Journal of Plant Physiology 204 (2016) 27–35

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Agrobacterium rhizogenes rolB gene affects photosynthesis and chlorophyll content in transgenic tomato (*Solanum lycopersicum* L.) plants

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ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 15 July 2016
Accepted 18 July 2016
Available online 25 July 2016

Keywords:
Tomato
Agrobacterium rhizogenes rolB gene
Suppression subtractive hybridization
Photosynthesis

ABSTRACT

Insertion of *Agrobacterium rhizogenes rolB* gene into plant genome affects plant development, hormone balance and defence. However, beside the current research, the overall transcriptional response and gene expression of *rolB* as a modulator in plant is unknown. Transformed *rolB* tomato plant (*Solanum lycopersicum* L.) cultivar Tondino has been used to investigate the differential expression profile. Tomato is a well-known model organism both at the genetic and molecular level, and one of the most important commercial food crops in the world.

Through the construction and characterization of a cDNA subtracted library, we have investigated the differential gene expression between transgenic clones of *rolB* and control tomato and have evaluated genes specifically transcribed in transgenic *rolB* plants. Among the selected genes, five genes encoding for chlorophyll *a/b* binding protein, carbonic anhydrase, cytochrome b_6/f complex Fe-S subunit, potassium efflux antiporter 3, and chloroplast small heat-shock protein, all involved in chloroplast function, were identified. Measurement of photosynthesis efficiency by the level of three different photosynthetic parameters (F_v/F_m, rETR, NPQ) showed *rolB* significant increase in non-photochemical quenching and *a*, *b* chlorophyll content. Our results point to highlight the role of *rolB* on plant fitness by improving photosynthesis.

1. Introduction

The soil bacterium *Agrobacterium rhizogenes* is the causal agent of the hairy root disease of Dicotyledonous plants. Virulence is associated with the transfer to the plant cell of the T-DNA region of the Ri plasmid, containing several loci crucial for disease development such as the rooting-locus or *rol* genes, *rolA, B, C* and *D* (Costantino et al., 1994; Nilsson and Olsson, 1997). The *rolB* gene was found to be, alone, essential and sufficient for the induction of hairy roots on different plant species (Altamura, 2004).

rolB-transgenic plants show increased rooting, alterations in leaf and flower morphology, adventitious root formation, reduction in internode length and apical dominance (Casanova et al., 2005). This observation, together with the reported increase in auxin sensitivity of *rolB*-transformed protoplasts (Maurel et al., 1991), suggested an altered response to auxins in transgenic plants and tissues. The increase in auxin sensitivity could determine the induction of *de novo* meristem formation by *rolB* both in tissue culture and *in planta* (Koltunow et al., 2001; Altamura 2004). It has been proposed that

Abbreviations: CA, carbonic anhydrase; CAB, chlorophyll *a/b* binding protein; Chl, chlorophyll; Cyt b_6/f, cytochrome b_6/f; ΔF, increase in fluorescence yield induced by a light-saturating pulse during the exposure to actinic light; DIG, digoxigenin; F_m, maximum fluorescence yield obtained with a light-saturating pulse after dark adaptation; F_m', maximum fluorescence in light-adapted state; F_v, variable fluorescence; F_v/F_m, maximum photochemical efficiency of PSII; KEA, potassium efflux antiporter; μE, microeinstein; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PEc, photosynthesis-irradiance curve; rETR, relative electron transfer rate; RLC, rapid light curve; sHSP, small heat shock protein; SSH, suppression subtractive hybridization.

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http://dx.doi.org/10.1016/j.jplph.2016.07.010
0176-1617/

rolB could function in the signal transduction and/or perception pathways of auxins, an hypothesis supported by the finding that it encodes a protein with tyrosine phosphatase activity localized in the plasma membrane of transformed plant cells (Filippini et al., 1996).

As for other *rol* genes, *rolB* has an effect on plant secondary metabolism. Increased anthraquinone and resveratrol production, positively correlated with *rolB* expression, was observed, respectively, in transgenic *Rubia cordifolia* and *Vitis amurensis* callus (Shkryl et al., 2008; Kiselev et al., 2007). In *rolB*-transformed ginseng tissues, otherwise, the production of ginsenosides was decreased with respect to the untransformed controls (Bulgakov et al., 1998).

rolB has also been involved in tolerance to both abiotic and biotic stress. The expression of *rolB* could promote scavenging of reactive oxygen species via enhanced expression of genes encoding antioxidant enzymes (Bulgakov et al., 2012), increased production of defence-related secondary metabolites such as phenolics (Arshad et al., 2014), and augmented activity of pathogenesis-related proteins (Veremeichik et al., 2012). Recently, *rolB* has been found to induce the expression of genes encoding components of the miRNA processing machinery, indicating a possible interaction in RNA-silencing network (Bulgakov et al., 2015).

To our knowledge no data are currently available on the overall transcriptional response of the plant to *rolB*, despite current research efforts in clarifying *rolB* gene function by morpho-physiological parameters, metabolism and stress response in different plant species.

The aim of the present study was therefore to isolate genes differentially expressed in tomato plants upon transformation with *rolB*, using the Suppression Subtractive Hybridization method (SSH) to generate a cDNA library enriched in sequences expressed in the transgenic plants versus the untransformed, *in vitro* regenerated controls.

