MOS1 Negatively Regulates Sugar Responses and Anthocyanin Biosynthesis in Arabidopsis

Ning Zhang 1, Maike Wang 1, Jie Huang 1, Leiyun Yang 2, Zhixue Wang 1,2, Dianxing Wu 1,* and Xiaoli Shu 1,*

1 State Key Laboratory of Rice Biology and Key Lab of the Ministry of Agriculture for Nuclear Agricultural Sciences, Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou 310029, China; 11216028@zju.edu.cn (N.Z.); 21716012@zju.edu.cn (M.W.); 21716002@zju.edu.cn (J.H.);
21316004@zju.edu.cn (Z.W.)
2 Plant Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA; ly293@cornell.edu
* Correspondence: dxwu@zju.edu.cn (D.W.); shuxl@zju.edu.cn (X.S.)

Received: 12 July 2020; Accepted: 11 September 2020; Published: 26 September 2020

Abstract: Sugars, which are important signaling molecules, regulate diverse biological processes in plants. However, the convergent regulatory mechanisms governing these physiological activities have not been fully elucidated. MODIFIER OF sncl-1 (MOS1), a modulator of plant immunity, also regulates floral transition, cell cycle control, and other biological processes. However, there was no evidence of whether this protein was involved in sugar responses. In this study, we found that the loss-of-function mutant mos1-6 (mos1) was hypersensitive to sugar and was characterized by defective germination and shortened roots when grown on high-sugar medium. The expression of MOS1 was enhanced by sucrose. Hexokinase 1, an important gene involved in sugar signaling, was upregulated in the mos1 mutant compared to wild-type Col-0 in response to sugar. Furthermore, the mos1 mutant accumulated more anthocyanin than did wild-type Col-0 when grown on high-sugar concentration medium or under high light. MOS1 was found to regulate the expression of flavonoid and anthocyanin biosynthetic genes in response to exogenous sucrose and high-light stress but with different underlying mechanisms, showing multiple functions in addition to immunity regulation in plant development. Our results suggest that the immune regulator MOS1 serves as a coordinator in the regulatory network, governing immunity and other physiological processes.

Keywords: Arabidopsis; sugar signaling; anthocyanin biosynthesis; MOS1

1. Introduction

Sugars not only serve as energy sources in plants but also as hormone-like molecules in regulating many important physiological processes, including metabolism [1,2], seed germination [3], and biotic and abiotic stress responses [4,5]. Many sucrose-insensitive or -hypersensitive mutants have been screened to identify genes involved in sugar signaling [6–9]. By studying these mutants, it has been recognized that sugars have crosstalk with other signals, such as light [10], hormones [11,12], stresses [12], and nutrients [13,14]. Sugar signaling is usually triggered by glucose [15], although sucrose is the main type of sugar for systemic transport in plants [16].

Sugar signaling pathways are conserved in eukaryotes [17]. Hexokinases (HXKs), a group of identified glucose sensors, also govern glucose phosphorylation and regulate sugar responses [10]. In Arabidopsis, HXK1 mutants are insensitive to glucose, and HXK1 has been reported to coordinate sugar, light, and hormones to control plant growth [10]. TREHALOSE-6-PHOSPHATE SYNTHASE (TPS), which is involved in the HXK-dependent glucose signaling pathway, catalyzes the biosynthesis of...
trehalose-6-phosphate from UDP-glucose and glucose-6-phosphate [18]. Overexpression of \( \text{AtTPS1} \) in Arabidopsis reduces the sensitivity to glucose [10,18]. G-protein-coupled receptors (GPCRs) identified in sugar signaling pathways can perceive sugar. Regulators of G-protein signaling (RGS) can activate the GTPase to drive G-protein into the inactive heterotrimer [19]. In Arabidopsis, glucose alters the interaction between G Protein Alpha Subunit1 (GPA1) and RGS1, consequently activating the hydrolysis of GTP and mediating sugar signal transduction [20]. Plant SNF1-RELATED KINASE (SnRK) proteins belong to a conserved SUCROSE-NONFERMENTING 1 (SNF1)/AMP-activated protein kinase (AMPK)/SnRK1 family, which plays important roles in metabolism regulation by sensing cellular energy charge [21–23]. SNF1 KINASE HOMOLOG 10 (AKIN10) and AKIN11, two Arabidopsis SnRK proteins, are reported to have important roles in sugar signaling pathways [12]. Recently, an evolutionarily conserved energy sensor TARGET OF RAPAMYCIN (TOR) complex has been demonstrated to link sugar signaling with meristem activation in Arabidopsis [24]. Sugar signals tightly coordinate the production and mobilization of sugars to regulate plant metabolism and development [25].

Moreover, environmental stresses would increase the accumulation of soluble sugars. Sucrose is necessary for producing anthocyanin, and sugars are closely associated with the regulation of anthocyanin biosynthesis [26,27]. As a class of secondary metabolites of flavonoids, anthocyanins are widely found in plants [28]. Anthocyanins absorb light in a certain wavelength range and play roles in the prevention of photoinhibition [29]. Anthocyanins are also antioxidants and confer multiple tolerances against abiotic and biotic stresses, including cold, UV, pathogens, and insects, by ROS scavenging [30]. The biosynthesis of anthocyanin in plants begins with the conversion of phenylalanine into coumarate-CoA by phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase, and 4-coumarate: CoA ligase, which are common steps shared by many secondary metabolic pathways [31]. The subsequent biosynthesis processes can be divided into early and late stages. In the early biosynthesis stage, coumarate-CoA is catalyzed consecutively by chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3-hydroxylase (F3H), flavonoid-3′-hydroxylase (F3′H), and flavonoid-3′5′-hydroxylase (F3′5′H) to form three types of dihydroflavonols [32]. In the late biosynthesis stage, dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose flavanol 3-O-glucosyl transferase are specific enzymes mediating anthocyanin biosynthesis from dihydroflavonols [32]. The \text{FLAVONOL SYNTHASE (FLS)} gene encodes a flavonol synthase that catalyzes the formation of flavonols from dihydroflavonols [33]. Genes participating in these two steps are called anthocyanin biosynthesis genes (\text{ABGs}). MYB-type transcription factors (TFs), basic helix–loop–helix (bHLH)-type TFs, and WD40-repeat TFs form MBW complexes to regulate the biosynthesis of anthocyanins [34]. The expression of genes encoding the MBW complex subunits is strongly induced by such factors as sugars, hormones, and environmental stresses, which subsequently activate the transcription of \text{ABGs} [35,36].

