Plant Biotechnology 26, 29–38 (2009)

Minireview

Manipulation of plant metabolic pathways by transcription factors

Akira Iwase, Kyoko Matsui, Masaru Ohme-Takagi*

Gene Regulation Research Group, Research Institute of Genome-based Biofactory, National Institute of Advanced Industrial Science and Technology (AIST), Central 4, Tsukuba, Ibaraki 305-8562, Japan
* E-mail: m-takagi@aist.go.jp Tel: +81-29-861-2641 Fax: +81-29-861-3026

Received October 24, 2008; accepted January 20, 2009 (Edited by H. Suzuki)

Abstract Plant metabolites are produced through complex processes that include multiple enzymatic steps, branched pathways and regulation by a number of functionally redundant transcription factors. In addition, plants synthesize and accumulate each metabolite, especially secondary metabolites, in specific tissues and cells during development. Therefore, manipulation of both transcription factors that regulate enzymatic steps of a metabolic pathway (metabolic regulators) and/or that regulate cellular differentiation (developmental regulators) would be an effective strategy for controlling plant metabolites, quantitatively and qualitatively. In this review, we describe the advantages of using transcription factors for metabolic engineering in plants. Transcriptional activators and repressors, including the chimeric repressors generated by CRES-T, are useful tools for the genetic engineering of metabolic pathways. In addition, we propose that the use of both developmental regulators and plant tissue culture technology, in combination with metabolic regulators, would be an effective strategy to increase the productivity of metabolites. We summarize the strategies that have been applied for the detection of regulators and enzyme genes involved in metabolic pathways.

Key words: CRES-T system, metabolic engineering, metabolome, transcription factor, transcriptome.

Using inexhaustible light energy, plants produce large numbers of metabolites, which include not only primary metabolites but also secondary metabolites, through various metabolic pathways. Secondary metabolites play integral roles in growth, development, symbiosis, reproduction and tolerance to biotic and abiotic stresses in plants (Kutchan 2001). More than 200,000 different metabolites are estimated to be produced in the plant kingdom (Dixon and Strack 2003; Trethewey 2004). On the other hand, the number of metabolites that prokaryotes and animals produce is estimated to be 5000 to 25000, which is much less than that of plants (Trethewey 2004). For more than thousands years, mankind has been utilizing plant metabolites as foods, pharmaceutical compounds and raw materials for industry (Oksman-Caldentey and Saito 2005). In addition, mankind uses plant metabolites for relaxation, e.g. flower color and fragrance. Therefore, plant biosynthetic pathways have been intensively studied, and genetic engineering to control the biosynthetic pathways has been applied for the effective production of useful metabolites (Capell and Christou 2004; Dixon 2005).

As well as metabolites, it is likely that plants have many, and various, families of transcription factors compared with other organisms (Riechmann et al. 2000). Transcription factors are defined as proteins that recognize a specific DNA sequence on the promoter region of genes and regulate the expression of the genes positively (activator) or negatively (repressor). Each plant metabolic pathway consists of multiple enzymatic steps, and each enzyme gene is under the regulation of transcription factors (Figure 1A). Therefore, the diversity of plant transcription factors seems to be tightly linked to the diversity of plant metabolites. This linkage might be a consequence of evolution, which is responsible for their sessile lifestyle and interactions with various environmental stresses (Grotewold 2005).

It has become evident that a number of transcription factors act as master regulators of various plant functions, and several of them have been identified to be key regulators for metabolic pathways (Broun 2004; Grotewold 2008). These transcription factors (referred to as metabolic regulators) activate or repress the expression of enzyme genes in specific metabolic pathways. Manipulations of such transcription factors appear to be more effective for the control of metabolic pathways than that of single enzyme genes in plants (Braun et al. 2001; Capell and Christou 2004), because plant metabolic pathways are composed of multiple and complicated steps, and various enzymes are involved at each step. A transcription factor often regulates the expression of multiple genes involved in a biosynthetic

Abbreviations: CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; EAR, ERF-associated amphiphilic repression; GC, gas chromatography; HPLC, high performance liquid chromatography; TF, transcription factor; RNAi, RNA interference

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pathway, simultaneously. It has been estimated that the *Arabidopsis* genome contains around 2000 genes for transcription factors (Guo et al. 2005). However, those that are involved in the regulation of metabolic pathway have not been well characterized. Identification of transcription factors that affect metabolite syntheses is necessary not only for understanding plant biosynthetic pathways in more detail, but also for applying them to the control of metabolites, both quantitatively and qualitatively.

