Primary Metabolism and Transcriptional Regulation in Higher Plants

Natsuki Hayami 1 and Yoshiharu Y. Yamamoto 1,2,3*

1 The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan
2 Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan
3 RIKEN CSRS, 1-7-22 Suehiro-cho, Tsurumi-ku, Kanagawa, 230-0045, Japan

ABSTRACT
Metabolism, which is a flow of metabolites, is orchestrated by gene expression, and it is in turn regulated by metabolites. Some of the metabolites are known to act as signaling molecules that regulate metabolism and also developmental processes in plants. Here we summarize findings about metabolites-directed gene regulation, focusing on primary metabolites including sugars and organic acids. Abundant soluble sugars such as sucrose and glucose are known to induce genes involved in starch storage, synthesis and degradation. These sugars also activate anthocyanin biosynthesis and suppress photosynthetic genes, both of which promote photoprotection responses. Several kinases, like hexokinase, sucrose non-fermenting1-related kinase 1 and target of rapamycin kinase have been identified, which are the major regulators of transcriptional regulation by these sugars. Although less studied than sugars, metabolites constituting the TCA cycle are good candidates of signaling molecules, because they are located in the primary metabolism adjacent to the respiratory electron transport system and thus are reflected by both primary metabolism and redox status of the electron transport system. Some organic acids as citrate, malate and 2-oxoglutarate have also been known to be implicated in the regulation of gene expression. Deficiency or over accumulation of some primary metabolites not only modulate gene expression of the local metabolic enzymes to supplement distorted metabolism but produce signals to balance between bigger metabolic blocks like carbon and nitrogen metabolisms. Status of primary metabolites can also produce signals of overall energy levels of plants. Relationship of sugar signaling to reactive oxygen species signaling is also suggested.

Keywords
2-oxoglutarate, citrate, glucose, primary metabolism, sucrose, transcriptional regulation

1. Introduction
Ability of plants to adapt to changing environmental conditions is one of the main determinants of crop yield. Typical mechanisms of the adaptation are composed of perception of environmental change by specific environmental sensors and subsequent signal transduction to lead alteration of gene expression. A major characteristic of this type of the mechanisms is specificity in both environmental perception and response. Higher plants have additional regulatory mechanisms which respond to internal conditions within cells, not to external ones. These include transcriptional responses to reactive oxygen species (ROS) and to primary metabolites. Accumulation of ROS is caused by the unbalanced metabolic flow or energy production processes and observed as a symptom of all the known environmental stresses in higher plants. Therefore, it is reasonable that ROS is a general signal for all the environmental stresses [1].

Regarding transcriptional regulation by primary metabolites, substantial examples have been reported by feeding experiments. Potential role of this type of regulations would be restoring metabolic balance after it’s
distortion due to environmental stresses or alteration of metabolic flow by developmental programs. It is expected that a broad role of these regulation is to cover a wide range of stress responses by enabling ROS signaling. However, the absolute role of these metabolites is largely unknown because there have been few studies that identify the physiological roles of primary metabolites in gene regulation.

Here we summarize the findings about metabolites-directed gene regulation, focusing on primary metabolites including sugars and organic acids. Although studies on metabolites-directed gene regulation in the field of molecular plant biology have been started more than 30 years ago, as mentioned above, those studies often failed to properly explore their roles. We hope summarization of the previous works would help re-interpretation of the results and support upcoming studies on environmental adaptation.

2. Transcriptional regulation by sucrose and glucose

Sugars are well-known signaling molecules that regulate growth, development and metabolism by controlling transcription, translation, enzyme activity and protein stability in plants [2, 3]. Sucrose is one of the most common and abundant carbon forms in plants. It is known to induce many genes involved in starch storage, biosynthesis and degradation. These include genes for sporamin [4], patatin [5], ADP-glucose pyrophosphorylase (AGPase) [6], β-amylase [7] and Granule-bound starch synthase [8]. Genes for sporamin and β-amylase are activated via Ca signal where Ca-dependent protein kinases are involved [9, 10]. Two maize genes, SHRUNKEN-1 (SH1) and SUCROSE SYNTHASE 1 (SUS1), encode sucrose synthase isozymes with very similar characteristics [11]. Depending on concentration of applied glucose, it is reported that low concentration of glucose activates SH1 while high concentration does SUS1. These examples demonstrate differential induction depending on glucose concentration. In some cases, the tissue specificity of their gene expression is altered by glucose concentration, even though overall enzymatic activity remains unchanged [12]. It has been reported that soybean vegetative storage protein genes, VSPA and VSPB, are activated by soluble sucrose, although the activation requires methyl jasmonate in addition to sucrose [13].

