Enhanced Salt Stress Tolerance in Transgenic Potato Plants Expressing IbMYB1, a Sweet Potato Transcription Factor

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Introduction

Plants have to fight against abiotic stresses from the environment, such as drought, extreme temperature, salt, and UV irradiation, for growth and crop production [36, 52]. Low-molecular-weight antioxidants and antioxidant enzymes play important roles in scavenging and controlling the production and accumulation of reaction oxygen species (ROS) when plants are exposed to environmental stresses [5, 22]. Therefore, various metabolic pathways using secondary metabolites have evolved in plants to help them adapt to changing environments [16]. Flavonols, anthocyanins, and proanthocyanidins are secondary plant metabolites that are known collectively as flavonoids. Flavonoids are found to directly scavenge $Q^2$ - and ·OH through single electron transfer [54], and the total flavonoid level was found to be highly correlated to a plant’s antioxidant capacity [2]. Anthocyanin is also linked to stronger abiotic stress resistance [38, 48, 46]. Phenols, including flavonoids (flavonols, flavones, anthocyanins, etc.) and several classes of non-flavonoids (phenolic acids, lignins, stilbenes, terpenoids, etc.), have been found to determine the antioxidant activity of plants [13]. The flavonoid biosynthetic pathways appear to be mostly regulated at the transcriptional level [39, 47].

IbMYB1, a transcription factor (TF) for R2R3-type MYB TFs, is a key regulator of anthocyanin biosynthesis during storage of sweet potatoes. Anthocyanins provide important antioxidants of nutritional value to humans, and also protect plants from oxidative stress. This study aimed to increase transgenic potatoes’ (Solanum tuberosum cv. LongShu No.3) tolerance to environmental stress and enhance their nutritional value. Transgenic potato plants expressing IbMYB1 genes under the control of an oxidative stress-inducible peroxidase (SWPA2) promoter (referred to as SM plants) were successfully generated through Agrobacterium-mediated transformation. Two representative transgenic SM5 and SM12 lines were evaluated for enhanced tolerance to salinity, UV-B rays, and drought conditions. Following treatment of 100 mM NaCl, seedlings of SM5 and SM12 lines showed less root damage and more shoot growth than control lines expressing only an empty vector. Transgenic potato plants in pots treated with 400 mM NaCl showed high amounts of secondary metabolites, including phenols, anthocyanins, and flavonoids, compared with control plants. After treatment of 400 mM NaCl, transgenic potato plants also showed high DDPH radical scavenging activity and high PS II photochemical efficiency compared with the control line. Furthermore, following treatment of NaCl, UV-B, and drought stress, the expression levels of IbMYB1 and several structural genes in the flavonoid biosynthesis such as CHS, DFR, and ANS in transgenic plants were found to be correlated with plant phenotype. The results suggest that enhanced IbMYB1 expression affects secondary metabolism, which leads to improved tolerance ability in transgenic potatoes.

Keywords: IbMYB1, potato, salt tolerance, secondary metabolite, transgenic
The MYB regulators are divided into four types, MYB1R, R2R3-MYB, R1R2R3 MYB (MYB 3R), and 4R MYB, based on the repeats of their specific DNA-binding domains. Numerous R2R3-MYB proteins, which were mainly found in plants, have been proved to be involved in the control of plant-specific processes, including secondary metabolism and response to biotic and abiotic stresses [12, 44]. In many plant species, MYB proteins were reported to be capable to regulate the biosynthesis of flavonoids and anthocyanin, and be induced by abiotic stress, exemplified by Arabidopsis MYB75 (PAPI), A1MYB90 (PAP2) [4], MYB12[35], and MYB12 [34], petunia AN2 [40] and PH4 [39], grape MYB1A and MYB2A [27, 28], MYB5a [11], and MYB1 [9], sweet potato MYB1 [33], apple MYB10/MYB1/MYBA [3, 15, 45], legume LAP1 [37], Persimmon MYB4 [1], and Epimedium sagittatum MYB1A [19].