In this review, we discuss how to utilize transcriptional activators and repressors for plant metabolic engineering, and how to discover transcription factors that affect plant metabolic pathways, using a novel method of artificial transcription repressor designated Chimeric REpressor gene-Silencing Technology (CRES-T; Hiratsu et al. 2003). We also emphasize that transcription factors that regulate plant development (referred to as developmental regulators) are also attractive for the manipulation of metabolites production from a biotechnological perspective as well as the metabolic regulators. Finally, methods of screening and identification of regulators for the metabolic pathways and enzyme genes involved in plant metabolism are summarized from recent studies in order to assist in the discovery of new regulators involved in plant metabolism.

Manipulation of transcription factors for the quantitative control of metabolites

Transcription factors are roughly classified into transcriptional activators and repressors by their positive or negative effect on the expression of their target genes. Recent studies have revealed that, in some metabolic pathways, both transcriptional activators and repressors were involved in the same pathway, for example, the phenylalanine-derived pathway in *Arabidopsis* (Borevitz 2000; Jin et al. 2000; Gonzalez et al. 2008; Matsui et al 2008; Dubos et al. 2008). For the quantitative control of a target metabolite by manipulation of transcription

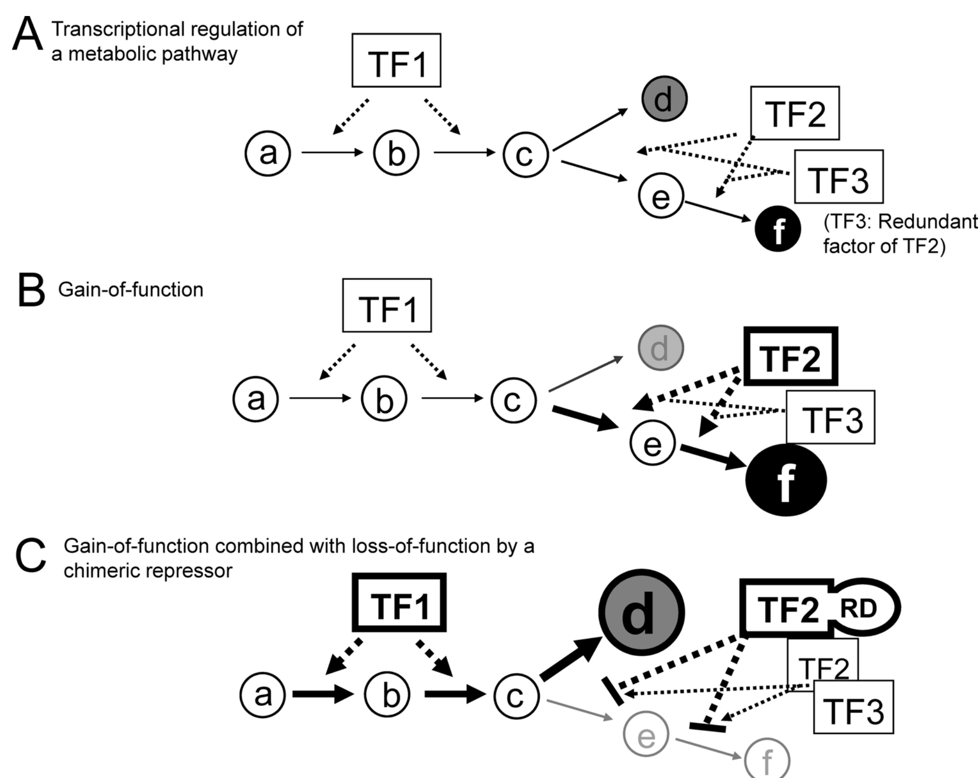

Figure 1. Strategies for plant metabolic engineering by manipulation of transcription factors that regulate enzymatic steps of a metabolic pathway (metabolic regulators). Arrows with solid line indicate enzymatic steps in a metabolic pathway. Arrows and T-bars with dashed line indicate activation and repression activity of a transcription factor on expression of a target enzyme gene, respectively. (A) Hypothetical branched biosynthetic pathway with several metabolites (a to f) and metabolic regulators (TF1 to TF3). Each transcription factor regulates expression of enzyme gene. TF3 is functionary redundant transcription factor of TF2. (B) Gain-of-function of TF2. The amount of metabolite f increases by activation of expression of genes for enzyme of steps from c to e, and e to f, while the amount of metabolite d decreases due to shortage of the precursor at the branching point. Thus, gain-of-function strategy of TF can be applied for the increase of metabolite f and decrease of d. (C) Gain-of-function of TF1 and loss-of-function of TF2 by applying TF2 chimeric repressor (CRES-T; Hiratsu et al. 2003). TF2RD is the chimeric repressor in which TF2 was fused to the EAR-repression domain (RD) and suppressed target genes dominantly over endogenous transcription factor (TF2) and functionally redundant transcription factor (TF3), resulting in suppression of the metabolic pathway from e to f. Similar effects are expected in the defective line (knockdown or knockout) of the genes for TF2 and TF3. In combination with gain-of-function of TF1, accumulation of metabolite d and reduction of metabolite f is to be expected.

