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Expressing the sweet potato orange gene in transgenic potato improves drought tolerance and marketable tuber production

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ABSTRACT

Potato (*Solanum tuberosum* L.) is generally considered to be sensitive to drought stress. Even short periods of water shortage can result in reduced tuber production and quality. We previously reported that transgenic potato plants expressing the sweet potato orange gene (*IbOr*) under the control of the stress-inducible *SWPA2* promoter (referred to as SOR plants) showed increased tolerance to methyl viologen-mediated oxidative stress and high salinity, along with increased carotenoid contents. In this study, in an effort to improve the productivity and environmental stress tolerance of potato, we subjected transgenic potato plants expressing *IbOr* to water-deficient conditions in the greenhouse. The SOR plants exhibited increased tolerance to drought stress under greenhouse conditions. *IbOr* expression was associated with slightly negative phenotypes, including reduced tuber production. Controlling *IbOr* expression imparted the same degree of drought tolerance while ameliorating these negative phenotypic effects, leading to levels of tuber production similar to or better than those of wild-type plants under drought stress conditions. In particular, under drought stress, drought tolerance and the production of marketable tubers (over 80 g) were improved in transgenic plants compared with non-transgenic plants. These results suggest that expressing the *IbOr* transgene can lead to significant gains in drought tolerance and tuber production in potato, thereby improving these agronomically important traits.

1. Introduction

Drought stress is a global, major environmental stress that reduces crop yields and restricts plant distribution. Depending on the extent of water availability, plants can be affected by drought stress in different ways, ranging from the inhibition of normal physiological activities and cellular damage to cell death [1,2]. Therefore, many land plants have evolved structural and physiological

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mechanisms to protect them from mild drought stress. In most crop plants, water deficit severely reduces plant growth, yields, and crop quality due to increased osmotic and oxidative damage. Due to the dramatic increase in the human population, together with increasingly serious environmental problems, it will be difficult for world agriculture to meet worldwide food and energy requirements in the future [3]. Therefore, it is crucial to develop crop cultivars with excellent environmental stress tolerance for use in sustainable agriculture.

Potato (*Solanum tuberosum* L.) is one of the four staple foods worldwide. The average per capita consumption of potato is gradually increasing due to its well-known health benefits. Moreover, the potato-harvesting period is relatively short, with a high production rate. However, potato is vulnerable to several environmental stresses, including drought stress. Even short periods of drought stress can result in serious damage and cause a significant reduction in tuber yields [4]. Therefore, in view of global warming and desertification, developing a potato line tolerant to water deficit has become a major task for potato breeders. Recently, several attempts were made to create potato cultivars possessing enhanced tolerance to drought stress by overexpressing genes for osmotic metabolites [5–7], transcription factor genes in the drought-signaling pathway involved in phyto-hormone signaling or biosynthesis [8], and genes responsive to antioxidant proteins [9].

The Orange gene (*Or*), which is involved in carotenoid accumulation, shares high levels of sequence homology in many crop plants such as cauliflower, rice, tomato, and sweet potato, as well as the model plant *Arabidopsis thaliana* [10–12]. Introducing the cauliflower *Or* gene induced the formation of chromoplasts and increased the carotenoid contents in transgenic potato tubers [13]. Recently, overexpressing the sweet potato *Or* (*IbOr*) gene also increased the carotenoid contents in transgenic potato tubers and sweet potato storage roots [14,15]. *IbOr* expression in sweet potato plants rapidly increases after NaCl, PEG, and H_2O_2 treatment [11]. Transgenic sweet potato calli overexpressing *IbOr* exhibit enhanced tolerance to salt stress, with increased carotenoid contents and antioxidant activity [11]. Therefore, *IbOr* is not only involved in carotenoid accumulation, but it also functions in the response to multiple abiotic stresses in transgenic potato and alfalfa plants, as well as in sweet potato callus [11,14,16]. Thus, *IbOr* may be useful for developing valuable crops with enhanced tolerance to multiple environmental stresses, along with increased nutrient contents.

When designing an efficient expression system, it is important to select the proper promoter, such as stress-inducible, tissue-specific, or constitutively expressed promoters. We previously isolated and characterized the strong oxidative stress-inducible sweet potato peroxidase anionic 2 (*SWPA2*) promoter [17]. The *SWPA2* promoter induces higher levels of exogenous gene expression than the *35S* promoter from cauliflower 1 virus (*CaMV 35S* promoter) in response to various stress treatments, and was successfully applied to several transgenic plants such as poplar, potato, sweet potato, alfalfa, and rice [16,18–21]. The use of the stress-inducible *SWPA2* promoter to drive transgene expression can minimize the negative effects of transgene overexpression on plant growth. Therefore, the stress-inducible *SWPA2* promoter is highly suitable for generating transgenic plants with enhanced tolerance to environmental stresses.

In the present study, over a 2-year period (2013–2014), we assessed the capacity of the sweet potato *IbOr* genes to increase drought tolerance in transgenic potato under greenhouse conditions and determined whether negative agronomic growth qualities were associated with high levels of transgene activity. Expression of *IbOr* increased drought stress tolerance in transgenic potato, thereby conferring high-quality tuber production.

2. Materials and methods

2.1. Plant materials

Transgenic potato plants (*S. tuberosum* L. cv. Atlantic) expressing *IbOr* under the control of the oxidative stress-inducible *SWPA2* promoter (SOR plants) were generated by *Agrobacterium*-mediated transformation. In a previous study [14], the introduced *IbOr* gene in transgenic SOR plants was confirmed by genomic and RT-PCR analysis. Transformed potato plants were maintained in vitro by sub-culturing every four weeks at 23 °C under 16-h day (4000 Lux light) and 8-h night conditions, as described by Goo et al. [22]. Ten plants of each line were then grown in 10 cm diameter pots containing commercial mineral-mixed soil in a greenhouse at approximately 28/22 °C (day/night) with daily watering; 6-week-old plants were used for water-deficient treatments.

2.2. Genomic PCR and RT-PCR

Genomic DNA from regenerated plants was isolated with a GeneAll Exgene™ Plant SV kit (Seoul, Korea) as described by the manufacturer. PCR was carried out with purified genomic DNA and primer sets for the *IbOr* and *bar* genes. To purify total RNA from the plants, leaves or tubers were macerated in liquid N_2, and Trizol reagent (Invitrogen, CA, USA) was used to extract the RNA, as described in the manufacturer's protocol. The RNA was further purified with an RNeasy Plant Mini kit (Qiagen, Germany), followed by cDNA synthesis with a kit (Toyobo, ReverTra Ace-αE-, Osaka, Japan). PCR and RT-PCR products were fractionated in a 1.5% agarose gel. The sequences of the primers used for amplification of the *IbOr* and *bar* genes were previously described [14].

2.3. Drought stress treatment

For dehydration treatment, the potato plants were irrigated with similar quantities of water through trays placed underneath the pots for 60 days, followed by withholding water for 15 days. The plants were then watered and allowed to recover from drought conditions. Tests for visible damage caused by dehydration were repeated in triplicate. Survival rates and wilting grades caused by dehydration conditions were evaluated after treatment and expressed as percentage survival rate and wilting grade level.