

1-1-2021

Manipulation of lipophilic antioxidants to enhance oxidative stress tolerance and nutritional quality in transgenic sweetpotato

HOSOO KIM

RENGİN ÖZGÜR UZİLDAY

BARIŞ UZİLDAY

SOEUN KIM

CHANJU LEE

See next page for additional authors

Follow this and additional works at: <https://dctubitak.researchcommons.org/botany>

 Part of the [Botany Commons](#)

Recommended Citation

KIM, HOSOO; UZİLDAY, RENGİN ÖZGÜR; UZİLDAY, BARIŞ; KIM, SOEUN; LEE, CHANJU; PARK, SULLU; BIAN, XIAOFENG; XIE, YIZHI; and KWAK, SANG-SOO (2021) "Manipulation of lipophilic antioxidants to enhance oxidative stress tolerance and nutritional quality in transgenic sweetpotato," *Turkish Journal of Botany*: Vol. 45: No. 7, Article 3. <https://doi.org/10.3906/bot-2109-50>

Available at: <https://dctubitak.researchcommons.org/botany/vol45/iss7/3>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Botany by an authorized editor of TÜBİTAK Academic Journals.

Manipulation of lipophilic antioxidants to enhance oxidative stress tolerance and nutritional quality in transgenic sweetpotato

Authors

HOSOO KIM, RENGİN ÖZGÜR UZİLDAY, BARIŞ UZİLDAY, SOEUN KIM, CHANJU LEE, SULU PARK, XIAOFENG BIAN, YIZHI XIE, and SANG-SOO KWAK

Manipulation of lipophilic antioxidants to enhance oxidative stress tolerance and nutritional quality in transgenic sweetpotato

Ho Soo KIM¹, Rengin Ozgur UZILDAY², Baris UZILDAY², So-Eun KIM^{1,3},
Chan-Ju LEE^{1,3}, Sul-U PARK^{1,3}, Xiaofeng BIAN⁴, Yizhi XIE⁴, Sang-Soo KWAK^{1,3,*}

¹Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea

²Department of Biology, Faculty of Science, Ege University, Izmir, Turkey

³Department of Environmental Biotechnology, KRIBB School of Biotechnology, University of Science and Technology (UST), Republic of Korea

⁴Institute of Food Crops, Provincial Key Laboratory of Agrobiolgy, Jiangsu Academy of Agricultural Sciences, Jiangsu, China

Received: 30.09.2021 • Accepted/Published Online: 09.11.2021 • Final Version: 28.12.2021

Abstract: Overproduction of reactive oxygen species (ROS) in plant cells under environmental stress cause oxidative stress, which is one of major factors limiting the plant productivity. To prevent this limitation by stress conditions, a possible strategy is to strengthen the antioxidant defense of plants by gene manipulation of antioxidant enzymes and low molecular-weight (LMW) antioxidants. LMW antioxidants are important for the plant protection to environmental stress and also have nutritional merits for humans as antiaging and antidisease molecules. Sweetpotato [*Ipomoea batatas* (L.) Lam] as a nutritional food crop has many advantages in terms of the cultivation on the global marginal lands. Thus sweetpotato is considered as an emerging multifunctional food crop ensuring the food and nutrition security in the face of climate crisis, since it contains high levels of LMW antioxidants, minerals, and dietary fiber. This review describes the current status and prospects of metabolic engineering of two lipophilic antioxidants, carotenoids and tocopherols, in transgenic sweetpotato. In addition, the potentiality of an *orange* (Or) gene involved in enhanced tolerance to various abiotic stresses by high accumulation of carotenoids is introduced in detail. The rational metabolic engineering of LMW antioxidants can be applicable to all plant species to cope with oxidative stress in face of climate change and nutritional problems in aging society.

Key words: Antioxidants, carotenoids, climate change, *Ipomoea batatas*, oxidative stress, tocopherols, sweetpotato

1. Introduction

After industrial revolution, demands on food and energy supplies dramatically increased due to growth of global human population and industrialization. By 2050, The Food and Agriculture Organization (FAO) predicts a global population of 9.7 billion (FAO, 2018a) and, due to these expectations, global energy requirements will increase more than 3.5 fold while need for food supplies will exceed more than 1.7 folds as compared the current state. To cope with upcoming food and energy demand, new plant cultivars adapted to marginal lands with suboptimal environments such as drought, salinity, and pollution are desired for establishment of a sustainable future. For this purpose, biotechnological tools can be used to increase plant productivity by improving environmental stress tolerance (Kwak, 2018; Kim and Kwak, 2020a).

Reactive oxygen species (ROS) are by-products of aerobic life that are generated in all aerobic cells during metabolism (Asada, 1999). Excessive production and

accumulation of ROS leads to oxidative stress, and it is among the main detrimental factors for plant cells during various environmental stresses. Plants as a sessile organisms have a potent ROS-scavenging antioxidant mechanism including enzymatic antioxidants such as catalases, ascorbate peroxidases, other peroxidases, and superoxide dismutase. Additionally, plant cells utilize nonenzymatic antioxidants, also known as low molecular-weight (LMW) antioxidants, including ascorbate, glutathione, carotenoids, and tocopherols (Kwon et al., 2002). In particular, LMW antioxidants are important for both the plant protection against environmental stress and nutritional merits for human being as antiaging and antidisease agents. LMW antioxidants in plants can be divided into lipid-soluble antioxidants such as carotenoids and tocopherols (vitamin E) and water-soluble antioxidants (ascorbic acid, glutathione and polyphenols). Among LMW antioxidants, carotenoids and tocopherols play an important role to protect oxidative stress derived from the environmental

* Correspondence: sskwak@kribb.re.kr

stress in chloroplasts and cell membranes, respectively. In this respect, it is possible to fortify the plants by metabolic engineering of LMW antioxidants ensuring the global food and nutrition security.

Sweetpotato [*Ipomoea batatas* (L.) Lam] is the sixth most important starch crop in the world and a rich source of various nutrients (Kwak, 2019). Asian countries produce two of the three parts of the global sweetpotato, which is originated from tropical area (FAO, 2018b). Sweetpotato has high carbohydrate content and can easily be adapted to utilize marginal lands with a potential to be a tool for food and energy security. In addition, sweetpotato can be utilized as a bioreactor for manufacturing of distinct materials by molecular breeding and biotechnological tools. Sweetpotato is also characterized as one of the ten superfoods because of high levels of LMW antioxidants such as carotenoids, ascorbic acid, tocopherols, and various polyphenols in addition to minerals including potassium and dietary fiber (CSPI 2016). Moreover, higher water use efficiency of sweetpotato as compared to other starch crops is a crucial factor for its use in drought affected lands (Isabirye et al., 2007).