factors, it is necessary to identify the transcription factors that are involved in the metabolic pathway and to characterize them, e.g. whether they are transcriptional activators or repressors. Enhancement of an activation activity of a transcription factor (gain-of-function), for example, by the ectopic expression or fusion of an external activation domain (such as the VP16 activation domain) can be used as a strategy for the quantitative control of a metabolite of interest. It would be expected that the enhancement (gain-of-function) of a transcriptional activator would promote the production of a metabolite in the pathway (Figure 1B, metabolite f). However, at the same time, the amount of the end product or its precursors on the branching pathway may decrease by a shortage of a precursor at the branching point (Figure 1B, metabolite d). Such reductions are often observed when an enzyme gene is ectopically expressed (Okinaka et al. 2003; Lanot et al. 2006, 2008). In case of transcriptional repressors, adverse effects would be expected to occur in a metabolic pathway when the transcriptional repressor is ectopically expressed. This has prompted the application of both a transcriptional activator and repressor included in a chimeric repressor (described below), which would increase the amount of the end products by the combination of activating and suppressing each branching pathway (Figure 1C, metabolites d). Such strategy, namely combination both activation and repression of enzyme gene expression, was applied to alter flower pigmentation in *Petunia hybrida* and several species (Tsuda et al. 2004; Tanaka and Ohmiya 2008). It should be pointed out that manipulation of a sole transcription factor is sometimes insufficient for the alteration of an amount of a metabolite of interest (van der Fits and Memelink 2000). Manipulation of a gene for a metabolic enzyme in combination with that of a transcription factor would be more efficient in increasing the amount of target products (Capell and Christou 2004; Xie et al. 2006; Grotewold 2008), as feeding the precursor(s) of the metabolic pathway (van der Fits and Memelink 2000).

Inhibition of branching pathways would be also effective for enhancing the metabolic pathway to the product of interest (Devic et al. 1999). For this purpose, a loss-of-function strategy using gene-silencing technology, such as gene-knockout lines, antisense technology and RNA interference, have been applied. However, attempts at suppressing gene expression by these methods were often less effective when compared with that of a gain-of-function strategy, mainly because of the genetic redundancy caused by genome duplication and/or polyploidy (Blanc et al. 2000; Simillion et al. 2002, Moor and Purugganan 2005; Handa et al. 2008). For example, *Arabidopsis* MYB transcription factors, PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1), PAP2, MYB113 and MYB114, are positive regulators of the anthocyanin metabolic pathway and are functionally redundant factors (Borevitz et al. 2000; Gonzalez et al. 2008). In order to obtain a loss-of-function phenotype in such a case, multiple genes for transcription factors have to be knocked out or knocked down simultaneously (Gonzalez et al. 2008). Thus simpler and more effective silencing methods that function on redundant genes are necessary for the manipulation of plant metabolic pathways.

In this respect, CRES-T (Hiratsu et al. 2003), a novel method for gene silencing, would be a powerful tool for altering metabolic pathways by suppressing expression of target enzyme genes in the pathway through modification of a metabolic regulator. The CRES-T system can convert a transcriptional activator into a dominant repressor simply by fusion of the EAR-repression domain with the activator, referring as chimeric repressor (Schematic diagram of the CRES-T system, see Shikata and Ohme-Takagi 2008). An outstanding advantage of the CRES-T system to other gene-silencing technologies, namely antisense, RNAi, and T-DNA insertion, is that the repressive activity of the chimeric repressor can overcome those of the endogenous and functionally redundant transcription factors, resulting in the induction of a dominant negative phenotype (Hiratsu et al. 2003; Matsui et al. 2004; Mitsuda et al. 2007; Koyama et al. 2007; Shikata and Ohme-Takagi 2008). With this system, we can simply induce phenotype similar to its loss-of-function alleles, which was hard to be obtained by the other gene-silencing technologies. Concretely, when this system was applied to *PAP1*, in the resultant plant that expressed PAP1 chimeric repressor, accumulation of anthocyanin and condensed tannin were reduced by suppression of the expression of the enzyme genes that regulate flavonoid biosynthesis, namely *CHS* and *DFR*, even though the endogenous *PAP1* and *PAP2* were expressed (Hiratsu et al. 2003; Matsui et al. 2004).