Sucrose induces NITRATE REDUCTASE (NR) gene expression in etiolated Arabidopsis seedlings, so that they assimilate nitrogen when sufficient carbohydrates are present [14]. Expression of nitrate transporter, NRT1.1 and NRT2.1 increases in the light period and decreases in the dark period but is strongly expressed in the dark due to the additional sucrose in Arabidopsis. This suggests that regulation of nitrogen uptake and photosynthesis is, at least in part, mediated by the transcriptional regulation of NRT1.1 and NRT2.1 by sucrose [15]. Sucrose also induces NRT2.4, NRT1.5, peptide transporter (PTR) family protein, At3g16180 and At5g62680, ammonium transporter AMT1.3, Zinc transporter ZIP11, inorganic phosphate transporter PHT3.1 and PHT1.4, potassium ion transporter KUP2 and HAK5, cyclic nucleotide gated channels 11 (CNGC11), yellow stripe-like transporter 4 (YSL4) and sulfate transporter SULTR1.1 and SULTR3.5 gene expression in Arabidopsis [16, 17]. In addition, assimilation of various mineral nutrients into amino acids is also regulated by sucrose.

Sugar-induced anthocyanin accumulation for enhanced photoprotection under sufficient sugar has been observed in many plant species. In petunia, one of the two genes for chalcone synthase (CHS-A) that expressed actively in the floral organs, is induced by sucrose, glucose and fructose. The CHS-A promoter was revealed to contain consensus sequences for sugar responses which was identified from promoter regions of the sporamin gene family [4, 18]. Teng et al. showed that sucrose is the most effective inducer of anthocyanin biosynthesis in Arabidopsis seedlings [19]. Note that sucrose activation of the Arabidopsis dihydroflavonol reductase gene (DFR) requires induction of a key transcription factor, MYB75/PAP1 [19]. Recent researches showed that sucrose suppresses DELLA, which is a signal transducer of the gibberellin signaling, activates MYB75/PAP1, CHS and DFR.
expression [20]. These regulations lead to the inhibition of cell expansion and anthocyanin biosynthesis by a single regulatory gene.

In 1990, seven photosynthetic genes were shown to be repressed specifically and coordinately by sucrose, glucose and acetate in a transient expression assay in maize mesophyll protoplasts [21]. Notably, sugars and acetate are shown to suppress transcription through distinct cis-regulatory elements within the promoter regions of these regulated genes. Subsequently, Krapp et al. [22] reported that not only carbohydrate feeding, but also accumulation of carbohydrate due to drift inhibition suppressed ribulosebisphosphate carboxylase small subunit (rbcS) gene expression in Chenopodium cells, potato and tobacco plants. Expression of rbcS was not suppressed by glucose analogue like 6-deoxyglucose or 3-O-methyl-glucose, which are not metabolized but have potential to be recognized as signal molecules. These examples indicate that glucose is not the direct signal but metabolic flow after glucose is required for regulation of rbcS transcript level [22]. Furthermore, Sucrose suppresses plastocyanin (PC) gene expression transiently in Arabidopsis [23].

Expression of α-amylase gene in rice suspension culture was repressed by sucrose, glucose and fructose. The synthesis and secretion of α-amylase are also found dependent on the level of sucrose. The derepression or repression of α-amylase synthesis can be readily reversed by deprivation or replenishment of sucrose [24]. The sugar beet BvSUT1 gene encoding the proton-sucrose symporter was specifically repressed by sucrose and not by glucose [25]. Regulation of apical dominance by sucrose in pea is also reported [26]. Artificial increasing of sucrose levels repressed expression of branched1 (BRC1) transcription factor and promoted axillary bud outgrowth. Application of sugar was sufficient for buds to be released from apical dominance without decapitation.

Hexokinase (HXK) is the first enzyme in the hexose assimilation pathway and also acts as a sensor for sugar responses in plants [27]. Glucose induces phenylalanine ammonia-lyase (PAL) and cell-wall invertase (CIN) gene expression in Chenopodium, and this induction is achieved through the HXK-independent pathway [28]. An AtHXK1-dependent pathway in Arabidopsis for sugar response regulates photosynthesis-related genes and other genes, including chlorophyll a/b-binding protein (CAB1), enhanced response to ABA (ERA1), plastocyanin (PC), phospholipase (PLD), small subunit of Rubisco, NRT2.1, NRT2.4, NRT1.1 NRT1.5, At3g16180 (PTR), AMT1.3, SULTR1.1, SULTR3.5, ZIP11 and KUP2. An AtHXK1-independent pathway for the sugar response was also identified, and it was revealed to regulate pathogenesis-related1 (PR1), PR5, AGPase, CHS, PAL1, asparagine synthase1 (ASI), HAK5, At5g62680 (PTR), PHT3.1, PHT1.4 and CNGC11 [17, 29]. Regulation of biosynthesis and degradation of indole-3-acetic acid (IAA) by sugars requires transcriptional regulation of multiple genes linked to several IAA biosynthetic pathways. Expression of these genes has been suggested to be regulated in both HXK1-dependent and -independent pathways, and also by phytochrome-interacting factor (PIF) transcription factors [30].

SNF1-RELATED KINASE 1 (SnRK1) is a key regulator of plant responses to cellular energy status associated with deprivation of nutrients [31]. It has been reported that expression of Arabidopsis basic-region leucine zipper 3 (bZIP3) was repressed by sugar through a SnRK1-dependent pathway [32]. Plants overexpressing bZIP3 showed aberrant shaped cotyledons, suggesting that bZIP3 plays a role in leaf morphology in addition to sugar response.