In this study, we aimed to develop transgenic potatoes (cv. LongShu No.3) that express IbMYB1, which was isolated from sweet potato by Kim et al. [23] and under control of an oxidative stress-inducible peroxidase (SWPA2) promoter [24], in order to increase the anthocyanin content in leaf and in tuber, and to subsequently enhance the resistant ability of potato to abiotic stress.

Materials and Methods

Plant Transformation and Gene Expression Analysis

The transformation construct was obtained by inserting the 750 bp IbMYB1 gene (Accession No. AB258984) fragment into the Agrobacterium tumefaciens strain EHA 105 harboring the pCAMBIA2300 binary vector. The T-DNA region also contained the kanamycin resistance gene of phosphotransferase II (nptII) and the IbMYB1 gene was driven by the oxidative stress-inducible SWPA2 promoter [24].

Potato plants (Solanum tuberosum L. cv. LongShu No.3) were taken into Agrobacterium-mediated transformation, where the protocols of potato propagation, transformation, and gene expression analysis all followed the method described by Kim et al. [25]. For the transgenic lines selection, the primers for genomic DNA of the IbMYB1 gene were 5'-CTTAGGCAACAGGTGGTCGCTT-3' and 5'-GTGAAATTTACGGCTTAGCCTTTAACA-3' and the primers of RT-PCR were 5'-CCATGCGCTCTCCAGGCAAGA-3' and 5'-ACGTTGTTCGGTGTGTCGTG-3'.

Treatments

Sterile potato seedlings were cultured in 0 or 100 mM NaCl-containing MS medium, root fresh weight and dry weight were measured at 20 days, and the expression of gene IbMYB1 was assayed. Potato seedlings from in vitro bottles were then transplanted to pots in a greenhouse with normal growth condition (a 16 h photoperiod with light intensity of 100 µmol photons m-2 s-1, 60% (w/v) relative humidity at day 25°C/ night 20°C). Four-week-old healthy potato plants with similar height in pots were watered with 0 or 400 mM NaCl for 6 days. Samples were taken at 0, 2, 4, and 6 days after treatment and kept in a deep freezer at −80°C for further analysis and RNA extraction. For UV-B stress, four-week-old potato plants were supplied with normal growth light plus 8 h UV-B radiation (308 nm; +2.7 kJ m−2 day−1) according to the method of Yin et al. [53], and for drought stress, plants were restricted from water for 6 days.

Physiological Analysis for Abiotic Stress Tolerance

The determination of total phenols and flavonoids were performed according to the methods described previously [7]. The extraction and quantification of the total anthocyanin were performed according to the pH differential method [30] with slight modification; two reagents of pH 1.0 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4 M) were prepared in advance, the extracts were mixed with 4-fold each reagent in volume, the mixture was incubated in dark for 15 min, and then the absorbance of 530 nm and 700 nm were measured immediately.

The assay of DPPH radical scavenging ability followed the method of Erkan et al. [14], where 100 µl of 5 times diluted 80% acidified methanol extracts was incubated with 0.2 mM methanolic DPPH solution for 15 min, and the absorbances at 515 nm were recorded. The ferric-reducing antioxidant power (FRAP) assay was according to Zhang et al. [54]. The assay of the chlorophyll content followed the method of Daneshmand et al. [10] and in the meantime the content of carotene was calculated. The determination of Fv/Fm and photosynthetic electron transport rate (ETR) was done using the by pulse amplitude modulated chlorophyll fluorescence system (Imaging PAM, Walz, Effeltrich, Germany).

Statistical Analysis

Data were statistically analyzed with Duncan’s multiple range test using Statistical Package for the Social Sciences (SPSS 12). Means refer to statistical significance at P < 0.05.

Results

Generation of Transgenic Potato Plants and Their Effects on Root Growth of Potato In Vitro

In this study, we used Agrobacterium-mediated transformation to develop transgenic potatoes (cv. LongShu No.3) with enhanced tolerance to environmental stress and enhanced nutritional value. We employed transgenic potato plants that expressed the IbMYB1 gene under control of an oxidative stress-inducible peroxidase (SWPA2) promoter (referred to as SM plants). After 12 transgenic lines were created, the potatoes were run through a high temperature selection and RT-PCR analysis. Two lines, namely, SM5