., 2017; Kang et al., 2018). Abiotic stresses, especially salt and drought, seriously affect the productivity and cultivation expansion of crop plants worldwide, accordingly, to develop their high tolerance to salt and drought is highly desirable (Zhu, 2002; Lindemose et al., 2013; Zhai et al., 2016; Li et al., 2017). As the precursor of abscisic acid (ABA), carotenoids have functional roles in development and environmental adaptation of plants (Schwartz et al., 2003; Nambara and Marion-Poll, 2005; Mehrotra et al., 2014; Li, 2015; Moreno et al., 2016). Thus, increasing the contents of carotenoids helps to enhance the adaptation of plants to harsh environments.

Auxin response factors (ARFs) constitute a family of plant specific transcription factors. A typical ARF protein contains a B3-DNA binding domain in the highly conserved N-terminal
region (Ulmasov et al., 1997; Hagen and Guilfoyle, 2002; Mei et al., 2018). ARFs mediate responses to auxin and have been shown to be implicated in senescence (Ellis et al., 2005), hormone signaling (Li et al., 2006) and developmental programs (Krokan et al., 2012). In rice, OsARF1 was auxin-regulated and classified as a primary auxin responsive gene (Waller et al., 2002). In Arabidopsis, ARF2 mediated ABA response (Wang et al., 2011); MP/ARF5 regulated embryo and flower patterning and vascular differentiation (Hardtke and Berleth, 1998; Krokan et al., 2012); ARF6 and ARF8 promoted jasmonic acid production and flower maturation (Nagpal et al., 2005); NPH4/ARF7 and ARF19 controlled leaf expansion and lateral root growth (Okushima et al., 2005; Wilmoth et al., 2005). In tomato, SIARF2 regulated lateral root formation and flower senescence (Ren et al., 2017); ARF4 controlled sugar metabolism (Sagar et al., 2013); ARF10 increased chlorophyll and sugar accumulation during fruit development (Mei et al., 2018); ARF5 regulated fruit set and development (Liu et al., 2018). However, the roles of ARFs in plant carotenoid biosynthesis and abiotic stress responses are rarely known to date.

Sweetpotato (Ipomoea batatas (L.) Lam.) is an important food crop worldwide, which provides rich carbohydrates and carotenoids for human consumption (Teow et al., 2007; Zhai et al., 2016). This crop can also be used for bioenergy production on marginal lands due to its high adaption to harsh environments (Liu et al., 2014). Sweetpotato breeders are focusing on improving carotenoid contents and abiotic stresses tolerance of this crop. Kang et al. (2017) summarized the improvement of carotenoids by gene engineering in sweetpotato. Overexpression of the genes related to carotenoid biosynthesis have been shown to increase the contents of carotenoids and enhance the tolerance to abiotic stresses in sweetpotato (Kim et al., 2012, Kim et al., 2013b; Yu et al., 2013; Kim et al., 2014; Li et al., 2017; Kang et al., 2018). To date, ARFs have not been reported in sweetpotato. In this study, we found that the IbARF5 gene from storage roots of sweetpotato is involved in carotenoid biosynthesis and salt and drought tolerance in transgenic Arabidopsis.

**MATERIALS AND METHODS**

**Plant Materials**

Sweetpotato line HVB-3 with high carotenoid content was employed to clone the IbARF5 gene in this study. The expressed sequence tag (EST) for IbARF5 was obtained from the transcriptome data of HVB-3 developed by Li et al. (2015). Arabidopsis wild type (Columbia-0, WT) was used for characterizing the IbARF5 gene.

**Isolation and Sequence Analysis of IbARF5**

Total RNA was extracted from freshly harvested storage roots of HVB-3 and transcribed into first-strand cDNA according to the method of Kang et al. (2018). The full-length cDNA of

![Phylogenetic tree of ARF5 in different plant species (A) and comparison of exon and intron constituents between IbARF5 and NtARF5 (B). Exons are represented by boxes and introns by lines.](image-url)
FIGURE 2 | Subcellular localization of IbARF5 in onion leaf hypodermal cells. Confocal scanning microscopic images show localizations of IbARF5-GFP fusion proteins to nuclei in the right column vs. GFP as control in the left column. Bars = 20 µm.