Further research showed that the target of rapamycin (TOR) kinase that is considered as a key regulator of growth, metabolism and stress responses in yeasts, plants and animals is regulated by diverse nutrient, energy, hormone and stress [31]. Analyses with chemical inhibitors, like a glycolysis blocker (2-deoxyglucose), a mitochondrial electron transport inhibitor (antimycin A) and a mitochondria uncoupers (2,4-dinitrophenol and carbonylcyanide m-chlorophenylhydrazone 5) revealed that glycolysis and active electron transport chain in the mitochondria are required to activate the TOR signaling. Glucose-TOR signaling regulates gene expression involved in glycolysis, TCA cycle, ribosome biogenesis, and synthesis of proteins, amino acids, lipids, and nucleotides [33].
Transcriptome analyses are also introduced to elucidate the metabolite regulation of gene expression. cDNA microarray analysis was performed in *Arabidopsis* seedlings to observe effect of sucrose on 11 genes including ones associated with carbohydrate and amino acid metabolism and found that those genes were upregulated in the absence of sucrose [34]. GeneChip analysis using *Arabidopsis* suspension cultured cells revealed that genes for carbohydrate, amino acid, protein and lipid catabolism as well as for autophagy were upregulated by sucrose starvation. These identified genes would be utilized for extensive nutrient salvage for survival under starvation. Although these cultures were non-photoautotroph, several photosynthesis-related genes were also upregulated. The down-regulated genes identified included ones for translation and cell division [35]. The *Arabidopsis* phosphorus-deficient 3 (*pho3*) mutant accumulates excess amount of sucrose and some other carbohydrates. Expression of many genes involved in primary carbon assimilation were decreased a little but significantly in the *pho3* mutant, suggesting that there is a limited feedback regulation on gene expression by sugars in mature *Arabidopsis* leaves [36]. The mutant showed a striking increase in expression of plastidic glucose 6-phosphate/phosphate translocator 1 and 2 (*GPT1* and *GPT2*). In the mutants, a large increase was also observed in the expression of transcription factors and enzymes involved in anthocyanin biosynthesis [18, 36]. Nitrogen is the most abundant inorganic element required by plants. Plants can sense and respond to changes in levels of carbon and nitrogen metabolites, which is called as C/N sensing and signaling [37, 38]. In typical experiments, seedlings were grown on a medium supplemented with glucose and/or nitrogen (ammonium or nitrate). Glucose had a greater effect on nitrogen metabolic gene regulation than nitrogen itself. Genes associated with carbohydrate metabolism, signal transduction, metabolite transport and stress response were also induced by glucose [39]. Phosphorus-starved or -replenished *Arabidopsis* plants were incubated with or without sucrose, and found that, several previously identified sugar responsive genes were regulated by phosphorus starvation. Likewise, phosphorus responsive genes were regulated by sugar [40]. Total 66 genes showed greater positive responses to simultaneous phosphorus and sucrose application, but less responses to individual applications, demonstrating synergetic effect of phosphorus-replenishment and sucrose addition for growth and development under ample nutrient conditions. In addition, 47 genes were up-regulated only under phosphorus-starved and sucrose treatment, revealing a special strategy under phosphate starvation with excess sugar [40]. Analyses of starchless phosphoglucomutase (pgm) mutants revealed that sugars modify expression of up to half of the clock-regulated genes, suggesting tight relationship between the sugar response and circadian regulation [41].

### 3. Transcriptional regulation by T6P

Trehalose 6-phosphate (T6P) is the precursor of trehalose. Because the amount of T6P changes in parallel with sucrose level, it was proposed that T6P is a signal molecule of sucrose availability to control amounts of sucrose and starch [42]. T6P suppresses SnRK1, which plays a central role in controlling energy level. There are multiple mechanisms for the suppression of SnRK1 by T6P in higher plants, and one of the mechanisms is suppression of gene expression of SnRK1 [43]. Genes located at the downstream of the SnRK1 signaling such as *FBPase*, *TPS8*, *TPS11*, *UDPGE*, and *KINγ*, were all down-regulated in transgenic potato plants with increased T6P levels, and these genes were up-regulated with decreased T6P levels [44]. In *Arabidopsis*, *ASNI*, *bGAL*, *AKINβ*, *TPS8*, and *TPS10*, which are up-regulated by the SnRK1 signaling, were down-regulated by T6P, and, *UDPGDH*, *MDH*, *bZIP11*, and *TPS5*, which are repressed by SnRK1, were up-regulated by T6P [45]. These results of *Arabidopsis* are consistent with the results of potato. These reports suggest T6P suppression of SnRK1 is the central part of regulation of the target genes by SnRK1.
Table 1: Reported gene regulation by sugars