Currently, emerging evidence has shown that sugars are involved in immunity [4,37]. MODIFIER OF \text{snc1-1} (MOS1), a modulator of plant immunity, positively regulates the NLR genes \text{SUPPRESSOR OF npr1-1} and \text{CONSTITUTIVE 1} (\text{SNCl}). MOS1 is responsible for the autoimmunity phenotype in \text{bonzai1} (\text{bon1}) and \text{snc1} [38,39] by binding to the promoter region of \text{SNCl} to activate its expression by interacting with TCP TFs [40]. MOS1 was also found to regulate floral transition by interacting with \text{Suppressor of FRIGIDA 4}, a transcriptional activator of \text{Flowering Locus C} [39]. Moreover, MOS1 plays roles in endoreduplication regulation [39]. These results show that MOS1 acts as an intermediary regulator to coordinate growth and defense in a complicated network. \text{CONSTITUTIVE EXPRESSION OF PR GENES5} (\text{CPR5}) is a regulator of growth and defense and acts in a resistance pathway dependent on Non-expresser of Pathogenesis-Related genes 1 (\text{NPR1}), a sugar hypersensitive mutant \text{hypesenescen1} allelic to the \text{cpr5} mutant [41]. However, there is no evidence of whether MOS1 is involved in sugar responses.
Mutant mos1 has been reported to have delayed flowering, increased ploidy level, and changed rosette size and other mutant phenotypes [39,40]. A previous study revealed that MOS1 antagonized MAD1 activity by interacting with MAD2 in endoreduplication regulation [40]. MAD2 loss-of-function mutants have defects in early seedling development, and these defects can be rescued by exogenous sugars [42]. Therefore, it is possible that exogenous sugar treatment also affects mos1 seedling development. To characterize the roles of MOS1 in plant development, in this study, we investigated the responses of mos1 mutants, wild-type Col-1, and mos1 complementation lines #1 and #2–9 under different sugar concentrations, including the germination rate, the expression of MOS1, a sugar-responsive gene Subunit 3 of ADP-Glucose Pyrophosphorylase (APL3) [43], and other genes involved in the sugar response pathway, i.e., HXK1 and TPS1 in the HXK1-dependent pathway [10,18], RGS1 and GPA1 in the RGS pathway [20,44], AKIN10 and AKIN11 in the SNF1-RELATED KINASE1 pathway [22,23,45,46], and SUGAR-INSENSITIVE 3 encoding an E3 ligase in an independent sugar-response pathway [47]. We found that the MOS1 knockout mutant mos1 exhibited hypersensitive responses to sugar. We hypothesized that MOS1 might participate in sugar signaling pathways and other processes related to sugar signaling. The HXK1 gene involved in sugar signaling pathways showed enhanced expression in the mos1 mutant in response to sugar. As sucrose is known as a positive factor in the accumulation of anthocyanin pigments [2] and high light stress can also boost the biosynthesis of anthocyanin [27], we speculated that MOS1 might also be involved in sugar- and light-induced anthocyanin biosynthesis. Then, we analyzed anthocyanin accumulation in wild-type Col-0 and mos1, #1, and #2–9 grown on medium supplemented with different concentrations of sugar and grown in the same soil medium but under different light intensities and found that MOS1 is involved in the regulation of anthocyanin biosynthesis triggered by sugar and light by affecting the expression of ABGs and FLS. This finding suggests that MOS1 has multiple roles in organizing sugar signaling and immune responses, thereby functioning as a coordinator in developmental, biotic, and abiotic stress responses.

2. Results

2.1. mos1 Mutant Was Hypersensitive to Sugar during Early Seedling Development

When mos1 mutant and wild-type Col-0 seeds were sown on medium supplemented with exogenous sugars, there was no difference in the germination rates of the mos1 mutant from that of wild-type Col-0 when sown on half-strength Murashige and Skoog (MS) medium supplemented with 0.8% sucrose (w/v) (normal medium; Figure S1). However, when seeds were sown on half-strength MS medium supplemented with 4% glucose, the germination rate of the mos1 mutant was reduced to 40% (Figure 1A,B), while the germination rate of wild-type Col-0 was 94% (Figure 1A,B). A similar germination phenotype was observed when seeds were sown on medium supplemented with 6% sucrose (Figure S1). However, when mos1 and wild-type Col-0 seeds were sown on half-strength MS medium supplemented with 4% mannitol (equimolar concentrations of glucose), no significant difference in germination rates was observed between them (Figure 1A,B), indicating that the defective germination of mos1 sown on medium supplemented with 4% glucose or 6% sucrose was not caused by osmotic stress but by sugars.

Furthermore, we investigated the expressions of APL3 under different sugar treatments. The expression level of APL3 in mos1 was similar to that of wild-type Col-0 when seedlings were grown on half-strength MS supplemented with 6% mannitol (Figure 1C), while it was 160% higher in mos1 when grown on half-strength MS supplemented with 6% sucrose (Figure 1C). These results were consistent with a previous study [48] and indicated that the mos1 mutant was sensitive to sugar.
To confirm whether the absence of MOS1 is responsible for sugar hypersensitivity in the mos1 mutant, we generated the MOS1 rescue construct pMOS1::MOS1::GFP and obtained two independent complementation lines, pMOS1::MOS1::GFP mos1–6 #1 (#1) and pMOS1::MOS1::GFP mos1–6 #2–9 (#2–9) (Figure S2). These two lines partially rescued the defect germination rate of mos1 grown on medium supplemented with sugar (Figure 1A,B and Figure S1), confirming that the knockdown of MOS1 is responsible for the defective germination rate and contributes to the sugar hypersensitivity of mos1.