FIGURE 3 | Transactivation activity of IbARF5 in the yeast. (A) the pBD-GAL4 vector as positive control; pBD-IbARF5; pBD-IbARF5-1; pBD-IbARF5-2; pBD-IbARF5-3; the empty pBD vector as negative control. The culture solution of the transformed yeast was drawn onto SD plate without tryptophan and histidine. (B) Different portions of IbARF5.
IbARF5 was amplified with specific primers (Supplementary Table S1) by rapid amplification of cDNA ends (RACE) method. Genomic DNA isolated from in vitro-grown plants of HVB-3 was used to amplify the genomic sequence of IbARF5. The IbARF5 cDNA was analyzed by an online BLAST. The open-reading frame (ORF) Finder was used to predict the ORF of IbARF5. The DNAMAN software was applied to align the amino acid sequence of IbARF5 with those of ARF proteins from different plant species. The MEGA 7.0 software was employed to conduct the phylogenetic analysis with the neighbor-joining (NJ) method. Exon–intron structure was constructed using Splign tool. The molecular weight and theoretical isoelectric point (pI) of IbARF5 were calculated at http://web.expasy.org/compute_pi.

Subcellular Localization of IbARF5

The IbARF5 ORF amplified with specific primers (Supplementary Table S1) was ligated into pMDC83. pMDC83-IbARF5-GFP and pMDC83-GFP (as control) were transiently expressed in the onion epidermal cells with a GeneGun (HeliosTM, Biorad, United States). After co-cultivation on Murashige and Skoog (MS) medium (pH 5.8) at 28°C for 24 h, the onion cells were examined under a laser scanning confocal fluorescence microscope (Nikon Inc., Melville, NY, United States).

Transactivation Activity Assay of IbARF5 in Yeast

Transactivation activity of IbARF5 in yeast (Saccharomyces cerevisiae) was assayed as described by Jiang et al. (2014). The corresponding regions of IbARF5 were PCR-amplified using specific primers (Supplementary Table S1) and integrated into the yeast expression vector pGBKT7 (pBD). Expression vectors pBD-IbARF5, pBD-IbARF5-1, pBD-IbARF5-2, pBD-IbARF5-3, pGAL4 (as positive vector), and pBD (as negative vector) were transferred into the yeast strain AH109, respectively. The transactivation activity was determined as described in the yeast protocols handbook (PT3024-1; Clontech, Mountain View, CA, United States).

Expression Analysis of IbARF5 in Sweetpotato

Total RNA was isolated from storage root, stem, and leaf tissues of the 100-day-old HVB-3 and used to analyze the expression of IbARF5 by quantitative real-time PCR (qRT-PCR) with its specific primers (Supplementary Table S1). Ibactin (AY905538) was served as an internal control. Comparative CT method was employed to quantify the gene expression (Schmittgen and Livak, 2008).

After cultured on MS medium for 4 weeks, the HVB-3 plants were treated in liquid MS media containing H2O (as control), 200 mM NaCl, 20% PEG6000 and 100 µM ABA, respectively, and sampled at 0, 2, 4, 6, 12, 24, and 48 h after treatment for analyzing the expression of IbARF5.

Production of the Transgenic Arabidopsis Plants

The overexpression vector pC3301-121-IbARF5 was constructed through inserting 35S-IbARF5-NOS into pCAMBIA3301. The recombinant vector was introduced into the Agrobacterium tumefaciens strain GV3101. The dipping flower method was applied to transform Arabidopsis and putatively transgenic Arabidopsis seeds were sown on MS medium with 12.5 mg L−1 phosphinothricin (PPT) for selecting transgenic plants. Histochemical GUS assay (Jefferson et al., 1987) and PCR analysis were used to identify the transgenic Arabidopsis plants. Transgenic Arabidopsis was planted in pots with a soil, vermiculite and humus mixture (1:1:1, v/v/v) to obtain T3 seeds.