| metabolite | regulation | gene | plant | memo | ref |
|------------|------------|------|-------|------|-----|
| sucrose    | up         | SPORAMIN | sweet potato |      | [4] |
| sucrose    | up         | PATATIN | potato |      | [5] |
| sucrose    | up         | ADP-glucose pyrophosphorylase (AGPase) | potato |      | [6] |
| sucrose,   | up         | beta-AMYLASE | sweet potato |      | [7] |
| glucose,   | up         | GRANULE-BOUND STARCH SYNTHASE | potato |      | [8] |
| fructose   | up         | SHRUNKEN1 (SH1), & SUCROSE SYNTHASE (SUS1) | maize |      | [11] |
| sucrose,   | up         | VEGITATIVE STRAGE PROTEIN A & B (VSPA & BSPB) | soybean | requirement of JA | [13] |
| glucose,   | up         | NITRATE REDUCTASE (NR) | Arabidopsis |       | [14] |
| fructose   | up         | NRT1.1, NRT2.1, NRT1.1, NRT2.4, NRT1.5, Aβ5g16180 (PTR), AMT1.3, ZIP11, SULTR1.1, SULTR3.5, KUP2 | Arabidopsis | HXK1-dependendent pathway | [16][17] |
| sucrose    | up         | Aβ5g62680 (PTR), PHT3.1, PHT1.4, CNGC11 | Arabidopsis | HXK1-independent pathway | [16][17] |
| sucrose    | up         | YSL4 | Arabidopsis |       | [16][17] |
| sucrose,   | down       | CHS-A | petunia |      | [18] |
| glucose,   | down       | MYB75/PAP1 | Arabidopsis |       | [19] |
| fructose   | down       | DELLA | Arabidopsis |       | [20] |
| sucrose    | up         | MYB75/PAP1, CHS, DFR | Arabidopsis |       | [20] |
| sucrose,   | down       | C4ppdkZm1, C4pepcZm1, C4meZm1, cabZm1, cabZm5, rbcSZm1, rbcSZm3 | maize |      | [21] |
| glucose,   | down       | C4ppdkZm1, C4pepcZm1, C4meZm1, cabZm1, cabZm5, rbcSZm1, rbcSZm3 | maize |      | [21] |
| fructose   | down       | C4ppdkZm1, C4pepcZm1, C4meZm1, cabZm1, cabZm5, rbcSZm1, rbcSZm3 | maize |      | [21] |
| acetate *  | down       | C4ppdkZm1, C4pepcZm1, C4meZm1, cabZm1, cabZm5, rbcSZm1, rbcSZm3 | maize |      | [21] |
| sucrose,   | down       | rbcS | Chenopodium, potato, tobacco | up-regulation by sucrose-deprivation | [22][23][24] |
| glucose,   | down       | PLASTCYANIN (PC) | Arabidopsis |       | [23] |
| fructose   | down       | alpha-amylase | rice |      | [24] |
| sucrose    | down       | BvSUT1 | sugar beet | no repression by glucose | [25] |
| Sugar/Glucose | Regulation | Gene/Pathway                                                                 | Plant/Species           | Source |
|--------------|------------|------------------------------------------------------------------------------|-------------------------|--------|
| sucrose      | down       | BRANCHED1 (BRC1)                                                            | pea                     | [26]   |
| sugar, glucose | up        | PHENYLALANINE AMMONIA-LYASE (PAL), CELL-WALL INVERTASE (CIN)               | Chenopodium             | [28]   |
| sugar, glucose | up        | ENHANCED RESPONSE TO ABA (ERA1), PHOSPHOLIPASE (PLD)                        | *Arabidopsis*           | [29]   |
| sugar, glucose | down      | CHLOROPHYLL a/b-BOINDING PROTEIN 1 (CAB1), PLASTOCYANIN (PC), SMALL SUBUNIT OF RUBISCO (rbcS) | *Arabidopsis*           | [29]   |
| sugar, glucose | up        | PR1, PR5, AGPase, CHS, PAL1                                                 | *Arabidopsis*           | [29]   |
| sugar, glucose | down      | ASPARAGINE SYNTHASE1 (AS1)                                                  | *Arabidopsis*           | [29]   |
| sucrose, glucose, fructose | down | bZIP3                                                                      | *Arabidopsis*           | [33]   |
| T6P          | down       | SnRK1                                                                        |                         | [43]   |
| T6P          | down       | FBPase, TPS8, TPS11, UDPGE, KINγ                                           | potato                  | [44]   |
| T6P          | down       | ASN1, bGAL, AKINβ, TPS8, and TPS10                                         | *Arabidopsis*           | [45]   |
| T6P          | up         | SnRK1, UDPGDH, MDH, bZIP11, and TPS5                                        | *Arabidopsis*           | [45]   |
| D-allose     | down       | OsGAE1, OsRPA1, cycOs2, OsXTR4, SLR1                                        | rice                    | [47]   |
| D-allose     | up         | OsNCED1, OsNCED2 and OsNCED3, OsABA8ox1, OsABA8ox2, and OsABA8ox3         | rice                    | [48]   |
| D-allose     | up         | OsABF1, WSI18                                                               | rice                    | [48]   |
| D-allose     | up         | AtABI5                                                                      | *Arabidopsis*           | [48]   |