The effectiveness of the CRES-T system has been demonstrated in various families of transcription factors, not only for altering accumulation of metabolites (such as anthocyanin, proanthocyanidin and lignin), but also for altering morphology, development, and response to plant hormones (Hiratsu et al. 2003; Matsui et al. 2004; Matsui et al. 2005; Mitsuda et al. 2005; Mitsuda et al. 2006; Koyama et al. 2007; Mitsuda et al. 2007; Heyl et al. 2008; Mitsuda and Ohme-Takagi 2008). Moreover, CRES-T system has been applied to other plant species including monocot and polyploid dicots such as *Oryza sativa* (Mitsuda et al. 2006), *Torenia fournieri* and *Chrysanthemum morifolium* (Narumi et al. 2008, Shikata and Ohme-Takagi 2008). Phenotypic information induced by CRES-T system in *Arabidopsis* and floricultural plants is available in the web-site

database (Mitsuda et al. 2008; http://www.cres-t.org/fiore/public_db/index.shtml).

It should be mentioned that, if a transcription factor has repressive activity, loss-of-function phenotype would not be induced by the CRES-T system but would exhibit similar phenotype to its ectopic expression lines. For example, in the case of the transcriptional repressor AtMYBL2, which acts as a negative regulator in the biosynthesis of anthocyanin, phenotype of AtMYBL2 chimera repressor lines obtained by CRES-T system resembled to that of its overexpressor, and showed opposite phenotype with its T-DNA tagged line (Matsui et al. 2008). The results indicate that other gene-silencing technologies such as RNAi should be required to induce loss-of-function phenotypes in which the transcription factor is a repressor. These indicate that CRES-T system could be used to identify novel repressors by comparison with phenotypes between lines of overexpression and chimeric repressor of a transcription factor.

Manipulation of developmental regulators for the control of metabolite production

It is well known that biosynthesis and accumulation of plant metabolites (especially secondary metabolites) take place in differentiated tissues and at different developmental stages. If the number of cells and/or tissues where a metabolite is produced could be increased, the productivity of target metabolites would be increased. Thus manipulation of developmental regulators that control cells and/or tissue identity would be a fascinating strategy for the promotion of metabolites production as well as that of regulators of metabolic pathways (Figure 2A, B, C). An example of the association of tissue specificity and production of metabolites was shown in the biosynthesis of pigmentations, namely anthocyanins, carotenoids and betalains, in flower petals (Grotewold 2006; Tanaka and Ohmiya 2008). In addition, recent studies using a molecular approach in combination with new dissection technologies, such as in situ RNA hybridization,

