A basic study toward metabolic engineering of sesquiterpenes

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Research aims

There are many useful sesquiterpenes, but their organic synthesis is often laborious and costly. Through a series of enzymatic reactions, microorganisms can produce various kinds of sesquiterpenes from farnesyl pyrophosphate (FPP). The initial step of these reactions is catalyzed by a pathway-specific cyclase. Microorganisms with an ability to produce a large amount of natural sesquiterpenes may be useful as a basis for foreign sesquiterpene cyclase gene expression, with the aim to produce large amounts of related sesquiterpene skeletons. Currently, these are difficult to provide at low cost using organic synthesis. In this study, we aimed to understand the regulatory mechanisms of sesquiterpene trichothecene (TCN) biosynthesis in *Fusarium graminearum*, focusing on a TCN biosynthesis transcription factor, *FgTri6*. The ultimate goal of this research is to develop the organism as a host for the mass production of heterologous sesquiterpenes.

Methods

We analyzed the mechanisms of *Tri6* regulation using the following approaches:

(1) Phylogenetically close *Fusarium sporotrichioides* does not require sucrose[1)] for TCN production

The promoter and coding region of the *F. sporotrichioides Tri6* (*FsTri6*) gene were introduced into the corresponding region of *F. graminearum*. In addition, *Tri6* was constitutively overexpressed in *F. graminearum*. The effects on TCN production in the absence of sucrose were examined using these strains.

(2) The promoter region of *Tri6* contains TRI6p and AreAp consensus binding sites[2)]

The two TRI6p binding sites on the *Tri6* promoter, 5′-AGGCCT-3′, were mutated to 5′-AGGgCT-3. Additionally, *Tri4* was constitutively overexpressed outside of the cluster under the control of a TEF promoter. Each of the 15 AreAp consensus-binding sites located on in *Tri6* promoter was mutated.

When the core region of TCN gene cluster is extended by introducing a vector fragment[3], or by moving the promoter region outside of the gene cluster[4], the *Tri* gene promoters do not function properly. Therefore, to engineer the *Tri6* locus, we used a positive and negative selection system that creates transgenic strains possessing only the preferred gene replacements, with mutations at the original locus[5].

Results

Each of the transgenic *F. graminearum* strains that carried either the promoter or coding region of *FsTri6* failed to produce TCN in the absence of sucrose (Fig. AB). Additionally, the *Tri6* overexpressor could not produce TCN in the absence of sucrose (Fig. 1C). Wild-type *F. graminearum* did not produce TCN in a medium supplemented with 100 μM sucrose (Fig. 1C left panel), although TCN did accumulate in the medium of the *Tri6* overexpressor (Fig. 1C right panel).

When the TRI6p-binding sites in the *Tri6* promoter were mutated, weak expression of *Tri6*, but not of other pathway *Tri* genes, was observed. Consistent with this observation, no TCN accumulation was detected in the mutant culture. After constitutively expressing *Tri4* outside of the gene cluster, a small amount of TCN became detectable. This indicates that, if sucrose is present in the medium, only a small amount of TRI6p is sufficient to activate other pathway *Tri* gene promoters and eventually produce TCN. The mutant strain, in which all the AreAp-binding sites on the *Tri6* promoter were modified, produced similar but slightly reduced amounts of TCN.

Conclusions

We found that neither the sequences of the *FgTri6* promoter nor the FgTRI6p protein were responsible for the sucrose-dependent induction of TCN production in *F. graminearum*. Sucrose has been suggested to be important for post-translational activation of TRI6p protein in *F. graminearum*, which is dependent on unknown signals activated by sucrose. Controlling the timing of sesquiterpene production so that it coordinates with the most appropriate stage of growth may mean that the fungus could be exploited as a host for the metabolic engineering of sesquiterpenes.