Measurement of Carotenoid Contents

Leaves (2-week-old) and seeds of the transgenic Arabidopsis plants were applied to extract α-carotene, lutein, β-carotene, β-cryptoxanthin, and zeaxanthin. High performance liquid chromatography (HPLC) system was used to determine their contents (Li et al., 2017).

Assay for Salt and Drought Tolerance

One-week-old in vitro-grown seedlings of transgenic Arabidopsis and WT were treated on MS media with 200 mM NaCl and 300 mM mannitol, respectively. After 2 weeks, their root length and fresh weight (FW) were investigated. Furthermore, the transgenic and WT seedlings were planted for 2 weeks in pots with a soil, vermiculite and humus mixture (1:1:1, v/v/v) and subsequently irrigated with a 33 mL of 300 mM NaCl solution for each pot once every 2 days for 2 weeks, or stressed by drought for 4 weeks followed by 2 days re-watering. The transgenic plants and WT grown for 6 weeks under normal condition were used as control. The proline and H2O2 contents and superoxide dismutase (SOD) activity in the transgenic plants and WT grown in pots for 4 weeks under normal condition, 1 week under 300 mM NaCl stress after 2 weeks of normal treatment,
and 2 weeks under drought stress after 2 weeks of normal treatment, respectively, were determined with Assay Kits (Comin Biotechnology Co., Ltd. Suzhou, China). The ABA content was measured as described by Gao et al. (2011). Twenty-seven plants in three pots with nine plants per pot were treated for each line.

For ABA sensitivity assay, the transgenic and WT seeds were sown on MS media with 0, 0.5, and 1 µM ABA, respectively. After 1 week, their germination and cotyledon opening and greening rates were investigated. Fifty seeds of each line on a plate were analyzed.

Expression Analysis of the Related Genes

Leaves (2-week-old) and seeds of the transgenic Arabidopsis plants and WT were applied to analyze the expression of the key genes in carotenoid biosynthesis. The leaves of the transgenic plants and WT potted for 4 weeks under normal condition, 1 week under 300 mM NaCl stress after 2 weeks of normal treatment, and 2 weeks under drought stress after 2 weeks of normal treatment, respectively, were used for analyzing the expression of the genes associated with ABA biosynthesis and abiotic stress responses. The specific primers of Atactin

![Graph showing expression analysis of IbARF5](image)

**Figure 5**: Expression analysis of IbARF5 in the in vitro-grown plants of HVB-3 after different times (h) in response to H2O, 200 mM NaCl, 20% PEG6000 and 100 µM ABA, respectively. Data are presented as means ± SE (n = 3). * and ** indicate a significant difference from that of WT at P < 0.05 and P < 0.01, respectively, by Student’s t-test.

**Table 1**: Carotenoid contents in leaves of the IbARF5-overexpressing Arabidopsis plants.

| Lines | α-carotene (μg g⁻¹ FW) | Lutein (μg g⁻¹ FW) | β-carotene (μg g⁻¹ FW) | β-cryptoxanthin (μg g⁻¹ FW) | Zeaxanthin (μg g⁻¹ FW) | Total (μg g⁻¹ FW) |
|-------|-------------------------|------------------|------------------------|-----------------------------|------------------------|------------------|
| WT    | 0.149 ± 0.005           | 13.275 ± 0.581   | 7.601 ± 0.467          | n.d.                        | 0.044 ± 0.010          | 21.070 ± 1.020   |
| L1    | 0.166 ± 0.011           | 17.475 ± 0.694** | 8.751 ± 0.552          | n.d.                        | 0.119 ± 0.007**        | 24.511 ± 1.038** |
| L4    | 0.151 ± 0.004           | 16.750 ± 1.221** | 8.649 ± 0.330          | n.d.                        | 0.147 ± 0.016**        | 23.896 ± 0.949*  |
| L5    | 0.145 ± 0.006           | 16.775 ± 0.315** | 6.657 ± 0.755          | n.d.                        | 0.147 ± 0.004**        | 23.993 ± 0.854*  |
| L6    | 0.153 ± 0.007           | 17.532 ± 1.210** | 6.717 ± 0.111          | n.d.                        | 0.126 ± 0.009**        | 24.529 ± 1.109** |