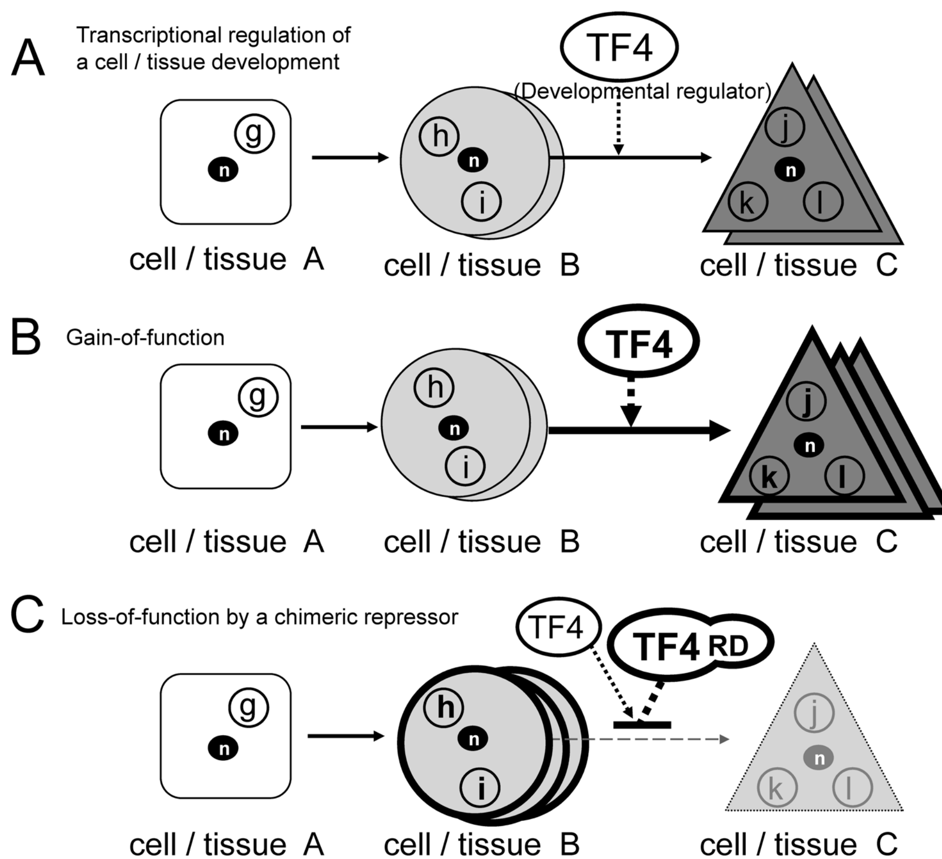

Figure 2. Strategies for plant metabolic engineering by manipulation of transcription factors that regulate cellular differentiation (developmental regulators). Arrows with solid line indicate developmental step. Arrows and T-bars with dashed line indicate activation and repression activity of a transcription factor to expression of a target gene for development, respectively. (A) Hypothetical developmental stage from cell/tissue A to cell/tissue C. Different metabolites (g), (h and i) and (j to l) are synthesized in cell/tissue A, cell/tissue B and cell/tissue C, respectively, during each of developmental stages. TF4 is a developmental regulator involved in determination of cell/tissue C identity. (B) Gain-of-function of TF4 increases number or mass of cell/tissue C and results in the promotion of the accumulation of metabolites j to l. (C) Loss-of-function of TF4. The TF4 chimeric repressor suppresses development cell/tissue B to cell/tissue C, resulting in the accumulation of metabolites h and i and reduction of metabolites j to l. Solid circle with a letter n represents a nucleus of the cell.

immunolocalization and microdissection, clarified that specific secondary metabolites were synthesized in specialized cells within a tissue, namely the vindoline (a terpenoid indole alkaloid) biosynthetic pathway in epidermal, laticifer and idioblast cells of *Catharanthus roseus* leaves (St-Pierre et al. 1999; Murata et al. 2005, 2008). A well-characterized example of relationships among cell differentiation, secondary metabolism and their regulation by transcription factors is the production and accumulation of proanthocyanidin (condensed tannin) in the seed coat of *Arabidopsis*, which occurs after fertilization within the innermost layer of the ovule integuments (Haughn and Chaudhury 2005; Broun 2005). Analyses of mutants with altered seed coat color, namely *transparent testa* (*tt*) mutants, revealed several transcription factors involved in the accumulation of proanthocyanidin (Haughn and Chaudhury 2005). Although both *tt8* and *tt16* mutants have a proanthocyanidin deficient phenotype, TT8 was identified as a metabolic regulator, while TT16 was found to be a global regulator of endothelial differentiation (Nesi et al. 2002; Haughn and Chaudhury 2005). This suggests that a developmental regulator that controls cell and/or tissue identities can also alter accumulation of metabolites as with metabolic regulators. Thus increase of cell number and tissues, where specific metabolites are synthesized and accumulated, would contribute to high yields of useful metabolites. A number of transcription factors that specify floral organ identities have been well characterized in several plant species and floral organs, namely sepals, petals, stamens and carpels, can be controlled by manipulation of transcription factors (reviewed in Shikata and Ohme-Takagi 2008). Increasing the number of carpels of the saffron plant (*Crocus sativus*) would be one of the targets for the production of useful metabolites.

In addition to the strategy of the manipulation of the developmental regulators for the spatial expansion of metabolite-producing areas, tissue culture techniques, such as cultures of multiple shoots (Miura et al. 1988), adventitious roots (Kusakari et al. 2000; Aoyagi et al. 2001; Yokoyama et al. 2003), hairy roots (Shanks and Morgan 1999; Seki et al. 2005; Guillon et al. 2006) and intact tissues (Iwase et al. 2005; Dörnenburg et al. 2008), would be an effective strategy, in combination with the manipulation of regulators that control metabolic pathways, to obtain higher productivity of useful metabolites. Moreover, both the availability of chemicals for promotion of biosynthesis, such as elicitors, and the utility of gene-inducible systems are also advantages of tissue culture technology for the efficient production of useful metabolites.