Genes regulated by sugars are summarized. In the column indicated as regulation, up means genes are induced by metabolites, down means genes are repressed by metabolites. The column indicated as ref shows reference numbers. T6P indicates trehalose 6-phosphate. *, Acetate is not sugars, but it suppress photosynthetic genes more strongly than sugars [21].
Furthermore, microarray analysis revealed that one of the rare sugars, D-allose, that is the D-glucose epimer at C3, upregulated many defense-related and PR protein genes and confers resistance to bacterial blight disease in rice [46]. This D-allose also induces growth arrest in rice, and this is caused by suppression of gibberellin-responsive genes, OsGAE1, OsRPA1, cycOs2 and OsXTR4, which are at downstream of SLR1. This suppression is achieved through the HXK-dependent pathway [47]. In addition, D-allose induces ABA biosynthesis genes, OsNCED1, OsNCED2 and OsNCED3. Inhibitor and mutant analyses revealed that induction of an ABA-responsive positive regulator of the ABA signaling, OsABF1, by D-allose is achieved through the HXK-dependent pathway. *Arabidopsis AtABI5* is known as the orthologue of rice OsABF1, is upregulated by D-allose, and requires phosphorylation of D-allose to D-allose 6-phosphate by hexokinase [48].

4. Transcriptional regulation by organic acids

Metabolites constituting the TCA cycle are good candidates of signaling molecules, because they are located in the primary metabolism adjacent to the respiratory electron transport system and thus are reflected by both primary metabolism and redox status of the electron transport system (ETS). Although some organic acids such as citrate, malate and 2-oxoglutarate (2-OG) have been known to be implicated in the regulation of gene expression [49, 50, 51, 52, 53], other organic acids have not been intensively studied in this point of view.

Nitrate assimilation consumes protons but produces malate to neutralize the alkalinization by proton consumption. Nitrate assimilation also consumes 2-OG for acceptance of ammonium in the GOGAT pathway. Muller *et al.* reported that the NITRATE REDUCTASE transcript was decreased after feeding with malate but not with 2-OG in tobacco [49]. As organic acids are integrated in the nitrate assimilation, crosstalk of signaling from organic acids with nitrate signaling is reasonable. Malate also activates gene expression of *Arabidopsis thaliana* dicarboxylate transporter (*AttDT*), which is a vacuolar malate transporter [50]. *Alternative oxidase (AOX)* is the terminal oxidase of the mitochondrial alternative respiratory pathway, and plays important roles when the cytochrome pathway of mitochondrial electron transport is reduced and stagnant under various stress conditions. Because of its expression profiles, *AOX* is a well-known molecular marker for ROS signal form mitochondrial ETS [54]. Supply of exogenous TCA cycle intermediates, citrate, malate and 2-OG caused rapid and dramatic accumulation of *AOX1* transcripts in tobacco cultured cells [51]. In *Arabidopsis*, *AOX1a* expression is increased by citrate accumulation [53], as in the case of tobacco [52]. These reports suggest a link between signaling of the organic acids and of ROS.

Effect of exogenously supplied malate and citrate on *Arabidopsis* gene expression was investigated by transcriptome analysis [21]. Gene Ontology (GO) analysis revealed that several categories for molecular function and biological processes such as photosynthesis, cell wall, biotic stress responses, and protein synthesis up- or down-regulated by both citrate and malate. On the other hand, TCA cycle, nitrogen metabolism, sulfur assimilation, and DNA synthesis and signaling were regulated by citrate and not by malate, whereas glycolysis and abiotic stress were regulated by malate and not by citrate. Regarding the effect of citrate feeding on metabolism, sugar contents (sucrose, glucose, fructose and galactose), amino acids (glutamate, tyrosine and phenylalanine) and organic acids (citrate and succinate) were found increased. Citrate repressed genes for photosynthesis. As mentioned before, these genes are also repressed by sucrose, so the effect of citrate on these genes may be caused through the increase in sugars. Gene expression of *Arabidopsis FERRITIN1 (FER1)* was activated by citrate and not by isocitrate, any other organic acids, or sugars, suggesting citrate-specific activation of *FER1* [55]. These findings demonstrate multiple pathways or flows for gene regulation by citrate feeding. 2-OG is a metabolic intermediate in both TCA cycle and nitrate assimilatory pathway, and is proposed as a key signaling molecule in plants [56]. The 2-OG dehydrogenase complex
(2-OGDHC) constitutes a mitochondrion-localized TCA cycle multienzyme system responsible for the conversion of 2-OG to succinyl-coenzyme A concomitant with NAD+ reduction. The 2-OGDHC activity is inhibited by the phosphonate analog, succinyl-phosphonate [57]. Genes associated with anabolic processes, protein synthesis, amino acid metabolism, nitrogen metabolism, RNA processing and histone methyl transferase were downregulated by succinyl-phosphonate. Up-regulated genes included chloroplast ribosomal proteins, adenylosuccinate lyase and an enzyme of the purine nucleotide cycle. Succinyl-phosphonate activates xyloglucan endotransglycosylase 6 (XTR6) involved in cell wall expansion [58]. This is consistent with previous reports of reduction of cell wall biosynthesis by inhibition of the TCA cycle [57, 59].