Leaves from 2-week-old Arabidopsis plants were sampled for the quantification of carotenoids. FW, fresh weight; n.d., not detectable. Data are presented as mean ± SE (n = 3). * and ** indicate a significant difference from that of WT at P < 0.05 and P < 0.01, respectively, by Student’s t-test.

**Table 2**: Carotenoid contents in seeds of the IbARF5-overexpressing Arabidopsis plants.

| Lines | α-carotene (μg g⁻¹ DW) | Lutein (μg g⁻¹ DW) | β-carotene (μg g⁻¹ DW) | β-cryptoxanthin (μg g⁻¹ DW) | Zeaxanthin (μg g⁻¹ DW) | Total (μg g⁻¹ DW) |
|-------|------------------------|------------------|------------------------|-----------------------------|------------------------|------------------|
| WT    | n.d.                   | 1.470 ± 0.041    | 0.178 ± 0.003          | 0.020 ± 0.005               | 0.089 ± 0.009          | 1.755 ± 0.048    |
| L1    | n.d.                   | 1.907 ± 0.028**  | 0.270 ± 0.031*         | 0.023 ± 0.006               | 0.119 ± 0.010**        | 2.318 ± 0.051** |
| L4    | n.d.                   | 2.519 ± 0.207**  | 0.262 ± 0.035*         | 0.038 ± 0.006               | 0.153 ± 0.008**        | 2.972 ± 0.241** |
| L5    | n.d.                   | 2.625 ± 0.150**  | 0.309 ± 0.057**        | 0.046 ± 0.002*              | 0.143 ± 0.005**        | 3.123 ± 0.194** |
| L6    | n.d.                   | 2.303 ± 0.287**  | 0.396 ± 0.059**        | 0.052 ± 0.003**             | 0.144 ± 0.023**        | 2.885 ± 0.313** |

Seeds from Arabidopsis plants were harvested for the quantification of carotenoids. DW, dry weight; n.d., not detectable. Data are presented as mean ± SE (n = 3). * and ** indicate a significant difference from that of WT at P < 0.05 and P < 0.01, respectively, by Student’s t-test.
Is Involved in Carotenoid Biosynthesis (NM112764) as internal control and the related genes were listed in Supplementary Table S1.

**Statistical Analysis**
All experiments were conducted with three biological replicates. Data presented as the mean ± SE were analyzed with Student’s t-test (two-tailed analysis) at $P < 0.05$ and $P < 0.01$.

**RESULTS**

**Cloning and Sequence Analysis of IbARF5**
The 3757-bp cDNA of the IbARF5 gene contained a 2841-bp ORF encoding a 946-aa polypeptide with a molecular weight of 104.84 kDa and a predicted pI of 5.17. The IbARF5 protein shared a high sequence identity with ARF5 proteins in *Nicotiana tabacum* (XP_016465083.1, 74%), *Capsicum annuum* (XP_016568464.1, 72%), *Sesamum indicum* (XP_011083507.1, 72%), *Solanum lycopersicum* (XP_006342026.1, 72%) and *Solanum tuberosum* (XP_00634382.2, 68%). It contained one plant-specific B3-DNA binding domain, one Auxin_res and one AUX_IAA (Supplementary Figure S1). Phylogenetic analysis showed that IbARF5 had a close relationship with that of *N. tabacum* (Figure 1A). The 4794-bp genomic DNA of IbARF5 contained 13 exons and 12 introns (Figure 1B).