Identification of transcription factors that affect plant metabolism

It will be necessary to identify transcription factors that regulate and control metabolic pathways in order to use them for the effective production of useful metabolites. However, identifications of phenotypes associated with metabolic changes are more difficult than those of morphological changes. This would be why fewer factors that regulate metabolic pathway have been characterized (Grotewold 2008). Among plant metabolic pathways, the pathway that leads to anthocyanin and condensed tannin biosynthesis has been extensively studied and the enzyme genes and regulators involved in the pathway have been identified. This was mainly due to the visibility of anthocyanin and condensed tannin, which allows detection of the alternation of accumulation of the metabolite (Table 1). Thus such visible clues for identifying metabolic alterations assist in the discovery of enzyme genes and transcription factors involved in a pathway. When a metabolic enzyme of a pathway of interest is characterized genetically, simplified transient expression assay for the analysis of interaction between a transcription factor and the promoter of the enzyme gene, such as co-transformation assay (Berger et al. 2007), should be efficient for identifying a transcription factor that regulates expression of the enzyme gene. This strategy can be applied to any factors and visible clues are activities of the reporter proteins, e.g. GUS, LUC and GFP. The HIGH ALIPHATIC GLUCOSINOLATE 1 (HAG1) was recently identified as an activator of aliphatic methionine-derived glucosinolate pathway by the co-transformation assay (Table 1; Gigolashvili et al. 2007). To date, many approaches to visualize metabolic alterations have been reported, as well as other plant traits (phenotypic changes) caused by alteration of metabolites accumulation (Table 1). Applying these methods and phenotypic changes to detect metabolic alternations would help to discover unidentified transcription factors that are involved in metabolic pathways.

Recently, the demand for bioenergy produced from plant materials has increased. Transcription factors that control biosynthesis of lignocellulose and lipids should be particularly investigated. Increase of the lipids content and modification of fatty acids in oil seeds have been required by the oleo-chemical industry. The *wrinkled1* mutant was isolated as a metabolically defect mutant with a phenotype of low seed-oil content, through the analysis of relative density of seeds using 1-bromohexan and 1, 6-dibromohexan (Table 1; Focks and Benning, 1998). Such a method, focusing on the difference in relative density, is efficient for identifying seeds with high oil content. Moreover, an interesting attempt was made to identify genes of castor (*Ricinus communis*) that

Table 1. Effective approaches to visualize metabolic alterations, and plant phenotypic changes caused by alteration of metabolites accumulation, mainly in *Arabidopsis thaliana*.

Metabolite	Metabolic alteration	Approaches to visualize metabolic alteration/ Phenotypic change	Relate TF gene/ enzyme gene or others*	Reference
Phenylalanine-derived metabolites	Anthocyanin accumulation/ reduction	Change of plant color	*PAP1*, *PAP2*, *MYB113*, *MYB114*, *MybL2*	Borevitz et al. (2000), Hiratsu et al. (2003), Gonzalez et al. (2008), Matsui et al. (2008), Dubos et al. (2008)
		High light (800 μmol m^{-2} sec^{-1})	*MybL2*	Gonzalez et al. (2008),
		High sucrose concentration (3 to 6%)	*PAP1*, *PAP2*, *MYB113*, *MYB114*, *MybL2*	Hiratsu et al. (2003), Gonzalez et al. (2008), Matsui et al. (2008)
	Proanthocyanidin accumulation/reduction	Seed color	*TT1*, *TT2*, *TT8*, *TT16*, *TTG1*, *TTG2*, *PAP1*, *MybL2*	Haughn and Chaudhury (2005; review), Matsui et al. (2004, 2008), Dubos et al. (2008)
		DMACA (dimethylamino cinnamaldehyde) staining	*ANR*	Xie et al. (2003, 2006)
	Leucoanthocyanidins (flavan-3,4-diols, Catechins (flavan-3-ol), Proanthocyanidin accumulation/reduction	Acidic vanillin staining	*TT2*, *PAP1*	Aastrup et al. (1984), Nesi et al. (2001), Matsui et al. (2004)
	Sinapoyl malate reduction	UV irradation (fluorescence)	*UGT72E1*, *UGT72E2*, *UGT72E3*	Lanot et al. (2008)
	Sinapoyl malate accumulation	UV-B irradation (survived plant)	MYB4	Jin et al. (2000)
	Lignin accumulation/reduction (Secondary wall)	Phloroglucinol staining	*IRX4*, *NST1*, *NST2*, *NST3*	Jones et al. (2001), Mitsuda et al. (2005), Mitsuda and Ohme-Takagi (2008)
		UV irradation(fluorescence)	*IRX4*, *NST1*, *NST2*, *NST3*	Jones et al. (2001), Mitsuda et al. (2005, 2007), Mitsuda and Ohme-Takagi (2008)
	Lignin (Secondary wall) reduction	Infloresence stem trait (easily bent)	*IRX4*, *NST1*, *NST3*	Jones et al. (2001), Mitsuda et al.(2007)
		Reduced fertility at elevated temperature (31°C)	*IRX4*	Jones et al. (2001)
		Anther indehiscence, siliques indehiscence	*NST1*, *NST2*, *NST3*	Mitsuda et al. (2005), Mitsuda and Ohme-Takagi (2008)
Methionine-derived metabolites	Methionine-derived glucosinolate	Co-transformation assay (Target enzyme gene promoter: GUS gene and 35S: TF gene, simultaneously expressed in cultured cells.)	*HAG1*	Gigolashvili et al. (2007)
Tryptophan-related/derived metabolites	Anthranilate accumulation	UV irradation(fluorescence)	*TRP1*	Rose et al. (1992), Bender and Fink (1998)
	Indole glucosinolate pathway activation	5-methyl-tryptophan resisant	*ATR1*	Bender and Fink (1998), Celenza et al. (2005)
	Indole alkaloid pathway activation	4-methyl-tryptophan resisant	*ORCA3* (*Catharanthus roseus*)	van der Fits and Memelink (2000)
Wax**	Wax accumulation	Glossier leaves	*WIN1*	Broun et al. (2004)
Lipid related	Neutral lipid accumulation	Sudan Red 7B, Sudan Black B staining	*HSI2*, *HSL1*, *Nud* (*Hordeum vulugare*)	Tsukagoshi et al. (2007), Taketa et al. (2008)
	Seed oil accumulation	Seed trait (wrinkled). Density test by centrifugation of seeds in a mixture of 1-bromohexan and 1,6-dibromohexan	*WRI1*	Focks and Benning (1998), Cernac and Benning (2004)