Among the selected genes, we found the up-regulation of genes involved in photosynthesis. These genes, directly or indirectly induced by *rolB,* participate in protection from light and oxidative stress, in capture and transfer of light energy, CO_2 diffusion, and cytochrome involvement in chloroplast electron transport chain. This suggested putative modification of photosynthesis in transgenic plants that were further explored through measurement of chlorophyll fluorescence.

The measure of chlorophyll variable fluorescence allows a non-destructive plant determination of photosynthetic efficiency and heat dissipation (Baker, 2008; Murchie and Lawson, 2013).

In normal physiological conditions photosynthetic pigments absorb more light energy than needed for driving photosynthesis. Excess light is either dissipated as heat or re-emitted as Chl fluorescence (Baker, 2008; Ruban, 2016). As a quantitative correlation exists between fluorescence changes observed upon exposure to light in dark-adapted leaves and changes in CO_2 assimilation, measurements of Chl fluorescence can be used to estimate photosynthetic efficiency and, in some conditions, also photosynthetic rate (Baker, 2008; Enriquez and Borowitzka, 2010).

Relative electron transfer rate (rETR) is used as a descriptor of relative changes in the photosynthetic rates (Enriquez and Borowitzka, 2010). A tight correlation, even if variable according to the conditions of photoacclimation, has been in fact observed between rETR and photosynthetic rate in terms of O_2 evolution rate.

Non-photochemical quenching of Chl *a* fluorescence (NPQ) is a short-term acclimation process that defends the photosynthetic apparatus against oxidative damage and is fundamental in preserving the integrity of the photosynthetic reaction centers and the antenna pigments (Ruban, 2016). This parameter describes the plant photoprotective capacity to dissipate as heat and/or to balance between photosystems the excess of energy absorbed (Enriquez and Borowitzka, 2010). Evaluation of NPQ has shown modifications upon stress in a bryophyte (Azzabi et al., 2013) and in tomato plants (Gerganova et al., 2016).

Light absorption is the first stage in photosynthesis, which is carried out by pigments such as Chl and accessory pigments (Liu et al., 2004). The efficiency of this process depends on pigments concentration and structure (Horton and Ruban, 2005), and changes in Chl *a* and *b* content are related to adaptation processes to maximize light-harvesting by chloroplasts (Björkman, 1981).

Taken together, these parameters obtained from the measure of Chl variable fluorescence allow assessing the photosynthetic performance of plants, gathering information on the efficiency of both the conversion of light into chemical energy by the photosynthetic apparatus and the mechanisms protecting it against excess light.

Table 1
Sequences of the PCR primers used in this study.

Primer	Sequence (5′ –>3′)
rolB-fw	ATGGATCCCAAATTGCTATTC
rolB-rev	TTAGGCTTCTTTCTTCAGGTT
rolB-rev2	GGAACTTGCGAAAATGGCGAT
A-5 fw	GAGGAGCAGCGGTAAGTTCAA
A-5 rev	TCACTGTCAGCACTCCATTCTC
A-6 fw	CCCAGGAGGTGCCTTTGAC
A-6 rev	CCTTCACCTTCAATTCAGCAAAA
C-43 fw	TTTCACTTGGAAATCTGCTTACGT
C-43 rev	AACCTCCCTTCAATGCCAATG
C-63 fw	GCTCAAAACTCATGCACCTGG
C-63 rev	TCCATCGTTCTCCCAACAACAAGG
E-6 fw	GAGGAGGATGATATGGAGAGTGC
E-6 rev	CAGAACCAGCTGAGCACCTAA
Tubulin-fw	CACATTGGTCAGGCCGGTAT
Tubulin-rev	GCCGTGCTCGAGGCAGTA

2. Materials and methods

2.1. Bacterial strains and media

rolB gene and its own promoter from *A. rhizogenes* pRi1855 was cloned into pBIN19 vector and transformed into *Agrobacterium tumefaciens* strain GV3101 via chemical transformation. Bacteria were grown at 28 °C in YEB medium supplemented with 50 mg L^{-1} rifampicin and 100 mg L^{-1} kanamycin.

2.2. Plant material

Agrobacterium-mediated transformation of *Solanum lycopersicum* cv. Tondino, kindly provided by Petoseed Italia (Parma, Italy), was performed as previously described (Bettini et al., 2003). Transgenic plants and the corresponding untransformed, *in vitro* regenerated controls were multiplied by micropropagation in Murashige and Skoog medium supplemented with Gamborg's B5 vitamins (Duchefa Biochemie B.V.) and grown *in vitro* at 25 ± 1 °C with a 16 h light–8 h dark photoperiod. Plants were transferred to a containment greenhouse for morphological analysis and self-fertilized to obtain first generation progeny.

2.3. Molecular analysis

DNA and RNA extractions were carried out by using the NucleoSpin Plant II and NucleoSpin RNA Plant kits (Macherey-Nagel GmbH & Co. KG), respectively. The presence of *rolB* in the transformed clones was assessed by PCR with gene-specific primers *rolB*-fw and *rolB*-rev (Table 1). Amplificability of the samples was evaluated by performing control reactions with primers for the β-1,3-glucanase gene (Bettini et al., 2015). One-step RT-PCR for