**IbARF5 Is Localized to Nuclei**
The images from onion epidermal cells indicated that the green fluorescence emitted by IbARF5-GFP was exclusively distributed over the nuclei of the cells (Figure 2). These results showed that IbARF5 was localized to nuclei.

**IbARF5 Shows Transactivation Activity in Yeast**
The yeast two-hybrid system was applied to identify a possible transactivation activity of IbARF5. The yeast cells harboring pBD-GAL4, pGBK7-IbARF5 and pGBK7-IbARF5-2, respectively, grew well on synthetic dropout (SD) plate without tryptophan and histidine and exhibited β-galactosidase activity, but the cells bearing pBD, pGBK7-IbARF5-1, and pGBK7-IbARF5-3, respectively, failed to grow (Figure 3A). These results demonstrated that IbARF5 might act as a transcription activator, and its transactivation activity was determined by the middle region, IbARF5-2 (Figure 3B).

**FIGURE 6** Responses of the transgenic Arabidopsis seedlings and WT cultured for 2 weeks on MS medium with 200 mM NaCl and 300 mM mannitol, respectively. Data are presented as mean ± SE ($n = 3$). ** indicates a significant difference from that of WT at $P < 0.01$ by Student’s t-test.
FIGURE 7 | Responses of the transgenic Arabidopsis plants and WT grown in pots under salt and drought stresses. (A) Phenotypes of transgenic plants vs. WT grown for 6 weeks under normal condition, 2 weeks under 300 mM NaCl stress after 2 weeks of normal treatment, and 4 weeks under drought stress followed by 2 days re-watering after 2 weeks of normal treatment, respectively. (B) ABA, proline and H$_2$O$_2$ contents and SOD activity in the transgenic plants and WT grown for 4 weeks under normal condition, 1 week under 300 mM NaCl stress after 2 weeks of normal treatment, and 2 weeks under drought stress after 2 weeks of normal treatment, respectively. Data are presented as mean ± SE (n = 3). ** indicates a significant difference from that of WT at $P < 0.01$ by Student’s t-test.
Expression Patterns of *IbARF5* in Sweetpotato

Quantitative real-time PCR analysis revealed that *IbARF5* exhibited higher expression level in the storage roots of HVB-3 than in its leaves and stems (Figure 4). Its expression in HVB-3 was strongly induced by NaCl, PEG6000 and ABA, and peaked (5.03-fold) at 4 h, (9.68-fold) at 24 h, and (12.18-fold) at 24 h, respectively (Figure 5).

Production of the Transgenic *Arabidopsis* Plants

Putatively transgenic *Arabidopsis* seeds formed the plants on MS medium with 12.5 mg L\(^{-1}\) PPT. GUS assay and PCR analysis confirmed that 6 of the randomly sampled 60 plants were transgenic plants, named L1, L2, . . . , L6, respectively, from which T\(_3\) were generated. *IbARF5* showed high expression levels in the transgenic *Arabidopsis* plants, especially L1, L4, L5, and L6 (Supplementary Figure S2).

*IbARF5* Increases Carotenoid Contents

The lutein and zeaxanthin contents were significantly increased, but \(\alpha\)-carotene and \(\beta\)-carotene contents were not changed and \(\beta\)-cryptoxanthin was not detected in leaves of L1, L4, L5, and L6 (Table 1). In seeds of these four transgenic plants, the lutein, \(\beta\)-carotene, and zeaxanthin contents were significantly increased, but \(\alpha\)-carotene was not detected and \(\beta\)-cryptoxanthin content was significantly increased only in L5 and L6 (Table 2). The total carotenoid contents in leaves and seeds were increased by 1.13–1.16 folds and 1.32–1.78 folds, respectively (Tables 1 and 2).

*IbARF5* Enhances Salt and Drought Tolerance

Four transgenic *Arabidopsis* plants, L1, L4, L5, and L6, and WT seedlings showed no significant differences in rooting and FW on MS medium without stresses (Figure 6). However, the transgenic plants exhibited good rooting and increased FW in contrast to WT on MS media with 200 mM NaCl and 300 mM mannitol, respectively (Figure 6).