Table 1. (Continued).

Metabolite	Metabolic alteration	Approaches to visualize metabolic alteration/ Phenotypic change	Relate TF gene/ enzyme gene or others*	Reference
Lipid related	Fatty acid β-oxidation defection	2,4-Dichlorophenoxybutyric acid-resistant root elongation and required sucrose for germination	PED1, PED2, PED3	Hayashi et al. (1998)
Hydroxy fatty acid	DsRed marker and accumulation in seed oils	high-throughput GC anaysis	Oleosin*** etc.	Lu et al. (2006)
	Oil reduction (through Oleosin*** protein reduction)	Seed shape and color	*LEC2*	Santos Mendoza et al. (2005)
Vitamin E	Tocopherol composition change	HPLC anaysis	VTE3, VTE4	Van Eenannaam et al. (2003)
Starch	Retain a high starch content	Iodine solution staining	SEX1	Yu et al. (2001)

* Underline shows enzyme gene or others. ** containing fatty acids, alcohols, aldehydes and ketones. *** A protein found in oil bodies, involved in seed lipid accumulation.

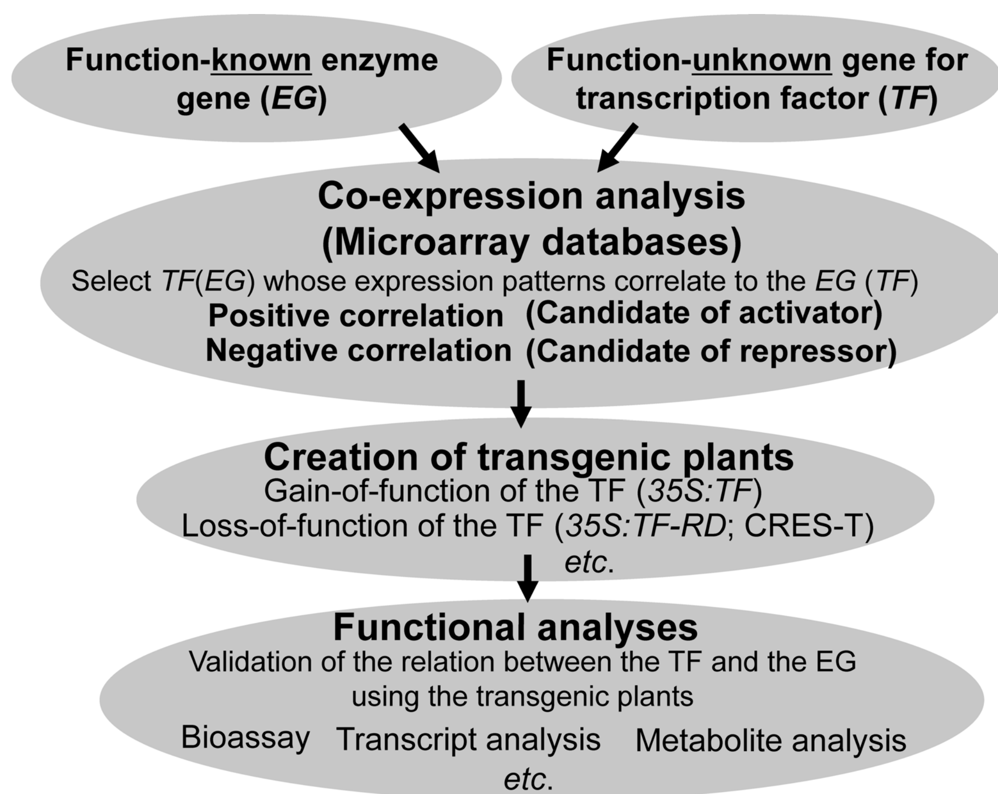

Figure 3. Schematic diagram of a strategy to discover transcription factors that affect metabolites accumulation. This strategy contains several screening methods, namely detection of co-expressed genes by the analysis of the transcriptome, loss-of function analysis by CRES-T, and methods for visualizing of metabolites alteration (e.g. metabolome analysis). The transcriptome analyses have enabled the prediction of transcription factors related to a metabolic pathway through the analysis of the co-expressed genes with the enzyme genes. CRES-T enables the induction of a dominant negative phenotype of a transcription activator in a simple and easy manner.

boosted hydroxy fatty acid accumulation (Table 1; Lu et al. 2006). In that report, castor cDNA was cloned into a binary vector that included a DsRed marker gene, which allowed the easy sorting of transgenic lines in seeds, and the fatty acids composition was analyzed by high-throughput gas-chromatography. Combined with these techniques, we may be able to find a new transcription factor and its target genes. By manipulation of them, it would be possible to obtain oilseeds with high-oil content and unique fatty acid compositions.

Genome-wide gene expression analysis and advanced bioinformatics allow the prediction of putative target genes of a transcription factor whose function is unknown, through analyses of its transcriptome and

genes co-regulated with the gene for the transcription factor (Obayashi et al. 2007). In combination with metabolic profiling analysis, MYB transcription factors, which regulate aliphatic glucosinolate biosynthesis, designated PRODUCTION OF METHIONINE-DERIVED GLUCOSINOLATE 1 (PMG1) and PMG2, were recently identified with ingenuity (Hirai et al. 2007). Such genetic screening has attracted attention because it would be possible to predict relationships among transcription factors and putative target enzyme genes bi-directionally, namely