Sweetpotato has the highest carbohydrate content among starch crops such as cassava, corn, potato, and sugarbeet. For example, it can yield 2-3 times more carbohydrates compared to maize (Ziska et al., 2009). High starch yield of sweetpotato and its resistance to stress conditions make it a very important crop in developing countries (Lebot, 2010). New sweetpotato cultivars with higher content of LMW antioxidants and tolerance to abiotic stresses can be achieved by metabolic engineering tools (Kang et al., 2017c), which is extensively studied and well-applied in other plant species to improve stress tolerance (El Sheikha and Ray, 2017). Gene manipulation of lipid-soluble antioxidants such as carotenoids and tocopherols were well studied under abiotic stress conditions in sweetpotato, and, here, we review the gene manipulation of major lipid soluble antioxidants in transgenic sweetpotato plants and their potential in the development cultivars of sweetpotato with superior traits, which will be invaluable to overcome the global crisis such as food, nutrition, and energy security. Additionally, the potential of the sweetpotato *Orange (IbOr)* gene is discussed as a target gene for regulation of carotenoid metabolism and for enhancement of plant stress tolerance (Kim et al., 2018a).

2. Regulation of carotenoid biosynthesis

Carotenoids are accessory pigments for photosynthesis and are important antioxidants that work to prevent damage of oxidative stress in plants. It is widely studied that carotenoids cope with detrimental effects of various abiotic stresses, such as high light, Ultraviolet-B (UV-B) radiation, high temperature, and drought stress by

scavenging excess ROS and prevent oxidative stress (Uarrota et al., 2018). Therefore, plant leaves contain high levels of carotenoids including lutein and β -carotene that are found in all plants and are a necessity for preventing the oxidative damage. Carotenoids are also crucial for humans due to their role in vitamin A synthesis, which poses public health threat due to its widespread chronic deficiency especially in developing countries. There have been several efforts to deal with this health issue, including engineered crop varieties with higher carotenoid content such as golden rice (Paine et al., 2005).

2-C-methyl-D-erythritol 4-phosphate (MEP) pathway is conserved in plant species for carotenoid biosynthesis (Giuliano, 2014; Kim et al., 2020b) (Figure 1). Geranylgeranyl pyrophosphate synthase (GGPS) catalyzes the conversion of geranylgeranyl diphosphate (GGPP) from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Following this, phytoene synthase (PSY) produces phytoene which is used to synthesize lycopene by the actions of several enzymes. At this point, several enzymes including lycopene ϵ cyclase (LCY- ϵ), lycopene β cyclase (LCY- β), and α -carotene ϵ -ling hydroxylase (CHY- ϵ) catalyze the conversion of lycopene to lutein in α -branch pathway while β -carotene hydroxylase (CHY- β), zeaxanthin epoxidase (ZEP) followed by neoxanthin synthase (NXS) enzymes catalyze the conversion of lycopene to neoxanthin in β -branch pathway. Finally, plant hormones and volatile compounds are formed from the apocarotenoids produced by carotenoid cleavage dioxygenase (CCD) enzymes (Giuliano, 2014). For example, abscisic acid (ABA), a vital plant hormone for stress response, is synthesized by 9-*cis*-epoxycarotenoid dioxygenase (NCED) from xanthoxin or violaxanthin.

Carotenoid biosynthetic pathway has been characterized to a great extent in sweetpotato (Kang et al., 2017c; Kim et al., 2020b). Leaves of sweetpotato consist of lutein, β -carotene, violaxanthin, and neoxanthin (Chen and Chen, 1993), while β -carotene, β -cryptoxanthin, violaxanthin, zeaxanthin, and other unidentified carotenoids can be found in the tuberous roots (Ishiguro et al., 2010; Kang et al., 2017c; Kim et al., 2020b). β -carotene is the main carotenoid in tuberous roots of sweetpotato. In this section, we try to review the current knowledge related to metabolic engineering of carotenoids in sweetpotato (Kang et al., 2017c; Kim et al., 2020b). The *IbGGPS* gene isolated from sweetpotato is localized primarily in chloroplasts of leaves (Chen et al., 2015). Enhanced levels of carotenoids were achieved in *IbGGPS* overexpressed *Arabidopsis* that exhibited increased tolerance to osmotic stress (Chen et al., 2015). The overexpressed *IbZDS* gene in sweetpotato resulted in improved salinity tolerance (Li et al., 2017).

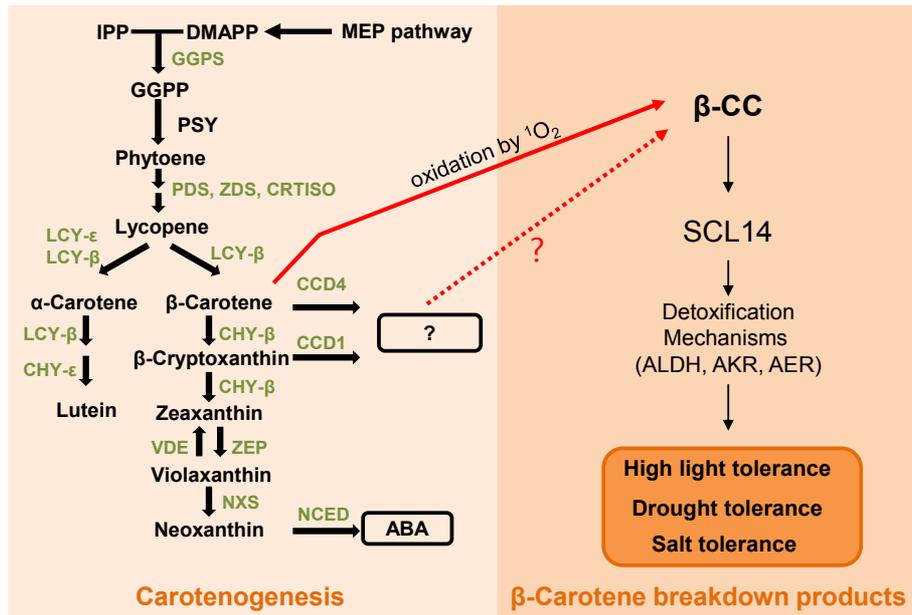


Figure 1. Scheme summarizing carotenogenesis, and action of β -carotene degradation product β -cyclocitral.

LCY-e and *LCY- β* genes are responsible for the branch composition of carotenoids in plants (Cazzonelli et al. 2009). RNA interference (RNAi) studies conducted in *IbLCY-e*, *IbLCY- β* , and *IbCHY- β* genes to enlighten the metabolism of carotenoids in sweetpotato. For example, down-expressed *IbLCY-e* by RNAi resulted in higher carotenoid levels in transgenic calluses of sweetpotato (Kim et al., 2013b). Action of *IbLCY-e* is indispensable for the α -branch pathway and downregulation of it is resulted an enhancement of the levels of carotenoids specific to β -branch. Moreover, *IbLCY-e* RNAi calluses were more tolerant to stresses such as salinity and oxidative stress (Kim et al., 2013b). In addition, transgenic sweetpotato seedlings obtained from these calluses were tolerant to salinity, drought, and oxidative stress when compared to NT plants (Ke et al., 2019). It has also been demonstrated that down-regulation of the *LCY- ϵ* gene in canola (*Brassica napus*) and tobacco (*Nicotiana tabacum*) similarly elevates the levels of carotenoids specific to β -branch (Shi et al., 2014).