| metabolite       | regulation | gene                                      | plant       | memo                        | ref  |
|------------------|------------|-------------------------------------------|-------------|-----------------------------|------|
| malate           | down       | NITRATE REDUCTASE (NIR)                   | tobacco     |                             | [49] |
| malate           | up         | vacuolar malate transporter (AttDT)       | Arabidopsis |                             | [50] |
| citrate, malate, | up         | ALTERNATIVE OXIDASE 1 (AOX1)              | tobacco     |                             | [51] |
| 2-oxoglutarate   |            |                                           |             |                             |      |
| citrate          | down       | AOX2                                      | Arabidopsis | ROS-activated gene          | [52] |
| citrate          | up         | AOX1a                                     | Arabidopsis | ROS-activated gene          | [53] |
| citrate          | up         | FERRITIN1 (FER1)                          | Arabidopsis | not activated by iso-citrate| [55] |

Genes regulated by sugars are summarized. In the column indicated as regulation, up means genes are induced by metabolites, down means genes are repressed by metabolites. The column indicated as ref shows reference numbers.

5. Conclusion

In this review, we summarize findings about metabolites-directed gene regulation, focusing on primary metabolites including sugars and organic acids. Although some reviews have been found on transcriptional regulation of metabolites of mammals [60, 61], information on plants is inadequate.

Accumulation of soluble sugars like sucrose or glucose promotes sugar utilization by inducing starch storage and biosynthesis and anthocyanin biosynthesis, and suppresses sugar synthesis by repressing primary carbon assimilation including photosynthesis. These are thought to be the feedback regulation mechanisms to keep the balance of sugar content in plants. However, these mechanisms have been observed in feeding experiments or mutants, but their physiological roles are not well understood in wild-type plants in the field. Sugar is known to be accumulated not only by light and CO2, but also dehydration and low temperature stress treatment [62], but to adapt these stresses, it is necessary to accumulate sugar which is a compatible solute. To understand the physiological roles of transcriptional regulation by soluble sugars, more data on sugar accumulation and gene expression under various stress conditions will be needed in the future.

In summary, deficiency or over accumulation of some primary metabolites not only modulate gene expression of the local metabolic enzymes to supplement distorted metabolism but also produces signals to balance between bigger metabolic blocks like carbon and nitrogen metabolisms, carbon metabolism and photosynthesis/photoprotection, and so on. Relationship of these metabolites to ROS is also suggested. Furthermore, status of primary metabolites can produce signals of overall energy levels of plants.

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REFERENCES

[1] Hieno A, Hushuna Ara N and Yamamoto YY (2019) H2O2-mediated biotic and abiotic stress responses in plants. In: Redox Homeostasis in Plants: From Signalling to Stress Tolerance (Panda PK and Yamamoto YY, ed.) pp.19–42. Springer.

[2] Rolland F, Baena-Gonzalez E and Sheen J (2006) Sugar sensing and signaling in plants: Conserved and novel mechanisms. Annu. Rev. Plant Biol., 57 (1): 675–709.

[3] Li L and Sheen J (2016) Dynamic and diverse sugar signaling. Curr. Opin. Plant Biol., 33: 116–125.

[4] Hattori T, Nakagawa S and Nakamura K (1990) High-level expression of tuberous root storage protein genes of sweet potato in stems of plantlets grown in vitro on sucrose medium. Plant Mol. Biol., 14(4): 595–604.

[5] Jefferson R, Goldsbrough A and Bevan M (1990) Transcriptional regulation of a patatin-1 gene in potato. Plant Mol. Biol., 14(6): 995–1006.

[6] Müller-Röber BT, Koßmann J, Hannah LC, Willmitzer L and Sonnewald U (1990) One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. MGG Mol. Gen. Genet., 224(1): 136–146.

[7] Nakamura K, Ohno MA, Yoshida N and Nakamura K (1991) Sucrose-induced accumulation of β-amylase occurs concomitant with the accumulation of starch and sporamin in leaf-petiole cuttings of sweet potato. Plant Physiol., 96(3): 902–909.

[8] Visser RGF, Stolte A and Jacobsen E (1991) Expression of a chimaeric granule-bound starch synthase-GUS gene in transgenic potato plants. Plant Mol. Biol., 17(4): 691–699.

[9] Ohno M, Hayashi K, Ishobe M and Nakamura K (1995) Involvement of Ca²⁺ signalling in the sugar-inducible expression of genes coding for sporamin and β-amylase of sweet potato. Plant J., 7(2): 297–307.

[10] Ohto M and Nakamura K (1995) Sugar-induced increase of calcium-dependent protein kinases associated with the plasma membrane in leaf tissues of tobacco. Plant Physiol., 109(3): 973–981.

[11] Echt CS and Choure P (1985) A comparison of two sucrose synthetase isozymes from normal and shrunken-1 maize. Plant Physiol., 79(2): 530–536.

[12] Koch KE, Nolte KD, Duke ER, McCarty DR and Avigne WT (1992) Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell., 4(1): 59–69.

[13] Mason HS, DeWald DB, Creelman RA and Mullet JE (1992) Coregulation of soybean vegetative storage protein gene expression by methyl jasmonate and soluble sugars. Plant Physiol., 98(3): 859–867.