from enzyme genes to transcription factors that might regulate the expression of the genes, and from transcription factors to enzyme genes and a metabolic pathway that might be regulated by the transcription factors (Figure 3). When possible correlations between transcription factors and enzyme genes have been predicted by co-expression analyses, we could be evaluate and confirm them experimentally by gain- and loss-of-functional analysis, such as ectopic expression and CRES-T system. Combination of the analyses of transcriptome and metabolome with bioassays will allow the discovery of novel transcription factors that are possibly involved in the metabolic pathways (Figure 3).

Concluding remarks

It has been reported that 25% of the chemical materials, which are contained in pharmaceutical preparations used in western countries, are either of natural plant origin or their derivatives (Payne et al. 1991). This is partly because plants easily synthesize a vast number of chemicals with complicated structures by the activity of enzymes, even under normal temperature and pressure using inexhaustible light energy. These aspects emphasize the importance of genetic engineering, which has been a fascinating strategy for the alteration of productivity and quality of characterized metabolites in intact plants. Manipulation of plant metabolic pathway by transcription factors is eagerly anticipated because it can regulate several enzyme genes in a metabolic pathway simultaneously, or can induce drastic changes, such as altering identity or fate, in cells or tissues where the corresponding metabolites are produced. Applying either or both gain- or loss-of-function methods would be good strategies for metabolic engineering, although the loss-of-function method is difficult to apply due to the presence of genetically and functionally redundant factors. CRES-T is a novel method for gene silencing that can suppress the expression of target genes dominantly over the activation activity of the endogenous and functionally redundant transcription factors. Therefore, CRES-T is a powerful tool for altering metabolic pathways, tissue identity or spatial development. As well as the strategies of modifying of both metabolic regulators and developmental regulators, tissue culture technology also holds promise for enhancing productivity. The combination of genetic analysis, such as co-expression analysis, loss-of-function analysis by CRES-T, and methods for visualizing metabolic alterations will provide an avenue for the discovery of new transcription factors that affect plant metabolites accumulation.

Acknowledgements

This work was supported by the New Energy and Industrial Technology Development (NEDO) program, which is part of the "Development of Fundamental Technologies for Controlling the Material Production Process of Plants" project.

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