IbLCY- β gene is also important for both α - and β -branch pathways, and decrease of *IbLCY- β* by RNAi also changed the color of calluses and enhanced the total carotenoids of sweetpotato. Down regulation of *IbLCY- β* gene is also related to tolerance to abiotic stress such as salinity and drought (Kim et al., 2014). Recently, a novel allele of the *LCY- β* gene was isolated from sweetpotato (named *IbLCYB2*) and characterized (Kang et al., 2018b). *IbLCYB2* overexpressed sweetpotato seedlings showed enhanced contents of various carotenoids, which triggered the resistance to environmental stresses.

The β -branch pathway of carotenoids is controlled by *CHY- β* gene. RNAi of this gene enhanced the content of β -carotene in sweetpotato calluses, which resulted in color change and tolerance to stresses (Kim et al., 2012). Tuberos roots of *IbCHY- β* RNAi transgenic sweetpotato showed a color change and increased levels of β -carotene and total carotenoids. Moreover, in *IbCHY- β* RNAi, sweetpotato plants expression of *IbNCED* expression, which is important in ABA biosynthesis, was increased leading to enhanced ABA levels and salinity tolerance (Kang et al., 2017a). The other genes involved in biosynthesis and degradation of carotenoids should remain to be studied to understand the regulation of carotenoids in sweetpotato.

3. Regulation of sweetpotato orange (*IbOr*) gene

The regulation of carotenoids depends on *Orange (Or)* gene, which is essential for stress tolerance (Kim et al., 2018a, 2021b) and has been characterized in alfalfa (*Medicago sativa*) (Wang et al., 2018), *Arabidopsis* (Bai et al., 2014), carrot (*Daucus carota*) (Ellison et al., 2018), cauliflower (*Brassica oleracea*) (Lu et al., 2006), melon (*Cucumis melo*) (Tzuri et al., 2015), rice (*Oryza sativa*) (Endo et al., 2109), saffron (*Crocus sativus*) (Ahrazen et al., 2020), sorghum (*Sorghum bicolor*) (Yuan et al., 2015), and sweetpotato (Kim et al., 2013a). The mechanism of the *Or* gene is well studied and characterized especially in sweetpotato (Table). In particular, sweetpotato orange (*IbOr*) gene was well studied in terms of abiotic stresses such as drought, salinity, and high temperature (Osorio, 2019)

Brassica oleracea, cauliflower was the first plant that *Or* gene was characterized in (Li et al., 2001). The *IbOr* gene

of sweetpotato Shinhwangmi cultivar and its function was characterized in sweetpotato calluses (Kim et al., 2013a). *IbOr* expression is high among all sweetpotato cultivars in leaf tissue independent of the root flesh color (orange, purple or white). However, in tuberous roots *IbOr* gene expression is high only in orange-fleshed varieties. *IbOr* expression in purple-fleshed roots results in production of both anthocyanins and carotenoids in tuberous roots. Overexpression of *IbOr* in transgenic sweetpotato plants increased heat, oxidative and drought stress tolerance (Park et al., 2015; Park et al., 2016; Kang et al., 2017b; Kim et al., 2018a). On the other hand, overexpressing *IbOr* in alfalfa and potato plants conferred the resistance to salinity, drought, and oxidative stress (Goo et al., 2015; Wang et al., 2015; Cho et al., 2016). Moreover, the overexpression of *IbOr* gene in maize leads to accumulation of higher levels of carotenoids (Tran et al., 2017).

A cysteine (Cys)-rich zinc finger domain has been characterized in the Or proteins. This domain, which is also found in various chaperone proteins such as DnaJ, is fundamental for proper folding of proteins (Hennessy et al., 2005), and chaperon activity in *IbOr* was also determined (Park et al., 2016). Or protein was found to be a master regulator of phytoene synthase activity, which is rate-limiting enzyme in biosynthesis of carotenoids in *Arabidopsis* (Zhou et al., 2015). Moreover, AtOr was also interacted with transcription factor TCP14 and repress its activity and so mediate EARLY LIGHT-INDUCIBLE PROTEINS (ELIPs) expression, which decreased the biosynthesis of chlorophylls and repressed the establishment of thylakoid membranes (Sun et al., 2019). Recently, the interaction between TIC, the key translocon in inner membranes of chloroplasts, and Or protein was determined, and a unique role for Or protein in protein import in plastids was well characterized (Yuan et al., 2021).

IbOr is a multifaceted protein for controlling carotenoid accumulation and photosynthetic efficiency in plants. The chaperon activity of *IbOr* constitutes a defending role for *IbPSY*, which is essential for biosynthesis of carotenoids in chloroplasts under stress conditions. Accordingly, transgenic *Arabidopsis* plants that overexpressed *IbOr* also showed enhanced tolerance to high temperatures (Park et al., 2016). It can be concluded that chaperone activity of *IbOr* is involved in regulation of carotenoid biosynthesis and tolerance to abiotic stress in plants (Figure 2). CCDs catalyze the oxidative cleavage of carotenoids for apocarotenoid production (Walter and Strack, 2011). *NCED*, *CCD1*, and *CCD4* are the genes that are essential for carotenoid metabolism, and their expression is enhanced in plants that have *IbOr* overexpression (Park et al., 2015). Moreover, *IbOr* can interact with *CCD4* (Part et al., 2020), while it did not interact with *CCD1*. However,

the metabolites of *IbCCD4* remains to be studied for better understanding the role of this interaction. *IbOr* is a key regulator for carotenoid homeostasis because of its interaction of both carotenoid biosynthesis (*PSY*) and degradation (*CCD4*) enzymes (Figure 2). Transgenic plants that had *IbOr* showed enhanced chlorophyll content, and they are much more efficient PSII in response to abiotic stresses (Kang et al., 2017b; Tran et al. 2017) (Figure 2).