The transgenic plants and WT grown in pots showed similar growth trends under normal conditions (Figure 7A). Under NaCl and drought stresses, the transgenic plants showed good growth, while WT almost died (Figure 7A). Furthermore, it was found that the ABA and proline contents were increased, SOD activity

![Image of experiment results](image-url)
IbARF5 was enhanced and H$_2$O$_2$ content was decreased in the transgenic plants (Figure 7B).

No obvious differences in seed germination were observed between the transgenic plants and WT under normal condition (Figure 8). Under the treatment with different ABA concentrations, the germination of the transgenic seeds was more sensitive to ABA-elicited inhibition than that of WT though both germination rate and cotyledon opening and greening rate of the transgenic and WT seeds declined (Figure 8). These results demonstrated that IbARF5 might participate in the ABA signaling pathway.

**IbARF5 Up-Regulates the Genes Involved in Carotenoid and ABA Biosynthesis and Abiotic Stress Responses**

The genes encoding the key enzymes in carotenoid biosynthesis, eranylgeranyl pyrophosphate (GGPS), $\zeta$-carotene desaturase (ZDS), phytoene synthase (PSY), $\varepsilon$-carotene hydroxylase ($\varepsilon$-CHY), $\beta$-lycopene cyclase ($\beta$-LCY) and $\beta$-carotene hydroxylase ($\beta$-CHY) except for phytoene desaturase (PDS) and $\varepsilon$-lycopene cyclase ($\varepsilon$-LCY) were systematically up-regulated in leaves of the transgenic *Arabidopsis* plants (Figure 9). GGPS, $\varepsilon$-CHY, $\beta$-LCY, and $\beta$-CHY exhibited the increased expression levels, but ZDS, PSY, and $\varepsilon$-LCY showed no changes in expression level and PDS was down-regulated in the transgenic seeds (Figure 9). Under NaCl and drought stresses, the genes encoding the key enzymes in ABA biosynthesis, zeaxanthin epoxidase (ZEP), 9-cis-epoxycarotenoid dioxygenase (NCED), and xanthoxin dehydrogenas (ABA2) were up-regulated, and abiotic stress-responsive genes encoding pyrroline-5-carboxylate synthase (P5CS), SOD, ascorbate peroxidase (APX), and dehydroascorbate reductase (DHAR) were also found to be up-regulated (Figure 10).

**DISCUSSION**

**IbARF5 Increases Carotenoid Contents and Salt and Drought Tolerance**

In plants, ARFs encode important transcription factors which regulate the expression of genes in response to auxin (Guilfoyle and Hagen, 2007). Several ARF transcription factor genes have
been cloned from Arabidopsis, rice and tomato, and were found to play crucial roles in plant growth and developmental processes (Harper et al., 2000; Waller et al., 2002; Ellis et al., 2005; Okushima et al., 2005; Wilmoth et al., 2005; Wang et al., 2011; Ren et al., 2017; Liu et al., 2018). However, there is no report on the ARF transcription factors in improving carotenoid contents and abiotic stress tolerance in plants. Previous studies demonstrated that AtARF5 affected lateral organ development, primary root initiation, flower primordium initiation, and cotyledon development in Arabidopsis (Krogan et al., 2012) and SlARF5 controlled fruit set and development in tomato (Liu et al., 2018). In the present study, the IbARF5 gene was isolated from sweetpotato line HVB-3 with high carotenoid content. We found that its overexpression significantly increased the content of carotenoids and enhanced the tolerance to salt and drought in transgenic Arabidopsis (Tables 1, 2 and Figures 6, 7).