[14] Cheng CL, Acero GN, Cristinisi M and Conkling MA (1992) Sucrose mimics the light induction of Arabidopsis nitrate reductase gene transcription. Proc. Natl. Acad. Sci. U. S. A., 89(5): 1861–1864.

[15] Lejay L, Tillard P, Lepeitit M, Olive FD, Filleur S, Daniel-Vedele F and Gojon A (1999) Molecular and functional regulation of two NO₃⁻ uptake systems by N- and C-status of Arabidopsis plants. Plant J., 18(5): 509–519.

[16] Lejay L, Gansel X, Cerezo M, Tillard P, Müller C, Krapp A, Von Wirén N, Daniel-Vedele F and Gojon A (2003) Regulation of root ion transporters by photosynthesis: Functional importance and relation with hexokinase. Plant Cell., 15(9): 2218–2232.

[17] Lejay L, Wirth J, Pervent M, Cross JMF, Tillard P and Gojon A (2008) Oxidative pentose phosphate pathway-dependent sugar sensing as a mechanism for regulation of root ion transporters by photosynthesis. Plant Physiol., 146(4): 2036–2053.

[18] Tsukaya H, Ohshima T, Naito S, Chino M and Komeda Y (1991) Sugar-dependent expression of the CHS-A gene for chalcone synthase from petunia in transgenic Arabidopsis. Plant Physiol., 97(4): 1414–1421.

[19] Teng S, Keurentjes J, Bentsink L, Koornneef M, and Smeekens S (2005) Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the MYB75/PAP1 gene. Plant Physiol., 139 (4): 1840–1852.

[20] Li Y, Van den Ende W and Rolland F (2014) Sucrose induction of anthocyanin biosynthesis is mediated by DELLA. Mol. Plant., 7(3): 570–572.

[21] Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell, 2(10): 1027–1038.

[22] Krapp A, Hofmann B, Schafer C and Mark Stitt (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the “sink regulation” of photosynthesis? Plant J., 3(6): 817–828.

[23] Dijkwel PP, Kock PAM, Bezemer R, Weisbeek PJ and Smeekens SCM (1996) Sucrose represses the developmentally controlled transient activation of the plastocyanin gene in Arabidopsis thaliana seedlings. Plant Physiol., 110(2): 455–463.

[24] Yu SM, Kuo YH, Sheu G, Sheu YJ and Liu LF (1991) Metabolic derepression of α-amylase gene expression in suspension-cultured cells of rice. J. Biol. Chem., 266(31): 21131–21137.

[25] Tzyy-Jen Chiou and Bush DR (1998) Sucrose is a signal molecule in assimilate partitioning. PNAS April 14, 1998 95 (8): 4784–4788.
[26] Mason MG, Ross JJ, Babst BA, Wienclaw BN and Beveridge CA (2014) Sugar demand, not auxin, is the initial regulator of apical dominance. Proc. Natl. Acad. Sci. U. S. A., 111(16): 6092–6097.

[27] Jang JC, León P, Zhou L and Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell, 9(1): 5–19.

[28] Ehness R, Ecker M, Godt DE and Roitsch T (1997) Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. Plant Cell, 9(10): 1825–1841.

[29] Xiao W, Sheen J and Jang JC (2000) The role of hexokinase in plant sugar signal transduction and growth and development. Plant Mol. Biol., 44(4): 451–461.

[30] Sairanen I, Novák O, Pěnčík A, Ikeda Y, Jones B, Sandberg G and Ljung K (2013) Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. Plant Cell., 24(12): 4907–4916.

[31] Smeekens S, Ma J, Hanson J and Rolland F (2010) Sugar signals and molecular networks controlling plant growth. Current Opinion in Plant Biology, 13(3): 273–278.

[32] Sanagi M, Lu Y, Aoyama S, Morita Y, Mitsuda N, Ikeda M, Ohme-Takagi M, Sato T and Yamaguchi J (2018) Sugar-responsive transcription factor bZIP3 affects leaf shape in Arabidopsis plants. Plant Biotechnol., 35(2): 167–170.

[33] Xiong Y, McCormack M, Li L, Hall Q, Xiang C and Sheen J (2013) Glucose-TOR signalling reprograms the transcriptome and activates meristems. Nature, 496(7444): 181–186.

[34] Lee EJ, Koizumi N and Sano H (2004) Identification of genes that are up-regulated in concert during sugar depletion in Arabidopsis. Plant, Cell Environ., 27(3): 337–345.

[35] Contiento AL, Kim SJ and Bassham DC (2004) Transcriptome profiling of the response of Arabidopsis suspension culture cells to Suc starvation. Plant Physiol., 135(4): 2330–2347.

[36] Lloyd JC and Zakhleniuk O V (2007) Genome-wide analysis of the Arabidopsis leaf transcriptome reveals interaction of phosphate and sugar metabolism. Plant Physiol., 143(1): 156–171.

[37] Bläsing OE, Gibon Y, Günther M, Höhne M, Morcuende R, Osuna D, Thimm O, Usadel B, Scheible WR and Stitt M (2005) Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. Plant Cell., 17(12): 3257–3281.