PSII core complex contains β -carotene that are bound to D1 and D2 (Ferreira et al., 2004). Therefore, β -carotene has ability to scavenge singlet oxygen (1O_2) produced at PSII. Detoxification of 1O_2 by β -carotene can occur by energy transfer or by direct oxidation by 1O_2 . Oxidation of β -carotene by 1O_2 can generate various derivatives such as β -carotene endoperoxide (Ramel et al. 2013). Cleavage of β -carotene due to oxidation can generate compounds such as β -cyclocitral (β -CC), β -ionone or dihydroactinidiolide (dhA) (D'Alessandro and Havaux, 2019). Moreover, besides oxidation by 1O_2 , a set of β -carotene cleave products can be enzymatically produced by CCDs (Harrison and Bugg, 2014). Interestingly, these breakdown products can act as signals to convey messages from chloroplasts to the nucleus. β -CC and dhA were shown to induce expression of 1O_2 responsive genes (Shumbe et al., 2014). Among the genes related to stress response, genes related to growth and development were downregulated, indicating an adaptive transcriptomic response. Accordingly, β -CC and dhA treatment conferred tolerance to highlight stress through lower lipid peroxidation and better PSI photoprotection (Shumbe et al., 2014). Moreover, β -CC also conferred tolerance to drought stress in *Arabidopsis* (D'Alessandro et al., 2018). β -CC induces SCL14 (SCARECROW LIKE 14) dependent xenobiotic detoxification response, which includes induction of reactive carbonyl species (RCS) detoxification enzymes such as aldehyde dehydrogenases, 2-alkenal reductases and aldo/keto reductases (D'Alessandro et al., 2018). RCS are breakdown products of lipid peroxides (LOOHs) produced during oxidation of polyunsaturated fatty acids by ROS. RCS can modify proteins by oxidation of thiol groups, Michael addition or Schiff base formation (reviewed by Yalcinkaya et al., 2019). Interestingly, β -apocarotenoids are also α , β -unsaturated carbonyls similar to RCS. Accordingly, similar to RCS, toxicity of β -apocarotenoids originates from their ability to react with thiol groups. Therefore, oxidation of β -carotene prepares the plant for the upcoming lipid peroxidation and induces similar defense mechanisms.

In case of sweetpotato, increase of β -carotene might also contribute to increased β -CC levels, which might lead to induction of stress tolerance genes. Moreover, as mentioned before, CCDs might also lead to enzymatic breakdown of β -carotene generating breakdown products

Table Physiological functions of homologous Or proteins from various plant species (modified from Kim et al., 2018a).

Species	Physiological functions	Reference
Alfalfa (<i>Medicago sativus</i>)	Accumulation of carotenoid and tolerance to abiotic stress in tobacco	Wang et al., 2018
Arabidopsis (<i>Arabidopsis thaliana</i>)	Accumulation of carotenoid in rice	Bai et al., 2014, 2016
	Accumulation of carotenoid in <i>Arabidopsis</i> callus	Yuan et al., 2015
	Regulators of active PSY protein	Zhou et al., 2015
	Carotenoid accumulation in corn	Berman et al., 2017
	Carotenoid accumulation in tomato	Yazdani et al., 2019
	Regulation of chloroplast biogenesis interacting with TCP14	Sun et al., 2019
	Regulation of chromoplast number and carotenoid accumulation via interaction between Or ^{His} and ARC3	Sun et al., 2020
	Plastid protein import through interaction with Tic	Yuan et al., 2021
	Carotenoid accumulation	Ellison et al., 2018
Cauliflower (<i>Brassica oleracea</i>)	Carotenoid accumulation in cauliflower	Lu et al., 2006
	Carotenoid accumulation and chromoplast formation in potato	Lopez et al., 2008
	Petiole elongation	Zhou et al., 2011, 2015
	Postharvest storage in potato	Li et al., 2012
	Photo-oxidative responses	Men et al., 2013
Melon (<i>Cucumis melo</i>)	Carotenoid accumulation	Tzuri et al., 2015
Rice (<i>Oryza sativa</i>)	CRISPR/Cas9-mediated genome editing results in β -carotene accumulation in rice calluses	Endo et al., 2019
	Negative regulation of carotenoid accumulation	Yu et al., 2021
Saffron (<i>Crocus sativus</i>)	Metabolism of PSY in saffron	Ahrazem et al., 2020
Sorghum (<i>Sorghum bicolor</i>)	Carotenoid accumulation in <i>Arabidopsis</i> callus	Yuan et al., 2015
Sweetpotato (<i>Ipomoea batatas</i>)	Accumulation of carotenoids under salinity	Kim et al., 2013a
	Accumulation of carotenoid in tuberous roots of sweetpotatos	Park et al., 2015
	Accumulation of carotenoids in alfalfa and tolerance to abiotic stress	Wang et al., 2015
	Accumulation of carotenoids in potato and tolerance to abiotic stress	Goo et al., 2015, Cho et al., 2016
	Stabilization of PSY protein	Park et al., 2016
	Carotenoid accumulation in maize	Tran et al., 2017
	Regulation of photosynthesis Tolerance to drought in sweetpotato Carotenoid over-accumulation in callus of R96H sweetpotato Interaction with IbCCD4 Carotenoid over-accumulation in tuberous roots of R96H sweetpotato and heat tolerance	Kang et al., 2017b Kim et al., 2018a Kim et al., 2019a Park et al., 2020 Kim et al., 2021b

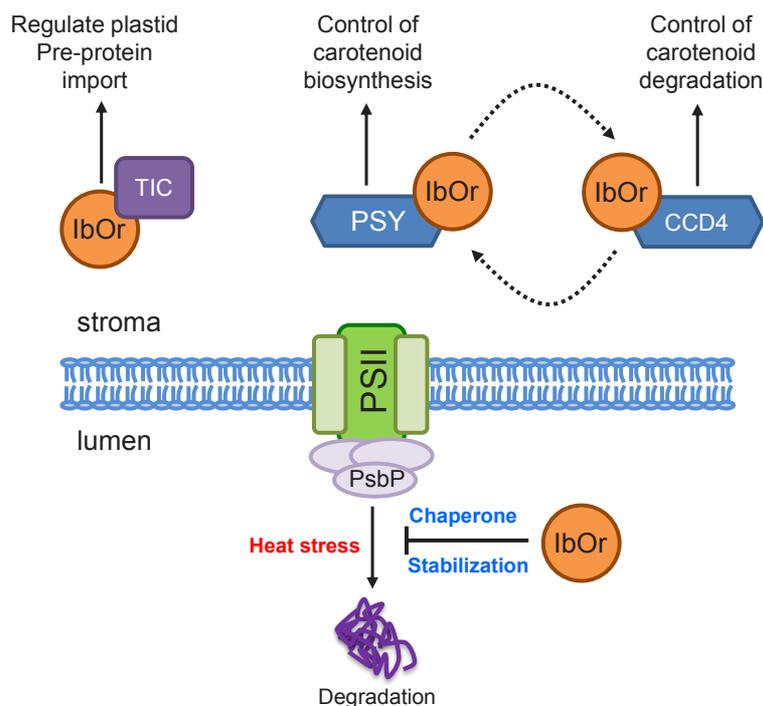


Figure 2. Hypothetical model summarizing roles of the sweetpotato *Or* (*IbOr*) gene. *IbOr* protein interacts with proteins (PsbP etc.) in photosystem II system. Moreover, *IbOr* protein interacts with proteins (PSY, CCD4) in biosynthesis and catabolism of carotenoids (modified from Kim et al. 2020a).

that have potential for retrograde signaling. Interaction of *IbOr* with CCDs might function for controlling their activity under stress linking β -carotene breakdown products to stress response. Finally, it would be interesting to test whether cysteine rich domain of *IbOr* is modified by β -apocarotenoids or not.