As high-sugar treatment also affected root elongation [25], we analyzed the root lengths of the wild-type Col-0 and mos1, #1, and #2–9 seedlings grown on medium containing different concentrations of sugar for 7 d. The root lengths of mos1 were comparable to the wild-type when seedlings were grown on normal medium or half-strength MS with 2% mannitol (Figure 1D). However, when seedlings were grown on half-strength MS with 2% glucose, the roots of mos1 were shorter than those of wild-type Col-0 (Figure 1D). Complementation lines #1 and #2–9 both showed normal root elongation, as with the wild-type (Figure 1D). This finding confirmed that the shortened root in mos1 was due to the loss of function of MOS1.
2.2. Expression of MOS1 Is Induced by Sucrose

As mos1 showed hypersensitivity to sugars, to explore the role of MOS1 in sugar signaling pathways, the expression of the MOS1 gene in wild-type Col-0 under different concentrations of sucrose was investigated. Compared to the seedlings grown on normal medium, the expression level of MOS1 in seedlings grown on medium supplemented with 6% sucrose increased by 270% (Figure 2A). To elucidate the expression pattern of MOS1 in response to exogenous sucrose, the transgenic plants pMOS1::GUS harboring the β-glucuronidase (GUS) reporter gene under the promoter and the first exon of MOS1 were used. Histochemical analysis showed that the expression of GUS driven by the MOS1 promoter was related to the developmental stages of leaves and sucrose concentrations. When seedlings were grown on normal medium, strong GUS signals were detected in the emerging tissues, but only notably weak GUS signals were detected in the mature tissues. However, when seedlings were transferred to medium containing 6% sucrose, the intensity of the GUS signal was stronger, with obvious GUS signals being detected in the mature tissues (Figure 2B). These results showed that the expression of MOS1 is promoted by exogenous sucrose.

![Figure 2](image)

**Figure 2.** MOS1 expression in response to sugar. (A) qRT-PCR analysis of MOS1 expression in 10-d-old Col-0 seedlings grown on half-strength MS medium with 0.8% sucrose (S), 6% mannitol (M), or 6% S. Quantification was normalized to ACTIN2. Error bars indicate the standard error (SE) of two independent biological replicates. The asterisk indicates a significant difference compared with seedlings grown on half-strength MS medium with 0.8% sucrose (one-way ANOVA/Bonferroni p < 0.001). (B) Representative images showing the β-glucuronidase activity of the pMOS1::GUS line grown on half-strength MS medium with 0.8% S or 6% S. Scale bars = 1 cm.

2.3. MOS1 Affects the Expression of HKX1 in Response to Sugar

According to the qRT-PCR results, the expression of seven genes in several well-established sugar-response pathways was unchanged in both wild-type Col-0 and the mos1 mutant in response to sucrose, except for HKX1 and AKIN11 (Figure 3). The transcription of AKIN11 was downregulated by sucrose in both wild-type Col-0 and mos1 mutants, which made it difficult to determine whether the transcription change was associated with MOS1. However, the transcription of HKX1 was significantly upregulated by sucrose in the mos1 mutant and unchanged in wild-type Col-1 (Figure 3). This finding suggested that the mos1 mutation may influence the HKX1-dependent sugar response pathway.
were 180%, 207%, and 124% higher than those in wild-type Col-0, respectively (Figure 6A, B). However, LDOX expression was similar to that in wild-type Col-0 under normal conditions. After high-light treatment, the accumulation of anthocyanin in response to high light in wild-type Col-0 and \textit{mos1} were 400% and 62% higher than those in wild-type Col-0, respectively, but there was no difference after treatment with 6% mannitol (Figure 6D).

2.4. MOS1 Represses Anthocyanin Biosynthesis Induced by Sugar and High-Light Stress

When seedlings were grown on normal medium, mutant \textit{mos1} had comparable anthocyanin content to wild-type Col-0. However, when seedlings were grown on medium supplemented with 3% glucose or 6% sucrose, \textit{mos1} accumulated 2.5- and 2-fold more anthocyanin than wild-type Col-0, respectively (Figure 4). In addition, complementation lines \#1 and \#2–9 accumulated similar amounts of anthocyanin pigments to wild-type Col-0 under all conditions (Figure 4).

Additionally, the accumulation of anthocyanin in response to high light in wild-type Col-0 and \textit{mos1}, \#1, and \#2–9 were analyzed concurrently. As shown in Figure 5, the anthocyanin content in \textit{mos1} was similar to that in wild-type Col-0 under normal conditions. After high-light treatment, \textit{mos1} accumulated anthocyanin pigments three times those in wild-type Col-0, while the contents of anthocyanin pigments in complementation lines \#1 and \#2–9 were similar to those in wild-type Col-0 (Figure 5). This finding indicated that MOS1 could repress anthocyanin biosynthesis induced by sucrose and high-light stress, although the mechanisms governing the effect warrant further analysis.