[38] Lunn JE, Delorge I, Figueroa CM, Van Dijck P and Stitt M (2014) Trehalose metabolism in plants. Plant J., 79(4): 544–567.

[39] Baena-González E and Lunn JE (2020) SnRK1 and trehalose 6-phosphate – two ancient pathways converge to regulate plant metabolism and growth. Curr. Opin. Plant Biol., 55: 52–59.

[40] Fukumoto T, Kano A, Ohtani K, Inoue M, Yoshihara A, Izumori K, Tajima S, Ishida Y, Tada Y, Nishizawa Y and Akimitsu K (2010) A rare sugar, D-allose, confers resistance to rice bacterial blight with upregulation of defense-related genes in Oryza sativa. Phytopathology, 100(1): 85–90.

[41] Fukumoto T, Kano A, Ohtani K, Yamasaki-Kokudo Y, Sato M, Fukumoto T, O’Hara LE, Primavesi LF, Delatle TL, Schluepmann H, Somsen GW, Silva AB, Fevereiro PS, Wingler A and Paul MJ (2013) The trehalose 6-phosphate/snRK1. signaling pathway primes growth recovery following relief of sink limitation. Plant Physiol., 162(3): 1720–1732.
[49] Müller C, Scheible WR, Stitt M and Krapp A (2001) Influence of malate and 2-oxoglutarate on the NIA transcript level and nitrate reductase activity in tobacco leaves. Plant, Cell Environ., 24(2): 191–203.

[50] Emmerlich V, Linka N, Reinhold T, Hurth MA, Traub M, Martinoia E and Neuhaus HE (2003) The plant homolog to the human sodium/dicarboxylic cotransporter is the vacuolar malate carrier. Proc. Natl. Acad. Sci. U. S. A., 100(19): 11122–11126.

[51] Gray GR, Maxwell DP, Villarimo AR and McIntosh L (2004) Mitochondria/nuclear signaling of alternative oxidase gene expression occurs through distinct pathways involving organic acids and reactive oxygen species. Plant Cell Rep., 23(7): 497–503.

[52] Clifton R, Lister R, Parker KL, Sappl PG, Elhafez D, Millar AH, Day DA and Whelan J (2005) Stress-induced co-expression of alternative respiratory chain components in Arabidopsis thaliana. Plant Mol. Biol., 58: 193–212.

[53] Gupta KJ, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, Bauwe H and Igamberdiev AU (2012) Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J. Exp. Bot., 63(4): 1773–1784.

[54] Hieno A, Naznin HA, Inaba-Hasegawa K, Yokogawa T, Hayami N, Nomoto M, Tada Y, Yokogawa T, Higuchi-Takeuchi M, Hanada K, Matsu M, Ikeda Y, Hojo Y, Hirayama T, Kusunoki K, Koyama H, Mitsuda N and Yamamoto YY (2019) Transcriptome analysis and identification of a transcriptional regulatory network in the response to H2O2. Plant Physiol., 180(3): 1629–1646.

[55] Finkemeier I, König A-C, Heard W, Nunes-Nesi A, Pham PA, Leister D, Fernie AR and Sweetlove LJ (2013) Transcriptomic analysis of the role of carboxylic acids in metabolite signaling in Arabidopsis leaves. Plant Physiol., 162(1): 239–253.

[56] Templeton GW and Moorhead GBG (2004) A renaissance of metabolite sensing and signaling: From modular domains to riboswitches. Plant Cell, 16(9): 2252–2257.

[57] Araújo WL, Nunes-Nesi A, Trenkamp S, Bunik VI and Fernie AR (2008) Inhibition of 2-oxoglutarate dehydrogenase in potato tuber suggests the enzyme is limiting for respiration and confirms its importance in nitrogen assimilation. Plant Physiol., 148(4): 1782–1796.

[58] Araújo WL, Tohge T, Nunes-Nesi A, Daloso DM, Nimick M, Krahnert I, Bunik VI, Moorhead GBG and Fernie AR (2012) Phosphonate analogs of 2-oxoglutarate perturb metabolism and gene expression in illuminated Arabidopsis leaf. Front. Plant Sci., 3(JUN): 1–19.

[59] van der Merwe MJ, Osorio S, Araújo WL, Balbo I, Nunes-Nesi A, Maximova E, Carrari F, Bunik VI, Persson S and Fernie AR (2010) Tricarboxylic acid cycle activity regulates tomato root growth via effects on secondary cell wall production. Plant Physiol., 153(2): 611–621.

[60] Desvergne B, Michalik L and Wahli W (2006) Transcriptional regulation of metabolism. Physiol. Rev., 86(2): 465–514.

[61] Vaulont S, Vasseur-Cognet M and Kahn A (2000) Glucose regulation of gene transcription. J. Biol. Chem., 275(41): 31555–31558.

[62] Maruyama K, Urano K, Yoshiwara K, Morishita Y, Sakurai N, Suzuki H, Kojima M, Sakakibara H, Shibata D, Saito K, Shinozaki K and Yamaguchi-Shinozaki K (2014) Integrated analysis of the effects of cold and dehydration on rice metabolites, phytohormones, and gene transcripts. Plant Physiol., 164(4): 1759–1771.