In *Cucumis melon*, a single nucleotide polymorphism referred to as golden-SNP caused by a change of arginine to histidine (R96H) in *CmOr* protein resulted in an orange phenotype indicating that *CmOr* is also related to regulation of β -carotene biosynthesis (Tzuri et al., 2015). Comparison of amino acid sequences of sweetpotato (*IbOr*), *Arabidopsis* (*AtOr*), and melon (*CmOr*) *Or* proteins revealed that the *CmOr* protein is related to the *IbOr* protein causing a phenotype that white-fleshed melon. Moreover, introduction of golden SNP to *IbOr* enhanced the carotenoid levels significantly in sweetpotato calluses (Kim et al., 2019a).

Sweetpotato plants overexpressing *IbOr-R96H* showed higher carotenoid accumulation in tuberous roots and heat stress tolerance than *IbOr* transgenic sweetpotato seedlings (Kim et al., 2021b). *IbOr-R96H* gene could be an effective tool to produce new cultivar of sweetpotato by CRISPR-Cas9-mediated base-editing techniques. Our results suggest that *IbOr-R96H* sweetpotato plants, with increased

carotenoid levels and abiotic stress resistance, would help mitigate the global food, nutrition and energy security issues in the face of global climate change, thus, facilitating the establishment of a sustainable society.

4. Regulation of tocopherol biosynthesis

Tocopherols are lipophilic LMW antioxidants that are collectively referred to as vitamin E, which have four forms, α -, β -, γ - and δ -tocopherol. Plastids are the main site for the production of tocopherols, which are important for plant growth and development. Moreover, they are important antioxidants that can prevent the damaging effects of ROS (Asensi-Fabado and Munne-Bosch, 2010; Akyol et al., 2020).

In the tocopherol biosynthetic pathway, α -tocopherol is synthesized from p-hydroxy phenylpyruvate (HPP), which is catalyzed by 4-HPP dioxygenase (HPPD), homogentisate phytyltransferase (HPT), 2-methyl-6-phytylbenzoquinol methyltransferase (MPBQ MT), tocopherol cyclase (TC), and γ -tocopherol methyltransferase (TMT) enzymes (Ji et al., 2016).

Isolation of five genes that take roles in the biosynthesis of tocopherol has been achieved from sweetpotato leaves, and, after treatments of abiotic stresses, their expressions have been determined (Ji et al., 2016). Among the enzymes

mentioned above, drought and oxidative stress triggered the induction of *IbHPPD* expression, while only drought stress enhanced the expression of *IbHPT*. Moreover, *IbMPBQ MT* and *IbTC* genes were only induced under salinity. Additionally, transient expression of these five genes involved in tocopherol biosynthesis in tobacco leaves elevates the level of α -tocopherol (Ji et al., 2016). *IbTC* overexpressing transgenic sweetpotato had higher α -tocopherol levels, and transgenic plants were more tolerant to salinity, drought, and oxidative stress (Kim et al., 2019b). Similarly, *IbHPPD* plants exhibited induced tolerance to environmental stresses (Kim et al., 2021a). Interestingly, following stress treatments, *IbHPPD* plants had higher ABA levels than NT plants. During dehydration, *IbHPPD* plants elevated α -tocopherol contents in their leaves. Properties of transgenic sweetpotato plants expressing *IbHPT*, *IbMPBQ MT*, *IbTMT* remain to be characterized to understand the regulation of tocopherols in detail and their performance under adverse conditions.

5. Conclusion

The current status of gene manipulation of two LMW lipophilic antioxidants such as carotenoids and tocopherols in transgenic sweetpotato was reviewed to enhance both abiotic stress tolerance and nutrition quality. RNAi technology is used to understand the carotenoid biosynthesis in sweetpotato. We emphasized the importance the roles of *IbOr* in the regulation of

carotenoid homeostasis and environmental stress tolerance. In particular, overexpression of the golden SNP-carrying *Or* gene (*IbOr-R96H*) showed significant increase in carotenoid accumulation and antioxidant activity in tuberous roots of sweetpotato. Thus, we expect that *IbOr-R96H* gene could be an effective tool to produce new cultivar of sweetpotato by site-specific mutagenesis of *IbOr* utilizing CRISPR-Cas9-mediated base-editing. In addition, *IbHPPD* and *IbTC* overexpressing transgenic sweetpotato plants showed an enhanced tolerance to various abiotic stress. As mentioned above, studies on the biosynthesis and degradation of LMW antioxidants in sweetpotato will lead to improvement of highly nutritional new cultivars, which will also cope with environmental stresses. This kind of improvement is urgently needed for sustainable crop production under climate change conditions. Taken together, it is anticipated that cultivars of sweetpotato that show enhanced contents of LMW antioxidants will be developed by combining genes that take part in biosynthesis and degradation of LMW antioxidants using new breeding techniques to increase stress tolerance and nutritional quality.

Acknowledgments

This research is supported by The National Research Foundation (NRF) of Korea funded by the Korean Government (MSIT) (2020R1A2C1004560) and The Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program (KGM5372113).