2.5. MOS1 Affects the Expression of Genes Related to Anthocyanin Biosynthesis in Response to Sugar and High Light

To discover the molecular regulatory mechanisms of MOS1 on anthocyanin accumulation, the transcription of six early ABGs (\textit{PAL}, \textit{C4H}, \textit{CHS}, \textit{CHI}, \textit{F3H}, \textit{F3’H}), three late \textit{ABGs} (\textit{DFR}, \textit{ANS}, \textit{LDOX}), \textit{FLS}, and two components of MBW complex, \textit{PAP} and \textit{TT8}, were analyzed. After treatment with 6% sucrose, the expression levels of \textit{PAL} and \textit{F3H} and \textit{F3’H} in \textit{mos1} were 50%, 96%, and 109% higher than those in wild-type Col-0, respectively, and the expression levels of \textit{DFR}, \textit{LDOX}, and \textit{UF3GT} in \textit{mos1} were 180%, 207%, and 124% higher than those in wild-type Col-0, respectively (Figure 6A, B). However, the expression levels of \textit{DFR}, \textit{LDOX}, and \textit{UF3GT} in \textit{mos1} treated with 6% mannitol were similar to those in wild-type Col-0 (Figure 6A, B). Moreover, after sucrose treatment, the expression level of \textit{FLS} was 60% lower in \textit{mos1} than in wild-type Col-0, although its expression was also lower upon treatment with 6% mannitol (Figure 6C). Correspondingly, after treatment with 6% sucrose, the transcript levels of \textit{PAP1} and \textit{TT8} were 400% and 62% higher than those in wild-type Col-0, respectively, but there was no difference after treatment with 6% mannitol (Figure 6D).
Figure 4. Anthocyanin accumulation induced by sugar in different lines. (A) Representative images of phenotypes of Col-0 and mos1, #1, and #2–9 grown on half-strength MS medium with 0.8% sucrose (S), 6% S, or 3% G. Scale bars = 1 cm. (B) Anthocyanin content in the seedlings of Col-0 and mos1, #1, and #2–9 grown on half-strength MS medium with 0.8% S or 6% S. (C) Anthocyanin content in the seedlings of Col-0 and mos1, #1, and #2–9 grown on half-strength MS medium with 0.8% S or 3% G. Error bars indicate SE of two independent biological replicates. The asterisks indicate significant differences compared with Col-0 under the same treatment (one-way ANOVA/Bonferroni p < 0.001).
The expression of most ABGs in mos1 was equivalent to that in wild-type Col-0 under both normal and high-light conditions, except for CHS. The expression abundance of CHS after high-light treatment in mos1 was 54% higher than that in wild-type Col-0 (Figure 7A,B). In addition, the expression of FLS in mos1 after high-light treatment was similar to that under normal light, while it was increased in wild-type Col-0 after high-light treatment (Figure 7C). This finding indicates that MOS1 affects the expression of FLS. Moreover, the expression of PAPI and TT8 was similar in the wild-type Col-0 and mos1 under both normal and high-light conditions (Figure 7D). Although the responses of these genes to high light and sucrose were different in mos1, we can still conclude that MOS1 might regulate the accumulation of anthocyanin under sugar and light treatment by influencing the expression of some ABGs but through different regulatory mechanisms.

3. Discussion

Sugar signaling plays important roles in plant development and abiotic and biotic stress responses [7]. In this study, we found that the absence of the MOS1 gene function caused intense responses to sugars, as characterized by a reduced germination rate and shortened roots. Correspondingly, the expression of the glucose-responsive marker gene APL3 was increased (Figure 1), and MOS1 could respond to exogenous sucrose (Figure 2). This finding indicated that MOS1 was a negative regulator of sugar responses and that there might be transcriptional feedback to control the responses within a certain range. The higher expression of HXK1 in response to sucrose in mos1 than in wild-type Col-0 (Figure 3) suggested that MOS1 may influence sugar responses by regulating the transcriptional level of HXK1. HXKs have been identified as glucose sensors in many plant species, and recently, HXK1 was discovered to have multiple functions [9], i.e., promoting anthocyanin biosynthesis in apple by stabilizing a bHLH TF [49]. In agreement with this finding, mos1 accumulated more anthocyanin than wild-type Col-0 when exposed to exogenous sugars (Figure 4).
transcript levels of PAP1 and TT8 were 400% and 62% higher than those in wild-type Col-0, respectively, but there was no difference after treatment with 6% mannitol (Figure 6D).

Figure 6. Expression analysis of anthocyanin biosynthesis genes in response to sucrose. qRT-PCR analysis of the expression of early ABGs (A), late ABGs (B), FLS (C), and TFs (D) in 10-d-old Col-0 and mos1 after 6% mannitol (CTRL) or 6% sucrose (6%S) treatment. Quantification was normalized to ACTIN2. Error bars indicate SE of two independent biological replicates. The asterisks indicate significant differences compared with the corresponding Col-0 (one-way ANOVA/Bonferroni p < 0.001).

The expression of most ABGs in mos1 was equivalent to that in wild-type Col-0 under both normal and high-light conditions, except for CHS. The expression abundance of CHS after high-light
treatment in mos1 was 54% higher than that in wild-type Col-0 (Figure 7A,B). In addition, the expression of FLS in mos1 after high-light treatment was similar to that under normal light, while it was increased in wild-type Col-0 after high-light treatment (Figure 7C). This finding indicates that MOS1 affects the expression of FLS. Moreover, the expression of PAP1 and TT8 was similar in the wild-type Col-0 and mos1 under both normal and high-light conditions (Figure 7D). Although the responses of these genes to high light and sucrose were different in mos1, we can still conclude that MOS1 might regulate the accumulation of anthocyanin under sugar and light treatment by influencing the expression of some ABGs but through different regulatory mechanisms.

Figure 7. Expression analysis of anthocyanin biosynthesis genes in response to light. qRT-PCR analysis of the expression of early ABGs (A), late ABGs (B), FLS (C), and TFs (D) in 14-d-old Col-0 and mos1 seedlings grown on soil under normal (CTRL) or 24 h high-light (HL) treatment. Quantification was normalized to ACTIN2. Error bars indicate SE of two independent biological replicates. The asterisks indicate significant differences compared with the corresponding Col-0 (one-way ANOVA/Bonferroni p < 0.001). NS, not significant.

In addition to sugars, anthocyanin biosynthesis is triggered by multiple stresses [2,5,27,50]. The overaccumulation of anthocyanin pigments in the mos1 mutant under sugar and high-light stress compared to wild-type Col-0 (Figures 4 and 5) indicated that MOS1 negatively regulates anthocyanin biosynthesis. Sugar activated the expression of several ABGs (Figure 6) [43], which was more pronounced in mos1 (Figure 6). As mos1 exhibited increased sensitivity to sugars, the overaccumulation
of anthocyanin pigments could be a consequence of the enhanced sugar response. Sugars also enhanced the expression of FLS [2,33] (Figure 6C); as a hypersensitive mutant, mos1 should have a higher expression of FLS. However, mos1 had a significantly lower expression level of FLS than did wild-type Col-0 (Figure 6C), and the lower expression level of FLS led to dihydroflavonol accumulation as substrates for subsequent anthocyanin biosynthesis. This finding indicates that MOS1 has other mechanisms independent of sugar signals in regulating anthocyanin biosynthesis.