![Figure 10](image-url)

**FIGURE 10** | Transcript levels of salt and drought responsive genes in leaves of transgenic Arabidopsis plants and WT pot-grown for 4 weeks under normal condition, 1 week under 300 mM NaCl stress after 2 weeks of normal treatment, and 2 weeks under drought stress after 2 weeks of normal treatment, respectively. Data are presented as mean ± SE (n = 3). ** indicates a significant difference from that of WT at P < 0.01 by Student’s t-test.
**IbARF5 Up-Regulates the Genes Involved in Carotenoid Biosynthesis**

It has been shown that carotenoid biosynthesis is mainly regulated at the transcript level of genes encoding the biosynthetic enzymes (Römer and Fraser, 2005; Sandmann et al., 2006; Li et al., 2017; Kang et al., 2018). In this study, we found that the key genes in carotenoid biosynthesis, GGPS, ZDS, PSY, ε-CHY, β-LCY, and β-CHY in leaves and GGPS, ε-CHY, β-LCY, and β-CHY in seeds of transgenic *Arabidopsis* were significantly up-regulated (Figure 9), which corresponded with the increased of carotenoid contents in transgenic *Arabidopsis* (Tables 1 and 2). These findings suggest that *IbARF5* positively controls the expression of carotenoid biosynthetic genes, which resulted in the increased carotenoid contents in transgenic *Arabidopsis*. Overexpression of the *Orange* gene (*IbOr*) from sweetpotato increased carotenoid accumulation and abiotic stress tolerance in transgenic sweetpotato, potato, and alfalfa (Kim et al., 2013a; Goo et al., 2015; Wang et al., 2015). Furthermore, it was proved that similar to AtOr of *Arabidopsis*, IbOr directly interacted with PSY and increased carotenoid accumulation (Park et al., 2016; Kim et al., 2018). Therefore, the precise underlying mechanisms of *IbARF5* in plant carotenoid accumulation need to be further investigated. In addition, we are developing the *IbARF5*-overexpressing sweetpotato plants for further analyzing its roles in carotenoid accumulation of the storage roots.

**IbARF5 Up-Regulates the Genes Involved in ABA Biosynthesis**

Carotenoids, especially β-branch carotenoids, serve as precursors for ABA biosynthesis and play a crucial role in plant tolerance and adaptation to abiotic stresses (Demmingadams and Adams, 2002; Xiong and Zhu, 2003; Sah et al., 2016). ABA regulates the expression of ABA-dependent stress-responsive genes and the increased level of ABA has been found to enhance the tolerance to salt and drought (Tuteja, 2007; Vishwakarma et al., 2017). Increased level of ABA has been found to enhance the tolerance expression of ABA-dependent stress-responsive genes and the 2002; Xiong and Zhu, 2003; Sah et al., 2016). ABA regulates the 2002; Xiong and Zhu, 2003; Sah et al., 2016). ABA regulates the 2002; Xiong and Zhu, 2003; Sah et al., 2016). ABA regulates the 

**CONCLUSION**

This study reveals, for the first time, that the *IbARF5* gene from sweetpotato is involved in carotenoid biosynthesis and salt and drought tolerance of plants. Its overexpression increased the contents of carotenoids and conferred the tolerance to salt and drought by up-regulating the key genes involved in carotenoid and ABA biosynthesis and abiotic stress responses in transgenic *Arabidopsis*. This study provides a novel ARF gene for improving carotenoid contents and salt and drought tolerance of sweetpotato and other plants.

**AUTHOR CONTRIBUTIONS**

QL and CK conceived and designed the experiments. CK and RL performed the experiments. QL, HZ, and NZ contributed reagents, materials, and analysis tools. QL and CK wrote the paper. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.01307/full#supplementary-material

**FIGURE S1** | Sequence alignment of *IbARF5* with its homologs from other plants. Characteristic regions of ARF5 are indicated above the *IbARF5* sequence. ←Plant-specific B3-DNA binding domain; ←Auxin_resp; ←ARF_JAA.

**FIGURE S2** | Expression analysis of *IbARF5* in the transgenic *Arabidopsis* plants. The *Arabidopsis* actin gene was used as an internal control. Data are presented as means ± SE (n = 3). ** indicates a significant difference from that of WT at P < 0.01 by Student’s t-test.

**TABLE S1** | Primers used in this study.
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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