References

- Ahrazem O, López AJ, Argandoña J, Castillo R, Rubio-Moraga Á et al. (2020). Differential interaction of *Or* proteins with the PSY enzymes in saffron. *Scientific Reports* 10: 552.
- Akyol TY, Yilmaz O, Uzilday B, Uzilday RÖ, Türkan İ (2020). Plant response to salinity: an analysis of ROS formation, signaling, and antioxidant defense. *Turkish Journal of Botany* 44, 1-13.
- Asada K (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 601-639.
- Asensi-Fabado MA, Munne-Bosch S (2010). Vitamins in plants: Occurrence, biosynthesis and antioxidant function. *Trends in Plant Science* 15: 582-592.
- Bai C, Capell T, Berman J, Medina V, Sandmann G, Christou P et al. (2016). Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor-product balance. *Plant Biotechnology Journal* 14: 195-205.
- Bai C, Rivera SM, Medina V, Alves R, Vilaprinco E et al. (2014). An *in vitro* system for the rapid functional characterization of genes involved in carotenoid biosynthesis and accumulation. *Plant Journal* 77: 464-475.
- Berman J, Zorrilla-López U, Medina V, Farré G, Sandmann G et al. (2017). The *Arabidopsis* ORANGE (*AtOR*) gene promotes carotenoid accumulation in transgenic corn hybrids derived from parental lines with limited carotenoid pools. *Plant Cell Reports* 36: 933-945.
- Cazzonelli CI, Cuttriss AJ, Cossetto SB, Pye W, Crisp P et al. (2009). Regulation of carotenoid composition and shoot branching in *Arabidopsis* by a chromatin modifying histone methyltransferase, SDG8. *The Plant Cell* 21: 39-53.
- Chen BH, Chen YY (1993). Stability of chlorophylls and carotenoids in sweet potato leaves during microwave cooking. *Journal of Agricultural and Food Chemistry* 41: 1315-1320.
- Chen W, He S, Liu D, Patil GB, Zhai H et al. (2015). A sweetpotato geranylgeranyl pyrophosphate synthase gene, *IbGGPS*, increases carotenoids content and enhances osmotic stress tolerance in *Arabidopsis thaliana*. *PLoS ONE* 10:e0137623.
- Cho KS, Han EH, Kwak SS, Cho JH, Im JS et al. (2016). Expressing the sweetpotato orange gene in transgenic potato improves drought tolerance and marketable tuber production. *Comptes Rendus Biologies* 339: 207-213.
- CSPI(2016). 10Bestfoods. Retrieved from <http://www.nutritionaction.com/get-download/download/?dtd=16511&n=1>

- Cunningham FX, Gantt E (1998). Genes and enzymes of carotenoids biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 49: 557-583.
- D'Alessandro S, Ksas B, Havaux M (2018). Decoding β -cyclocitral-mediated retrograde signaling reveals the role of a detoxification response in plant tolerance to photooxidative stress. *The Plant Cell* 30: 2495-2511.
- d'Alessandro S, Havaux M (2019). Sensing β -carotene oxidation in photosystem II to master plant stress tolerance. *New Phytologist* 223: 1776-1783.
- El Sheikh AF, Ray RC (2017). Potential impacts of bioprocessing of sweet potato. *Critical Reviews in Food Science and Nutrition* 57: 455-471.
- Ellison SL, Luby CH, Corak KE, Coe KM, Senalik D et al. (2018). Carotenoid presence is associated with the *Or* gene in domesticated carrot. *Genetics* 210: 1497-1508.
- Endo A, Saika H, Takemura M, Misawa N, Toki S (2019). A novel approach to carotenoid accumulation in rice callus by mimicking the cauliflower *Orange* mutation via genome editing. *Rice* 12: 1-5.
- Evans HM, Bishop KS (1922). On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science* 56: 650-651.
- FAO (2018a). The future of food and agriculture – Alternative pathways to 2050. Rome, Italy
- FAO (2018b). FAOSTAT, <http://faostat.fao.org/>
- Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004). Architecture of the photosynthetic oxygen-evolving center. *Science* 303(5665): 1831-1838.
- Foyer CH, Descourvieres P, Kunert KJ (1994). Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. *Plant Cell and Environment* 17: 507-523.
- Giuliano G (2014). Plant carotenoids: genomics meets multi-gene engineering. *Current Opinion in Plant Biology* 19: 111-117.
- Goo YM, Han EH, Jeong JC, Kwak SS, Yu J et al. (2015). Overexpression of the sweetpotato *IbOr* gene results in the increased accumulation of carotenoids and confers tolerance to environmental stresses in transgenic potato. *Comptes Rendus Biologies* 338: 12-20.
- Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB et al. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* 319: 330-333.
- Harrison PJ, Bugg TD (2014). Enzymology of the carotenoid cleavage dioxygenases: reaction mechanisms, inhibition and biochemical roles. *Archives of Biochemistry and Biophysics* 544: 105-111.
- Hennessy F, Nicoll WS, Zimmermann R, Cheetham ME, Blatch GL (2005). Not all J domains are created equal: implications for the specificity of Hsp40-Hsp70 interactions. *Protein Science* 14: 1697-1709.
- Howitt CA, Pogson BJ (2006). Carotenoids accumulation and function in seeds and non-green tissues. *Plant Cell and Environment* 29: 435-445.
- Isabiry MG, Ruyschaert L, Van-Linden JP, Maguada MK, Deckers J (2007). Soil losses due to cassava and sweet potatoes harvesting: a case study from low input traditional agriculture. *Soil and Tillage Research* 92: 96-103.
- Ishida H, Suzuno H, Sugiyama N, Innami S, Tadokoro T et al. (2000). Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas* *poir*). *Food Chemistry* 68: 359-367.
- Ishiguro K, Yoshinaga M, Kai Y, Maoka T, Yoshimoto M (2010). Composition, content and antioxidative activity of the carotenoids in yellow-fleshed sweet potato (*Ipomoea batatas* L.). *Breeding Science* 60: 324-329.
- Islam S (2006). Sweetpotato (*Ipomoea batatas* L.) leaf: Its potential effect on human health and nutrition. *Journal of Food Science* 71: R13-R21.
- Ji CY, Kim YH, Kim HS, Ke Q, Kim GW et al. (2016). Molecular characterization of tocopherol biosynthetic genes in sweetpotato that respond to stress and activate the tocopherol production in tobacco. *Plant Physiology and Biochemistry* 106: 118-128.
- Kang C, He S, Zhai H, Li R, Zhao N et al. (2018a). A sweetpotato auxin response factor gene (*IbARF5*) is involved in carotenoid biosynthesis and salt and drought tolerance in transgenic *Arabidopsis*. *Frontiers in Plant Science* 9: 1307.
- Kang C, Zhai H, Xue L, Zhao N, He S et al. (2018b). A lycopene β -cyclase gene, *IbLCYB2*, enhances carotenoid contents and abiotic stress tolerance in transgenic sweetpotato. *Plant Science* 272: 243-254.
- Kang L, Ji CY, Kim SH, Ke Q, Kim HS et al. (2017a). Suppression of β -carotene hydroxylase gene increases β -carotene contents and tolerance to abiotic stress in transgenic sweetpotato plants. *Plant Physiology and Biochemistry* 117: 24-33.
- Kang L, Kim HS, Kwon YS, Ke Q, Ji CY et al. (2017b). *IbOr* regulates photosynthesis under heat stress by stabilizing *IbPsbP* in sweetpotato. *Frontiers in Plant Science* 8: 989.
- Kang L, Park SC, Ji CY, Kim HS, Lee HS et al. (2017c). Metabolic engineering of carotenoids in transgenic sweetpotato. *Breeding Science* 67: 27-34.
- Kanwischer M, Porfirova S, Bergmüller E, Dormann P (2005). Alterations in tocopherol cyclase activity in transgenic and mutant plants of *Arabidopsis* affect tocopherol content, tocopherol composition and oxidative stress. *Plant Physiology* 137: 713-723.
- Ke Q, Kang L, Kim HS, Xie T, Liu C et al. (2019). Down-regulation of lycopene ϵ -cyclase expression in transgenic sweetpotato plants increases the carotenoid content and tolerance to abiotic stress. *Plant Science* 281: 52-60.
- Kim HS, Ji CY, Lee CJ, Kim SE, Park SC et al. (2018a) *Orange*: a target gene for regulating carotenoid homeostasis and increasing plant tolerance to environmental stress in marginal lands. *Journal of Experimental Botany* 69: 3393-3400.
- Kim HS, Kwak SS (2020a). Crop biotechnology for sustainable agriculture in the face of climate crisis. *Plant Biotechnology Reports* 14: 139-141.

