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Over-expression of bael quinolone synthase in tobacco improves plant vigor under favorable conditions, drought, or salt stress

Mohankumar Saraladevi Resmi, Padmanabhan Jayanthi Vivek, Eppurathu Vasudevan Soniya *

Plant Molecular Biology Division, Rajiv Gandhi Centre for Biotechnology, Thycaud (P.O.), Thiruvananthapuram 695 014, Kerala, India

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ABSTRACT

Type III polyketide synthases (PKSs) catalyze the biosynthesis of various medicinally important secondary metabolites in plants, but their role in growth and stress response is unclear. Here, we over-expressed quinolone synthase (*QNS*) from bael in tobacco. *QNS*-overexpressing plants showed an overall increase in growth, photosynthetic efficiency and chlorophyll content compared to wild type plants. Second-generation (T_2) transgenic plants grew to maturity, flowered early and set viable seeds under favorable conditions without yield penalty. An increased accumulation of flavonoids, phenols and alkaloids was associated with higher tolerance to drought and salinity stress in transgenic plants. Thus, bael *QNS* seems to function as a positive regulator of plant growth and stress response, and could be potentially used for engineering plants tolerant to abiotic stress.

1. Introduction

Type III PKS superfamily of enzymes catalyzes the biosynthesis of diverse type of secondary metabolites in plants such as chalcones, resveratrols, benzophenones, quinolones and diarylheptanoids like phenylphenalenones, curcuminoids etc. [1,2]. They are homodimer proteins where the active site in each subunit iteratively catalyze the priming, extension and cyclization reactions to generate an array of plant phenylpropanoids (of secondary biosynthetic origin) including flavonoids (Fig. 1). An interesting feature of plant type III PKSs is that it shows an extremely broad substrate specificities. They can readily accepts a wide variety of unnatural substrates, including aromatic and aliphatic thioesters and produces novel, unexpected products with profound medicinal importance [3].

The secondary metabolites synthesized by Type III PKS in plants provide protection against various environmental stresses. They played a key role in the early evolution of land plants by acting as UV-sunscreens [4]. Chalcone synthases (*CHS*), the most well-known representative of this family, are influenced by various stresses and environmental factors such as UV, wounding or pathogen attack, jasmonic acid, methyl jasmonate, low temperature and high intensity light [5]. A chalcone synthase from *Sorghum bicolor*, *SbCHS8* expression was reported to be induced in mesocotyls with the inoculation of *Cochliobolus heterotrophus* and *Colletotrichum sublineolum* [6]. Recent studies reported an increased accumulation of quercetin (a phenolic compound) derivatives in *Physcomitrella patens* following ultraviolet-B (UV-B) radiation [7]. Moreover, an increased expression of stilbene synthase (*STS*), another well characterized type III PKS catalyzing the biosynthesis of stilbenes (resveratrol) were reported in response to various biotic and abiotic stresses [8]. Xu et al. [9] reported the isolation of a stilbene synthase promoter from *Vitis pseudoreticulata* induced under pathogen, cold and salicyclic acid treatment.

Although the number of identified diverse type III PKS family of enzymes is growing exponentially, the role of only a few like CHS and STS were extensively studied in transgenic plants. *CHS* gene was over-expressed for increasing the production of lignin content in *Linumus itatissimum* [10], phenolic acids and anthocyanin in potato tubers [11] and flavonolignans in hairy root cultures of *Silybum marianum* [12]. Moreover, over-expression of *CHS* gene in tobacco plants resulted in white flowers [13]. The over-expression of *STS* gene in various plants like tobacco, tomato, and alfalfa resulted in the accumulation of either resveratrol or its glucoside which confers improved disease resistance to the plants [14–17].

* Corresponding author at: Plant Molecular Biology Division, Rajiv Gandhi Centre for Biotechnology, Thycaud (P.O.), Thiruvananthapuram 695 014, Kerala, India. Fax: +91 471 234 8096.

E-mail addresses: resmivivek@gmail.com (M.S. Resmi), vivekpj@gmail.com (P.J. Vivek), evsoniya@rgcb.res.in (E.V. Soniya).

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Quinolone synthase (*QNS*) is a type III PKS identified from *Aegle marmelos* Corr. which catalyze the biosynthesis of quinolones [2]. Quinolones are naturally occurring anthranilic acid-derived alkaloids found in a limited number of plant species of the family Rutaceae [18]. *QNS* is a unique type III polyketide synthase (PKS) that exhibits unusually broad substrate specificity to produce various aromatic polyketides. *QNS* catalyzes the condensation of N-methylanthraniloyl-CoA with one malonyl-CoA to produce 4-hydroxy-2(1H)-quinolone; but with p-coumaroyl-CoA and one molecule of malonyl-CoA to produce p-hydroxybenzalacetone [2] (Fig. 1). The present study reports the over-expression of *QNS* in *Nicotiana tabacum* var. petita hybrid and evaluation of its effect on the germination and physiological response of transgenic plants under favorable, salinity and drought stressed conditions. *QNS* over-expressing plants showed an overall increase in growth and other physiological responses under non-stressed conditions. Moreover, the ectopic expression of *QNS* conferred enhanced tolerance under salinity and drought stressed conditions.

2. Results

2.1. Over-expression of QNS improves plant vigor under favorable growth conditions

To study the effect of over-expression of *QNS* in a heterologous system, where its preferable substrates are likely un-available, we have transformed tobacco plants with the binary vector pMDC85-*QNS-GFP*, in which the *A. marmelos* quinolone synthase gene is under the control of 2X*35S* promoter of Cauliflower mosaic virus.