References

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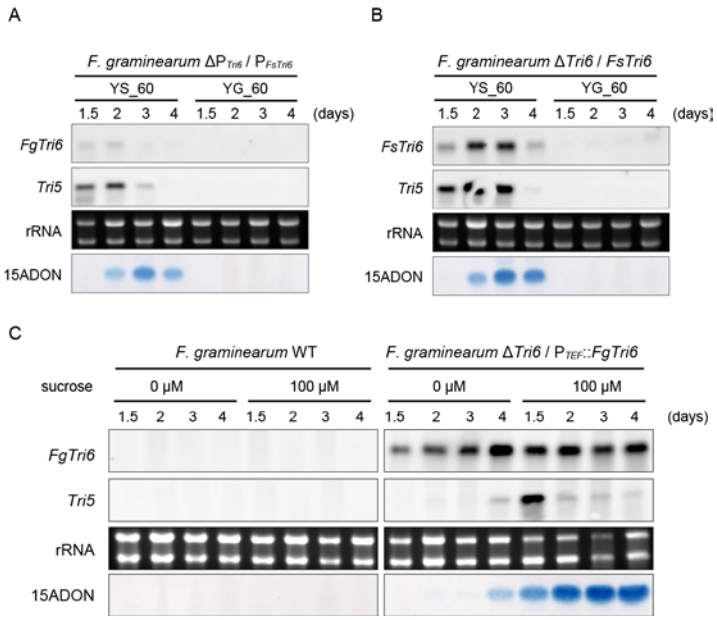

Fig. 1. Sucrose-dependent expression of *Tri* genes and TCN production in *F. graminearum*. YS_60 and YG_60 media contain sucrose and glucose as a carbon source, respectively. The results of northern blotting and TLC analyses are shown. (A) A transgenic strain in which the *FgTri6* promoter was replaced by the *FsTri6* promoter. (B) A transgenic strain in which the *FgTri6* coding region was replaced by the *FsTri6* coding region. (C) A transgenic strain in which *Tri6 was* constitutively overexpressed. The effects of 100 μM sucrose (in YG_60 medium) on *Tri* gene expression and TCN production were analyzed.

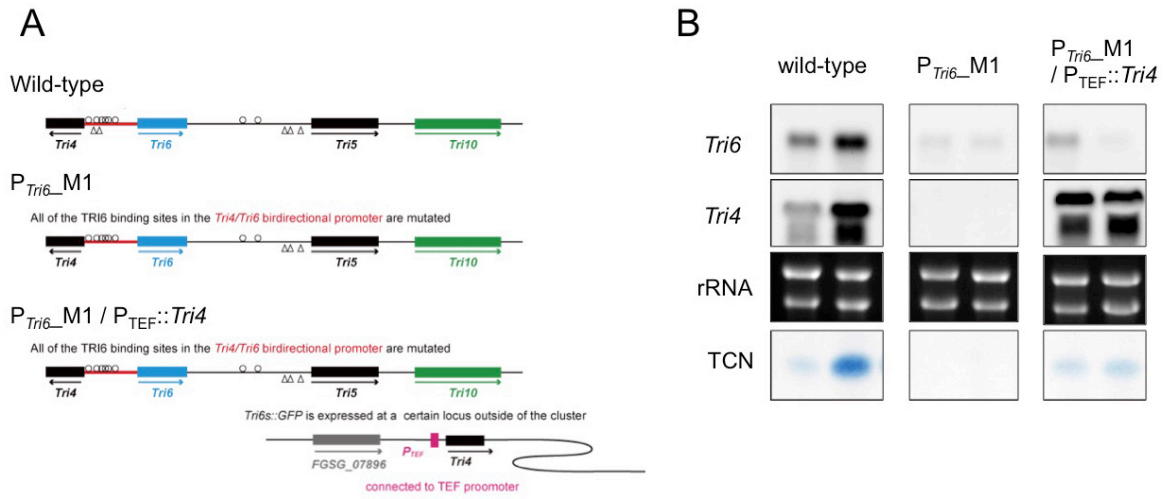

Fig. 2. Generation of transgenic strains carrying mutations on the *Tri6* promoter region. (A) P_{Tri6}_M1, a transgenic stain in which the two TRI6p-binding sites on the *Tri6* promoter were mutated; P_{Tri6}_M1/ P_{TEF}::*Tri4*, a transgenic strain in which the two TRI6p-binding sites on the *Tri6* promoter were mutated and *Tri4* was overexpressed from outside of the gene cluster. (B) *Tri* gene expression and TCN production by transgenic strains. The results of northern blotting and TLC analyses are shown.