- Kim HS, Lee CJ, Kim SE, Ji CY, Kim ST et al. (2018b). Current status on global sweetpotato cultivation and its prior tasks of mass production. *Journal of Plant Biotechnology* 45: 190-195.
- Kim HS, Wang W, Kang L, Kim SE, Lee CJ et al. (2020b). Metabolic engineering of low molecular weight antioxidants in sweetpotato. *Plant Biotechnology Reports* 14: 193-205.
- Kim SE, Bian X, Lee CJ, Park SU, Lim YH et al. (2021a). Overexpression of 4-hydroxyphenylpyruvate dioxygenase (*IbHPPD*) increases abiotic stress tolerance in transgenic sweetpotato plants. *Plant Physiology and Biochemistry* 167: 420-429.
- Kim SE, Kim HS, Wang Z, Ke Q, Lee CJ et al. (2019a). A single amino acid change at position 96 (Arg to His) of the sweetpotato Orange protein leads to carotenoid overaccumulation. *Plant Cell Reports* 38: 1393-1402.
- Kim SE, Lee CJ, Ji CY, Kim HS, Park SU et al. (2019b). Transgenic sweetpotato plants overexpressing *tocopherol cyclase* display enhanced α -tocopherol content and abiotic stress tolerance. *Plant Physiology and Biochemistry* 144: 436-444.
- Kim SE, Lee CJ, Park SU, Lim YH, Park WS et al. (2021b). Overexpression of the golden SNP-carrying *Orange* gene enhances carotenoid accumulation and heat stress tolerance in sweetpotato plants. *Antioxidants* 10: 51.
- Kim SH, Ahn YO, Ahn MJ, Jeong JC, Lee HS et al. (2013a). Cloning and characterization of an *Orange* gene that increases carotenoids accumulation and salt stress tolerance in transgenic sweetpotato cultures. *Plant Physiology and Biochemistry* 70: 445-454.
- Kim SH, Ahn YO, Ahn MJ, Lee HS, Kwak SS (2012). Down-regulation of β -carotene hydroxylase increases β -carotene and total carotenoids enhancing salt stress tolerance in transgenic cultured cells of sweetpotato. *Phytochemistry* 74: 69-78.
- Kim SH, Jeong JC, Park S, Bae JY, Ahn MJ et al. (2014). Down-regulation of sweetpotato lycopene β -cyclase gene enhances tolerance to abiotic stress in transgenic calluses. *Molecular Biology Reports* 41: 8137-8148.
- Kim SH, Kim YH, Ahn YO, Ahn MJ, Jeong JC et al. (2013b). Downregulation of the lycopene β -cyclase gene increases carotenoids synthesis via the β -branch-specific pathway and enhances salt-stress tolerance in sweetpotato transgenic calluses. *Physiologia Plantarum* 147: 432-442.
- Kwak SS (2018). Agroforestry biotechnology for sustainable agriculture on marginal lands. *Scientific Journal "Regional Problems"* 21: 51-53.
- Kwak SS (2019). Biotechnology of the sweetpotato: ensuring global food and nutrition security in the face of climate change. *Plant Cell Reports* 38: 1361-1363.
- Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY et al. (2002). Enhanced tolerance of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. *Plant Cell and Environment* 25: 873-882.
- Lebot V (2010). Sweet potato. Root and tuber crops: Handbook of plant breeding. London: Springer 97-125.
- Li L, Paolillo DJ, Parthasarathy MV, DiMuzio E, Garvin DF (2001). A novel gene mutation that confers abnormal patterns of β -carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *Plant Journal* 26: 59-67.
- Li L, Yang Y, Xu Q, Owsiany K, Welsch R et al. (2012). The *Or* gene enhances carotenoid accumulation and stability during post-harvest storage of potato tubers. *Molecular Plant* 5: 339-352.
- Li R, Kang C, Song X, Yu L, Liu D et al. (2017). A ζ -carotene desaturase gene, *IbZDS* increases β -carotene and lutein contents and enhances salt tolerance in transgenic sweetpotato. *Plant Science* 262: 39-51.
- Lopez AB, Van Eck J, Conlin BJ, Paolillo DJ, O'Neill J et al. (2008). Effect of the cauliflower *Or* transgene on carotenoids accumulation and chromoplast formation in transgenic potato tubers. *Journal of Experimental Botany* 59: 213-223.
- Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran et al. (2006). The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β -carotene accumulation. *Plant Cell* 18: 3594-3605.
- Men X, Sun T, Dong K, Yang Y (2013). *Or* mutation leads to photo-oxidative stress responses in cauliflower (*Brassica oleracea*) seedlings during de-etiolation. *Journal of Plant Research* 126: 823-832.
- Mene-Saffrane L, Pellaud S (2017). Current strategies for vitamin E biofortification of crops. *Current Opinion in Biotechnology* 44: 189-197.
- Osorio CE (2019). The Role of *Orange* gene in carotenoid accumulation: Manipulating chromoplasts toward a colored future. *Frontiers in Plant Science* 10: 1235.
- Ouyang SQ, He SJ, Liu P, Zhang WK, Zhang JS et al. (2011). The role of tocopherol cyclase in salt stress tolerance of rice (*Oryza sativa*). *Science China-Life Sciences* 54: 181-188.
- Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ et al. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nature Biotechnology* 23(4): 482-487.
- Park SC, Kang L, Park WS, Ahn MJ, Kwak SS et al. (2020). Carotenoid cleavage dioxygenase 4 (*CCD4*) cleaves β -carotene and interacts with *IbOr* in sweetpotato. *Plant Biotechnology Reports* 14: 737-742.
- Park SC, Kim HS, Jung YJ, Kim SH, Ji CY et al. (2016). Orange protein has a role in phytoene synthase stabilization in sweetpotato. *Scientific Reports* 6: 33563.
- Park SC, Kim SH, Park SY, Lee HU, Lee JS et al. (2015a). Enhanced accumulation of carotenoids in sweetpotato plants overexpressing *IbOr-Ins* gene in purple-fleshed sweetpotato cultivar. *Plant Physiology and Biochemistry* 86: 82-90.
- Prabawardani S, Suparno A (2015). Water use efficiency and yield of sweetpotato as affected by nitrogen and potassium application. *Journal of Agricultural Science* 5: 128-137.
- Ramel F, Mialoundama AS, Havaux M (2013). Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants. *Journal of Experimental Botany* 64: 799-805.