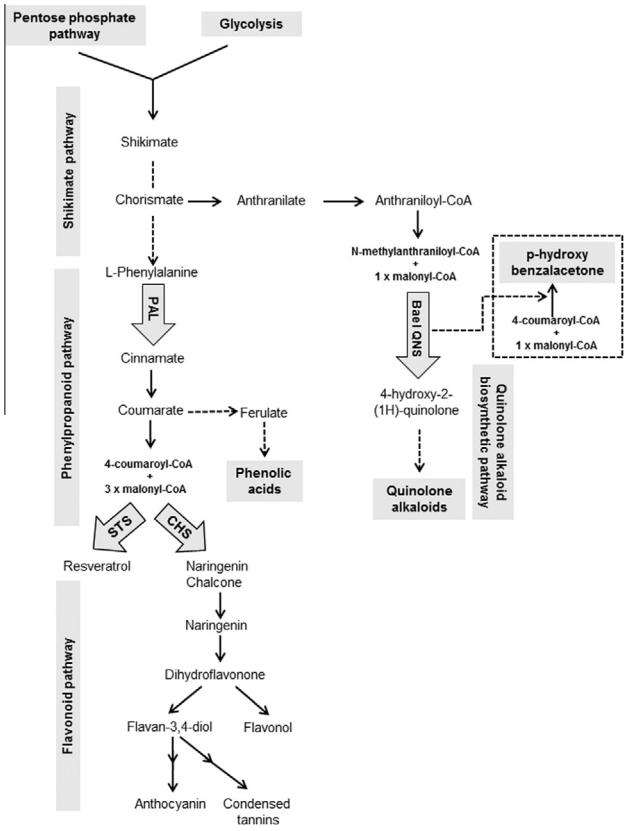

Fig. 1. Metabolic flow from shikimate to flavonoid and quinolone alkaloid via phenylpropanoid pathway. Key enzymes are indicated in arrow (upper case). PAL, phenylalanine-ammonia lyase; STS, stilbene synthase; CHS, chalcone synthase; QNS, quinolone synthase; CoA, coenzyme A. Hydroxybenzalacetone formation by bael QNS is shown in dotted square box.

The expressed protein will also contain a GFP epitope tag at its C terminus. Ten hygromycin-resistant independently transformed transgenic plants were selected (T_0 generation), grew them to maturity and rose up to T_2 generation. Three different lines (TG_1, TG_4 and TG_5) of T_2 generation were selected and used for further analysis. PCR of genomic DNA were used to identify the presence of transgene in the selected T_2 lines (Fig. 2A). Quantitative PCR analysis indicated the presence of *QNS* transcripts in all transgenic lines but not in the WT tobacco (Fig. 2B). In order to examine the presence of QNS protein in the transgenic lines, Western blot analysis with anti-GFP epitope antibodies was performed. A cross-reacting band matching to the size expected of *QNS-GFP* in all of the three transgenic lines was observed which was absent in the WT plant (Fig. 2C).

To evaluate whether over-expression of bael *QNS* have any effect on the overall plant growth and development, experiments were conducted to compare various growth parameters like shoot and root biomass, shoot length, root length, leaf area etc. in transgenic lines and WT plants grown in greenhouse. The transgenic plants exhibited a significantly altered growth rate and were taller with their average stem diameters enhanced by 11–13% than those of WT plants. The shoot biomass in transgenic plants was significantly higher with an overall increase of 2.5 fold in fresh weight and 1.7 fold in dry weight (Table 1). The physiological observations summarized in Table 1 suggested that under favorable growth conditions, the plants with *QNS* over-expression developed profuse root and shoot systems to support an increase in growth and biomass.

Surprisingly transgenic lines bolted earlier than WT plants (Fig. 3). The number of days required for flowering and seed weight or pod weight of the transgenic and WT were also recorded. The transgenic plants flowered much earlier with almost 50% reduction in flowering time as compared to WT plants and were also having a considerable increase in seed or pod weight (Table 2).

2.2. Physiological assessment of transgenic plants under favorable conditions

When seeds of T_2 generation transgenic plants (TG_1, TG_4 and TG_5 lines) were placed in MS medium, they showed 100% germination rate with an early response to germination as compared to WT plants (Fig. 4A and B). The seedlings also showed altered morphology with an overall increase in leaf size and root length (Fig. 4C). An overall increase in chlorophyll a, chlorophyll b and total chlorophyll contents were also observed in transgenic plants (Fig. 4D). To test whether the overall increased chlorophyll content in transgenic lines are in line with increased photosynthetic efficiency, we examined several physiological parameters that control plant vigor. The various parameters studied included the transpiration rate, photosynthesis rate, chlorophyll fluorescence parameter Fv/Fm (for testing the vitality of photosystem II); light response curve or net CO_2 uptake rate, intercellular CO_2 concentration (Ci) and leaf conductance. The *QNS* over-expressing plants showed significantly higher photosynthesis rates, transpiration rates, leaf conductance, net CO_2 uptake rate, Ci and Fv/Fm than non-transformed control plant (Fig. 5A–F) when grown under favorable growth conditions. The results suggest a positive relation between *QNS* over-expression and increased growth vigor.

In order to study the effect of *QNS* over-expression on the overall secondary metabolite production in tobacco, a phytochemical analysis was carried out. An overall increase of 1.75–2 fold in total flavonoid, 2.3–6.4% in total phenol and 64–85% in total alkaloid contents were noticed in transgenic plants as compared to WT plants (Table 3) under normal condition. The major in vitro product of QNS, 4-hydroxy-1-methyl (2H) quinolone (% w/w) was not detected in any of the transgenic sample tested. These findings