Moreover, the regulatory mechanisms of MOS1 in anthocyanin biosynthesis under sugar and light stresses might be different. Upon high-light treatment, only the expression of CHS in the mos1 mutant was higher than that in the wild-type, while under 6% sucrose, several ABGs but no CHS had different expression levels between the mos1 mutant and wild-type Col-0 (Figures 6 and 7). Moreover, the expression of FLS in CTRL under sugar treatment was different from that under light treatment (Figures 6 and 7), which might be due to the pretreatment in the dark before transport to medium containing different sugars compared to no pretreatment before transfer to chambers with different light intensities. Additionally, the expression of FLS was not induced by high-light treatment in mos1 (Figure 7C). All these pieces of evidence indicate the specific function of MOS1 in the transcriptional regulation of FLS. A MOS1-interacting protein [40], TCP15, represses anthocyanin biosynthesis under high light [51], suggesting that TCP15 and MOS1 might also be involved in anthocyanin biosynthesis as well as immune responses. TCP15 affects the expression level of PAP1, TT8, and DFR under high light [51], while MOS1 showed no influences on PAP1, TT8, and DFR under high light (Figure 7). That might be because the regulation mechanisms of TCP15 and MOS1 on anthocyanin synthesis do not overlap completely, just like in immune responses [40]. Additionally, we used different sampling time points from Vialo et al. [51], while the effects of TCP15 had been found to be related to the irradiation time [51]. However, MOS1 showed negative regulations on the expression of PAP1, TT8, and DFR under 6% sucrose (Figure 6). The expressions in the early part of the high-light treatment and the global gene expression changes with RNA-seq will be conducted in further studies, which will be beneficial for obtaining a better understanding of the regulation of MOS1 and the interactions of MOS1 and TCP15 on anthocyanin biosynthesis combined with genetic analysis.

Similar to anthocyanin biosynthesis, plant defense responses are affected by many factors, such as hormones, sugars, and light [52–55]. Recently, there has been increasing evidence supporting the contribution of sugar signals to plant immune responses. HXK1 plays positive roles in immune regulation, and the glucose phosphorylation capacity of HXK1 has been found to be essential for cell death and defense responses in the MIPS (myo-inositol 1-phosphate synthase) mutant [37]. MOS1 also plays positive roles in immunity, but the mos1 mutant has normal defense responses [37,39,40]. Thus, there is a possibility that the enhanced HXK1 expression in mos1 may be a compensation mechanism to maintain proper immune responses, and MOS1 might be the convergent regulator involved in the sugar-immunity regulation network.

Some studies also suggested that anthocyanin could take part in immunity in plants, but the precise underlying mechanism remains uncharacterized [56,57].

As MOS1 showed functions in anthocyanin accumulation (Figures 4 and 5), it may be worthwhile to identify convergent regulators in anthocyanin biosynthesis and immune response crosstalk, which may provide new insights into the coordinated network between immunity and other physiological processes.

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

Arabidopsis thaliana Col-0 ecotype (available in Arabidopsis information service, N1092), the mos1-6 mutant (mos1) derived from Col-0 by gamma irradiation, and pMOS1:GUS created in a previous study [38] were graciously provided by Dr. J Hua (Cornell University, USA). Two complementary lines, pMOS1::MOS1: GFP mos1–6#1 (#1) and pMOS1::MOS1: GFP mos1–6 #2–9 (#2–9), were created...
and identified in this study by Z.W. and L.Y. All plants were grown in chambers with 50% humidity at 22 °C and under 12-h light (light intensity: 150 µmol m⁻² s⁻¹) and 12-h dark.

To ensure that the plants grew normally, half-strength MS with 0.8% (w/v) sucrose was used as normal medium. For sugar treatment, 10-d-old seedlings grown on normal medium were transferred to the dark for 24 h to reduce intercellular sugar. After that step, the medium was replaced by half-strength MS medium with 3% (w/v) glucose, 6% (w/v) sucrose, 3% (w/v), and 6% (w/v) mannitol for an additional 3 h under light.

For the high-light treatment, plants were grown in soil under 150 µmol m⁻² s⁻¹ light (normal) for 14 d. Then, some plants were transferred to chambers with a light intensity of 450 mol m⁻² s⁻¹ (high light). Seedlings treated for 1 d were used for RNA isolation, and seedlings treated for 3 d were used to analyze the anthocyanin content.

4.2. Plasmid Construction and Generation of Transgenic Plants

A genomic fragment of the entire MOS1 coding region (without stop codon) and the 2680-bp sequence upstream of the ATG start codon were amplified by PCR from genomic DNA isolated from Col-0. The PCR product was cloned into the pDONR222 vector by BP reactions (Invitrogen, 11789020) and then cloned into the binary vector pGWB550 [58] to create pMOS1::MOS1:GFPCOM. The constructed vector was introduced into mos1 using Agrobacterium tumefaciens GV3101. Transgenic plants were selected on plates with hygromycin.

4.3. Germination Assay and Root Length Measurement

All seeds, harvested and stored identically, were sown on normal medium and medium containing 4% glucose or 4% mannitol. All plates were incubated at 4 °C for 2 d and then placed in a growth chamber for 7 d. The germination rate was scored by cotyledon greening. At least 50 seeds for each genotype were used for each independent biological repeat, and two repeats were conducted.

For root length measurement, seedlings were grown vertically on normal medium and medium supplemented with 2% glucose or 2% mannitol for 7 d. Images were captured by a digital camera, and the root lengths were calculated by ImageJ.

4.4. GUS Staining

To analyze GUS activity in response to sugar, pMOS1::GUS transgenic lines [30] were grown on the indicated medium. The seedlings were dipped into chilled 90% acetone and then stained in 100 mM sodium phosphate buffer (pH 7.2) containing 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, 2 mM K₃Fe(CN)₆, 2 mM K₄Fe(CN)₆, 10 mM EDTA, and 0.1% (v/v) Triton X-100 at 37 °C. After staining, 70% (v/v) ethanol was used to remove the chlorophyll. Images were recorded by a LEICA S9 stereoscope.