- Ren W, Zhao L, Wang Y, Cui L, Tang Y et al. (2011). Overexpression of homogentisate phytyltransferase in lettuce results in increased content of vitamin E. *African Journal of Biotechnology* 10: 14046-14051.
- Shi Y, Wang R, Luo Z, Jin L, Liu P et al. (2014). Molecular cloning and functional characterization of the *lycopene ϵ -cyclase* gene via virus-induced gene silencing and its expression pattern in *Nicotiana tabacum*. *International Journal of Molecular Sciences* 15: 14766-14785.
- Shumbe L, Bott R., Havaux M. (2014). Dihydroactinidiolide, a high light-induced beta-carotene derivative that can regulate gene expression and photoacclimation in *Arabidopsis*. *Molecular Plant* 7: 1248-51.
- Sun T, Yuan H, Chen C, Kadirjan-Kalbach DK, Mazourek M et al. (2020). OR^{HIS}, a natural variant of OR, specifically interacts with plastid division factor ARC3 to regulate chromoplast number and carotenoid accumulation. *Molecular Plants* 13: 864-878.
- Sun T, Zhou F, Huang XQ, Chen WC, Kong MJ et al. (2019). ORANGE represses chloroplast biogenesis in etiolated *Arabidopsis* cotyledons via interaction with TCP14. *Plant Cell* 31: 2996-3014.
- Tran TL, Ho TH, Nguyen DT (2017). Overexpression of the *IbOr* gene from sweet potato (*Ipomea batatas* 'Hoang Long') in maize increases total carotenoid and β -carotene contents. *Turkish Journal of Biology* 41: 1003-1010.
- Tsegaye Y, Shintani DK, DellaPenna D (2002). Overexpression of the enzyme p-hydroxyphenolpyruvate dioxygenase in *Arabidopsis* and its relation to tocopherol biosynthesis. *Plant Physiology and Biochemistry* 40: 913-920.
- Tzuri G, Zhou X, Chayut N, Yuan H, Portny V et al. (2015). A 'golden' SNP in CmOr governs the fruit flesh color of melon (*Cucumis melo*). *Plant Journal* 82: 267-279.
- Uarrota VG, Stefen DLV, Leolato LS, Gindri DM, Nerling D (2018). Revisiting carotenoids and their role in plant stress responses: From biosynthesis to plant signaling mechanisms during stress. In *Antioxidants and antioxidant enzymes in higher plants* (pp. 207-232). Springer, Cham.
- USDA Database (2018). National Nutrient Database for Standard Reference Legacy Release <https://ndb.nal.usda.gov/ndb/foods/show/301764?fgcd=&manu=&lfacet=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=raw+Van>. Accessed 23 Apr 2018
- Walter MH, Strack D (2011). Carotenoids and their cleavage products: Biosynthesis and functions. *Natural Products Reports* 28: 663-692.
- Wang S, Nie S, Zhu F (2016). Chemical constituents and health effects of sweet potato. *Food Research International* 89: 90-116.
- Wang Z, Ke Q, Kim MD, Kim SH, Ji CY et al. (2015). Transgenic alfalfa plants expressing the sweetpotato *Orange* gene exhibit enhanced abiotic stress tolerance. *PLoS ONE* 10: e0126050.
- Wang Z, Xu WH, Kang JY, Li M, Huang J et al. (2018). Overexpression of alfalfa *Orange* gene in tobacco enhances carotenoid accumulation and tolerance to multiple abiotic stresses. *Plant Physiology and Biochemistry* 130: 613-622.
- Williams R, Soares F, Pereira L, Belo B, Soares A et al. (2013). Sweet potato can contribute to both nutritional and food security in Timor-Leste. *Field Crops Research* 146: 38-43.
- Woo HJ, Sohn SI, Shin KS, Kim JK, Kim BG et al. (2014). Expression of tobacco tocopherol cyclase in rice regulates antioxidative defense and drought tolerance. *Plant Cell* 117: 257-267.
- Yalcinkaya T, Uzilday B, Ozgur R, Turkan I, Mano JI (2019). Lipid peroxidation-derived reactive carbonyl species (RCS): Their interaction with ROS and cellular redox during environmental stresses. *Environmental and Experimental Botany* 165: 139-149.
- Yazdani M, Sun Z, Yuan H, Zeng S, Thannhauser TW et al. (2019). Ectopic expression of ORANGE promotes carotenoid accumulation and fruit development in tomato. *Plant Biotechnology Journal* 17: 33-49.
- Ye X, Al-Babilli S, Kloti A, Zhang J, Lucca P et al. (2000). Engineering the provitamin a (β -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287: 303-305.
- Ytterberg AJ, Peltier JB, Van Wijk KJ (2006). Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes. *Plant Physiology* 140: 984-997.
- Yu B, Lydiate DJ, Young LW, Schäfer UA, Hannoufa A (2008). Enhancing the carotenoids content of *Brassica napus* seeds by downregulating lycopene epsilon cyclase. *Transgenic Research* 17: 573-585.
- Yu Y, Yu J, Wang Q, Wang J, Zhao G et al. (2021). Overexpression of the rice ORANGE gene OsOR negatively regulates carotenoid accumulation, leads to higher tiller numbers and decreases stress tolerance in Nipponbare rice. *Plant Science* 310: 110962.
- Yuan H, Owsiany K, Sheeja TE, Zhou X, Rodriguez C et al. (2015). A single amino acid substitution in an ORANGE protein promotes carotenoid overaccumulation in *Arabidopsis*. *Plant Physiology* 169: 421-431.
- Yuan H, Pawlowski EG, Yang Y, Sun T, Thannhauser TW et al. (2021). Arabidopsis ORANGE protein regulates plastid pre-protein import through interacting with Tic proteins. *Journal of Experimental Botany* 72: 1059-1072.
- Zhou X, Sun TH, Wang N, Ling HQ, Lu S et al. (2011). The cauliflower *Orange* gene enhances petiole elongation by suppressing expression of eukaryotic release factor 1. *New Phytologist* 190: 89-100.
- Zhou X, Welsch R, Yang Y, Alvarez D, Riediger M et al. (2015). *Arabidopsis* OR proteins are the major posttranscriptional regulators of phytoene synthase in controlling carotenoid biosynthesis. *Proceedings of National Academy of Sciences of United States of America* 112: 3558-3563.
- Ziska LH, Runion GB, Tomecek M, Prior SA, Torbe HA et al. (2009). An evaluation of cassava, sweetpotato and field corn as potential carbohydrate sources for bioethanol production in Alabama and Maryland. *Biomass and Bioenergy* 33: 1503-1508.