4.5. Measurement of Anthocyanin Content

Fresh seedlings grown on the indicated medium or after light treatment were used for measuring anthocyanin content. Leaf tissues of 20 mg were homogenized in 0.6 mL of methanol–HCl (1%, v/v) and then incubated at 4 °C for 1 d. After centrifugation, 0.4 mL chloroform and 0.4 mL ddH₂O were added to the supernatant and vortexed vigorously. Then, the samples were centrifuged, and the absorbance of the supernatant was measured at 530 and 657 nm. Relative anthocyanin concentrations were calculated with the equation

\[ \text{anthocyanin content} = \frac{(A530-A657)}{\text{fresh weight (g)}} \]

4.6. RNA Extraction and Quantitative Real-Time PCR Analysis

Total RNA was isolated from plants with RNAiso Plus (Takara, Shiga, Japan, 9108), according to the manufacturer’s instructions. cDNA was synthesized from 2 µg RNA by a PrimeScript™ RT reagent Kit with a gDNA Eraser Kit (Takara, Shiga, Japan, RR047). Quantitative RT-PCR was performed with a Bio-Rad CFX96™ Real-Time System (Bio-Rad, Hercules, USA) using TB Green Premix Ex
Taq™ II (Tli RNaseH Plus; Takara, Shiga, Japan, RR820). Primers for RT-PCR are listed in Table S1. Two independent biological replicates were performed.

5. Conclusions

We provide evidence that the immune regulator MOS1 represses sugar responses and anthocyanin biosynthesis in Arabidopsis, possibly at the transcriptional level. Our findings highlight the involvement of MOS1 in sugar signaling. In the future, identifying MOS1 genetic interacting regulators and studying the regulation of MOS1 in sugar and hormone signaling may not only help to characterize the roles of MOS1 in specific biological processes but also elucidate the mechanism governing the balance of growth and defense.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/21/19/7095/s1. Table S1: Primers sequences for RT-PCR in this study. Figure S1: Representative images of the germination of Col-0 and mos1, #1, and #2–9 grown on 1/2 MS medium with 0.8% sucrose, 6% Man, or 6% Suc. Figure S2: RT-PCR analysis of MOS1 expression in Col-0 and mos1, #1, and #2-9.

Author Contributions: Conceptualization, N.Z. and X.S.; methodology, M.W., J.H., L.Y., and Z.W.; validation, N.Z. and X.S.; formal analysis, N.Z. and X.S.; investigation, M.W.; resources, D.W.; data curation, N.Z. and M.W.; writing—original draft preparation, N.Z.; writing—review and editing, X.S.; visualization, X.S.; supervision, D.W.; project administration, D.W.; funding acquisition, N.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the China Postdoctoral Science Foundation (2019M652063).

Acknowledgments: We greatly appreciate the scientific advice from Jian Hua.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ABGs anthocyanin biosynthesis genes
AKIN SNF1 kinase homolog
APL3 Subunit3 of ADP-Glucose Pyrophosphorylase
ANS anthocyanidin
bHLH basic helix–loop–helix
bon1 bonzai1
CHI chalcone isomerase
CHS chalcone synthase
DFR dihydroflavonol
F3H flavanne-3-hydroxylase
FLS flavonol synthase
GUS β-glucuronidase
HEX1 Hexokinases 1
HL high-light
MAD1 mitotic arrest deficient1
MOS1 modifier of snc1-1
MS Murashige and Skoog
PAL Phenylalanine ammonia lyase
PAP1 Purple acid phosphatase 1
SNC1 suppressor of npr1-1, constitutive 1
TFs transcription factors
TT8 Transparent testa8

References

1. Ren, M.; Venglat, P.; Qiu, S.; Feng, L.; Cao, Y.; Wang, E.; Alexander, D.; Chalivendra, S.C.; Logan, D.C.; Mattoo, A.; et al. Target of rapamycin signaling regulates metabolism, growth, and life span in Arabidopsis. Plant Cell 2012, 24, 4850–4874. [CrossRef]
2. Solfanelli, C.; Poggi, A.; Loreti, E.; Alpi, A.; Perata, P. Sucrose-specific induction of the Anthocyanin Biosynthetic pathway in Arabidopsis. *Plant Physiol.* 2006, 140, 637–646. [CrossRef]
3. Li, Y.; Li, L.; Fan, R.; Peng, C.; Sun, H.; Zhu, S.; Wang, X.; Zhang, L.; Zhang, D. Arabidopsis sucrose transporter SUT4 interacts with cytochrome b5-2 to regulate seed germination in response to sucrose and glucose. *Mol. Plant* 2012, 5, 1029–1041. [CrossRef]
4. Van den Ende, W. Sugars take a central position in plant growth, development and stress responses. A focus on apical dominance. *Front. Plant Sci.* 2014, 5, 313. [CrossRef]
5. Bolouri Moghaddam, M.R.; Van den Ende, W. Sugars and plant innate immunity. *J. Exp. Bot.* 2012, 63, 3989–3998. [CrossRef]
6. Zhou, L.; Jang, J.; Jones, T.; Sheen, J. Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. *Proc. Natl. Acad. Sci. USA* 1998, 95, 10294–10299. [CrossRef]
7. Rolland, F.; Baena-Gonzalez, E.; Sheen, J. Sugar sensing and signaling in plants: Conserved and novel mechanisms. *Annu. Rev. Plant Biol.* 2006, 57, 675–709. [CrossRef]
8. Zheng, L.; Shang, L.; Chen, X.; Zhang, L.; Jing, H. Tang1, encoding a symplekin_C domain-containing protein, influences sugar responses in Arabidopsis. *Plant Physiol.* 2015, 168, 117–129. [CrossRef]
9. Li, L.; Sheen, J. Dynamic and diverse sugar signaling. *Curr. Opin. Plant Biol.* 2016, 33, 116–125. [CrossRef]
10. Moore, B.; Zhou, L.; Rolland, F.; Hall, Q.; Cheng, W.; Liu, Y.; Hwang, I.; Jones, T.; Sheen, J. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* 2003, 300, 332–336. [CrossRef]
11. Rook, F.; Hadingham, S.; Li, Y.; Bevan, W. Sugar and ABA response pathways and the control of gene expression. *Plant Cell Environ.* 2006, 29, 426–434. [CrossRef] [PubMed]
12. Baena-Gonzalez, E.; Rolland, F.; Thevelein, M.; Sheen, J. A central integrator of transcription networks in plant stress and energy signalling. *Nature* 2007, 448, 938–942. [CrossRef] [PubMed]
13. Sheen, J.; Cho, Y.; Baena, E.; Hall, Q.; Rolland, F.; Xiong, Y.; Yoo, S. Sugar and energy sensing and signalling networks in plants. *Photosynth. Res.* 2007, 91, 134.
14. Lei, M.; Liu, D. Sucrose regulates plant responses to deficiencies in multiple nutrients. *Plant Signal. Behav.* 2011, 6, 1247–1249. [CrossRef]
15. Ruan, Y. Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. *Annu. Rev. Plant Biol.* 2014, 65, 33–67. [CrossRef]
16. Zhang, C.; Han, L.; Slewinski, L.; Sun, J.; Zhang, J.; Wang, Z.; Turgeon, R. Symplastic phloem loading in poplar. *Plant Physiol.* 2014, 166, 306–313. [CrossRef]
17. Rolland, F.; Sheen, J. Sugar sensing and signalling networks in plants. *Biochem. Soc. Trans.* 2005, 33, 269–271. [CrossRef]
18. Avonce, N.; Leyman, B.; Mascorro-Gallardo, O.; Van Dijck, P.; Thevelein, M.; Iturriaga, G. The Arabidopsis trehalose-6-P synthase AtTPS1 gene is a regulator of glucose, abscisic acid, and stress signaling. *Plant Physiol.* 2006, 136, 3649–3659. [CrossRef]
19. Srinivasa, S.P.; Watson, N.; Overton, M.C.; Blumer, K.J. Mechanism of RGS4, a GTPase-activating protein for G protein alpha subunits. *J. Biol. Chem.* 1998, 273, 1529–1533. [CrossRef]
20. Johnston, A.; Taylor, P.; Gao, Y.; Kimple, J.; Grigston, C.; Chen, J.; Siderovski, P.; Jones, M.; Willard, F. Gtpase acceleration as the rate-limiting step in Arabidopsis protein-coupled sugar signaling. *Proc. Natl. Acad. Sci. USA* 2007, 104, 17317–17322. [CrossRef]
21. Hardie, D.G. AMP-activated/SNF1 protein kinases: Conserved guardians of cellular energy. *Nat. Rev.* 2007, 8, 774–785. [CrossRef]
22. Halford, N.; Hey, S.J. Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants. *Biochem. J.* 2009, 419, 47–259. [CrossRef]
23. Mohannath, G.; Jackel, J.N.; Lee, Y.H.; Buchmann, R.C.; Wang, H.; Patil, V.; Adams, A.K.; Bisaro, D.M. A complex containing SNF1-Related Kinase (SnRK1) and Adenosine kinase in Arabidopsis. *PLoS ONE* 2014, 9, e87592. [CrossRef] [PubMed]
24. Xiong, Y.; McCormack, M.; Li, L.; Hall, Q.; Xiang, C.; Sheen, J. Glucose-TOR signalling reprograms the transcriptome and activates meristems. *Nature* 2013, 496, 181–186. [CrossRef]
25. Sokolov, N.; Dejardin, A.; Kleczkowski, A. Sugars and light/dark exposure trigger differential regulation of ADP-glucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). *Biochem. J.* 1998, 336, 681–687. [CrossRef]
26. Schmitz, J.; Heinrichs, L.; Scossa, F.; Fernie, R.; Oelze, L.; Dietz, J.; Rothbart, M.; Grimm, B.; Flügge, I.; Häusler, E. The essential role of sugar metabolism in the acclimation response of Arabidopsis thaliana to high-light intensities. *J. Exp. Bot.* **2014**, *65*, 1619–1636. [CrossRef]

27. Mahmood, K.; Xu, Z.; El-Kereamy, A.; Casaretto, A.; Rothstein, J. The Arabidopsis transcription factor ANAC032 represses anthocyanin biosynthesis in response to high sucrose and oxidative and abiotic stresses. *Front. Plant Sci.* **2016**, *7*, 1548. [CrossRef]

28. Tanaka, Y.; Sasaki, N.; Ohmiya, A. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *Plant J.* **2010**, *54*, 733–749. [CrossRef] [PubMed]

29. Hughes, M.; Neufeld, S.; Burkey, O. Functional role of anthocyanins in high-light winter leaves of the evergreen herb Galax urceolata. *New Phytol.* **2005**, *168*, 575–587. [CrossRef]

30. Nakabayashi, R.; Yonekura-Sakakibara, K.; Urano, K.; Suzuki, M.; Yamada, Y.; Nishizawa, T.; Matsuda, F.; Kojima, M.; Sakakibara, H.; Shinozaki, K.; et al. Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids. *Plant J.* **2014**, *77*, 367–379. [CrossRef]

31. Yin, R.; Messner, B.; Faus-Kessler, T.; Hoffmann, T.; Schwab, W.; Hajirezaei, M.R.; Schäffner, A.R. Feedback inhibition of the general phenylpropanoid and flavonol biosynthetic pathways upon a compromised flavonol-3-O-glycosylation. *J. Exp. Bot.* **2012**, *63*, 2465–2478. [CrossRef]

32. Zhao, D.; Tao, J. Recent advances on the development and regulation of flower color in ornamental plants. *Front. Plant Sci.* **2015**, *6*, 261. [CrossRef]

33. Nguyen, N.; Kim, J.; Kwon, J.; Jeong, C.; Lee, W.; Lee, D.; Hong, S.; Lee, H. Characterization of Arabidopsis thaliana FLAVONOL SYNTHASE 1 (FLS1)—Overexpression plants in response to abiotic stress. *Plant Physiol. Biochem.* **2016**, *103*, 133–142. [CrossRef]

34. Zheng, T.; Yang, H.; Zhang, L.; Li, T.; Liu, B.; Zhang, D.; Lin, H. Regulation of anthocyanin accumulation via MYB75/HAT1/TPL-mediated transcriptional repression. *PLoS Genet.* **2019**, *15*, e1007993. [CrossRef]

35. Cominelli, E.; Gusmaroli, G.; Allegra, D.; Galbiati, M.; Wade, K.; Jenkins, I.; Tonelli, C. Expression analysis of anthocyanin regulatory genes in response to different light qualities in Arabidopsis thaliana. *J. Plant Physiol.* **2008**, *165*, 886–894. [CrossRef]

36. Teng, S.; Keurentjes, J.; Bentsink, L.; Smeekens, S. Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the MYB75/PAP1 gene. *Plant Physiol.* **2005**, *139*, 1840–1852. [CrossRef]

37. Bruggeman, Q.; Prunier, F.; Mazubert, C.; de Bent, L.; Garmier, M.; Lugan, R.; Benhamed, M.; Bergounioux, C.; Raynaud, C.; Delarue, M. Involvement of Arabidopsis hexokinase1 in cell death mediated by myo-inositol accumulation. *Plant Cell* **2015**, *27*, 1801–1814. [CrossRef]

38. Li, Y.; Tessaro, J.; Li, X.; Zhang, Y. Regulation of the expression of plant resistance gene SNC1 by a protein with a conserved BAT2 domain. *Plant Physiol.* **2010**, *153*, 1425–1434. [CrossRef]

39. Bao, Z.; Zhang, N.; Hua, J. Endopolyphenolization and flowering time are antagonistically regulated by checkpoint component MAD1 and immunity modulator MOS1. *Nat. Commun.* **2014**, *5*, 5628. [CrossRef]

40. Zhang, N.; Wang, Z.; Bao, Z.; Yang, L.; Wu, D.; Shu, X.; Hua, J. MOS1 functions closely with TCP transcription factors to modulate immunity and cell cycle in Arabidopsis. *Plant J.* **2018**, *93*, 66–78. [CrossRef]

41. Aki, T.; Konishi, M.; Kikuchi, T.; Fujimori, T.; Koncz-Kalmán, Z.; Stankovic-Stangeland, B.; Bakó, L.; Mathur, J.; Okrész, L.; Stabel, S.; et al. Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in Arabidopsis. *Genes Dev.* **1998**, *12*, 3059–3073. [CrossRef]

42. Ding, D.; Muthuswamy, S.; Meier, I. Functional interaction between the Arabidopsis orthologs of spindle assembly checkpoint proteins MAD1 and MAD2 and the nucleoporin NUA. *Plant Mol. Biol.* **2012**, *79*, 203–216. [CrossRef]

43. Baiar, M.; Hemmann, G.; Holman, R.; Corke, F.; Card, R.; Smith, C.; Rook, F.; Bevan, W. Characterization of mutants in Arabidopsis showing increased sugar-specific gene expression, growth, and developmental responses. *Plant Physiol.* **2004**, *134*, 81–91. [CrossRef]

44. Chen, J.; Jones, M. Atg8a1 function in Arabidopsis thaliana. *Method Enzymol.* **2004**, *389*, 338–350.

45. Németh, K.; Salchert, K.; Puttko, P.; Bhalerao, R.; Koncz-Kálmán, Z.; Stankovic-Stangeland, B.; Bakó, L.; Mathur, J.; Okrész, L.; Stabel, S.; et al. Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in Arabidopsis. *Genes Dev.* **1998**, *12*, 3059–3073. [CrossRef]

46. Bhalerao, R.; Salchert, K.; Bakó, L.; Okrész, L.; Szabados, L.; Muranaka, T.; Machida, Y.; Schell, J.; Koncz, C. Regulatory interaction of PRL1 WD protein with Arabidopsis SNF1-like protein kinases. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5322–5327. [CrossRef]
47. Huang, J.; Taylor, P.; Chen, J.; Uhrig, F.; Schnell, J.; Nakagawa, T.; Korth, L.; Jones, M. The plastid protein THYLAKOID FORMATION1 and the plasma membrane g-protein GPA1 interact in a novel sugar-signaling mechanism in Arabidopsis. Plant Cell 2006, 18, 1226–1238. [CrossRef]

48. Rook, F.; Corke, F.; Card, R.; Munz, G.; Smith, C.; Bevan, M.W. Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. Plant J. 2002, 26, 421–433. [CrossRef]

49. Hu, D.; Sun, C.; Zhang, Q.; An, J.; You, C.; Hao, Y. Glucose sensor MdHXK1 phosphorylates and stabilizes MdbHLH3 to promote anthocyanin biosynthesis in apple. PLoS Genet. 2016, 12, e1006273. [CrossRef]

50. Xu, W.; Grain, D.; Gourrierec, L.; Harscoët, E.; Berger, A.; Jauvion, V.; Scagnelli, A.; Berger, N.; Bidzinski, P.; Kelemen, Z.K.; et al. Regulation of flavonoid biosynthesis involves an unexpected complex transcriptional regulation of TT8 expression, in Arabidopsis. New Phytol. 2013, 198, 59–70. [CrossRef]

51. Viola, I.; Camoirano, A.; Gonzalez, H. Redox-dependent modulation of anthocyanin biosynthesis by the TCP transcription factor TCP15 during exposure to high light intensity conditions in Arabidopsis. Plant Physiol. 2016, 170, 74–85. [CrossRef]

52. Patricia, L.; Sheen, J. Sugar and hormone connections. Trend. Plant Sci. 2003, 8, 110–116.

53. Pieterse, M.; Leon-Reyes, A.; Van der Ent, S.; Van Wees, C. Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. 2009, 5, 308–316. [CrossRef]

54. Roden, C.; Ingle, A. Lights, rhythms, infection: The role of light and the circadian clock in determining the outcome of plant-pathogen interactions. Plant Cell 2009, 21, 2546–2552. [CrossRef] [PubMed]

55. Hua, J. Modulation of plant immunity by light, circadian rhythm, and temperature. Curr. Opin. Plant Biol. 2013, 16, 406–413. [CrossRef] [PubMed]

56. Harbone, B.; Williams, W. Cheminform abstract: Advances in flavonoid research since 1992. Phytochemistry 2000, 55, 481–504. [CrossRef]

57. Winkel-Shirley, B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 2001, 126, 485–493. [CrossRef]

58. Nakagawa, T.; Suzuki, T.; Murata, S.; Nakamura, S.; Hino, T.; Mako, K.; Tabata, R.; Kawai, T.; Tanaka, K.; Niwa, Y.; et al. Improved Gateway binary vectors: High-performance vectors for creation of fusion constructs in transgenic analysis of plants. Biosci. Biotechnol. Biochem. 2007, 71, 2095–2100. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).