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Rapid identification of potential drought tolerance genes from *Solanum tuberosum* by using a yeast functional screening method

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ABSTRACT

Identification of major stress tolerance genes of a crop plant is important for the rapid development of its stress-tolerant cultivar. Here, we used a yeast functional screen method to identify potential drought-tolerance genes from a potato plant. A cDNA expression library was constructed from hyperosmotic stressed potato plants. The yeast transformants expressing different cDNAs were selected for their ability to survive in hyperosmotic stress conditions. The relative tolerances of the selected yeast transformants to multiple abiotic stresses were also studied. Specific potato cDNAs expressed in the tolerant yeast transformants were identified. Sixty-nine genes were found capable of enhancing hyperosmotic stress tolerance of yeast. Based on the relative tolerance data generated, 12 genes were selected, which could be most effective in imparting higher drought tolerance to potato with better survival in salt and high-temperature stresses. Orthologues of few genes identified here are previously known to increase osmotic stress tolerance of yeast and plants; however, specific studies are needed to confirm their role in the osmotic stress tolerance of potato.

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1. Introduction

The actual productivity of crops usually falls far short of its maximum potential, mainly due to various environmental constraints, such as drought, extremes in temperature, salinity, etc. Such constraints are expected to augment in coming decades due to global warming and associated changes in climate. Thus, to sustain the present rate of increase in agriculture production, it is important to develop improved varieties of crop plants with higher tolerance to various stresses. Better understanding of the mechanisms by which a plant can cope with adverse environments is important for the development of stress-tolerant plants. Studies in various plant species, especially in model plants such as *Arabidopsis* and tobacco have revealed various mechanisms involved in stress tolerance of plants, and those mechanisms were summarized in various reviews [1–3]. To make use of accumulating information on stress tolerance mechanisms of plants for the development of stress-tolerant varieties of a particular agricultural crop, it is important to investigate the specific stress tolerance mechanisms of that particular crop too. Therefore, it is time for us to strengthen our understanding of the stress tolerance mechanisms working in agriculturally important plants, such as potato, tomato, rice, etc.

Potato (*Solanum tuberosum* L.) is highly sensitive to environmental stress. The effect of environmental constraints on potato cultivation is well reflected in the discrepancy between its average and record yields;

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according to Boyer [4], the maximum yield of potato is three times larger than its average yield, and this difference is mainly due to environmental constraints. Potato plants are very sensitive to drought stress [5]; hence, drought is a major constraint for potato cultivation in many parts of the world [6,7]. The adverse effect of drought stress on potato virtually occurs at all stages of the crop, from seedling emergence to tuber initiation and bulking, which ultimately results in reduced tuber yield [8,9]. Furthermore, prolonged water scarcity leads to several physiological disorders, such as tuber cracking, tuber malformation, hollow heart, vascular discoloration and reduction in the accumulation of total dry matter in tubers [10,11]. Overall, the effect of drought stress on potato cultivation is severe.

In future, it will not be economical to irrigate the increasing proportion of drought-struck agricultural land, and hence, the development and supply of drought-tolerant varieties of potato is increasingly important. There is also an urgent need to supply improved varieties to farmers so that they will continue potato cultivation in hot and dry areas. Faster and better understanding of drought-tolerance mechanisms in potato will help us to speed up crop improvement programs (through breeding and/or genetic engineering) for the development of drought-tolerant verities. Even though there were few studies to deduce the drought-tolerance mechanisms in potato, an understanding of the key genes and overall network of genes that are related to drought stress in potato is still inadequate.

Recently, a functional screening-based approach, using yeast or bacteria as an experimental system, is being utilized to identify genes that may play significant roles in the stress tolerance of plants [12–17]. Among them, a functional screening system that uses yeast have advantage over those that use bacteria, as yeast is a eukaryotic organism with relatively closer post-translational modification to that of higher plants [18]. Priyanka et al. [19] used a yeast system to screen for multiple abiotic stress tolerance abilities of a pigeon pea hybrid-proline-rich protein, and found that the yeast overexpressing the protein is tolerant to multiple stresses. Functional screening of yeast expressing a cDNA library from *Jatropha curcas* resulted in the isolation/identification of sequence orthologues of genes with known functions in stress tolerance, such as allene oxide cyclase, late embryogenesis abundant protein-5, metallothionein, thioredoxins, etc.; in addition to these, a number of uncharacterized genes are also identified to have potency to impart stress tolerance [16].

With the availability of a large amount of genome sequence, transcriptome, and proteome data, it is possible to predict potential stress tolerance genes of a crop plant. However, the functional screen-based methods for choosing/identifying potential stress tolerance genes of a crop plant hold their own advantages. These functional screening methods select the genes based on their relative ability in imparting higher stress tolerance to yeast or bacteria; hence, these methods can be extended further to select the most potent genes from a bigger list of potential genes. In addition, these methods are capable of identifying ability of unknown or uncharacterized genes, leading to the discovery of new stress tolerance genes.

In this study, we used a yeast-based functional screen to identify potential drought tolerance genes in potato. To enable the screening process, a cDNA expression library was constructed from drought stressed potato plants under the control of the yeast GAL1 system, and yeast transformants expressing potato cDNAs were selected for their ability to survive in hyperosmotic stress condition. The relative tolerances of the selected yeast transformants to multiple abiotic stresses were also studied to propose the most potent candidate genes for detailed investigations to explore their role in stress tolerance of potato.

2. Materials and methods

2.1. Plant material

Nodal explants of potato were grown in Murashige and Skoog (MS) medium [20], containing 2.5% sucrose and 0.7% agar for 28 days, then the regenerated plants were transferred to a liquid ½MS medium, containing 0.5% sucrose (L½MS) for a week. During these seven days, the culture bottles were fully closed for first two days, partially open for the third and fourth day and fully open for the last three days to make the plants acclimatize to the culture room conditions. During this acclimatization period, at every 24 h the L½MS medium in which plants were growing was replaced with the same fresh medium. Plants were found to remain strong and healthy in the standard culture room conditions (relative humidity of 50–60%, 25 ± 2 °C and a 16 h photoperiod). The plants with similar height and root mass (by visual observations) were used for stress experiments (Fig. 1).

2.2. Details of yeast strain (Saccharomyces cerevisiae) used for the functional study

Accession No: Y00000, Strain: BY4741, Genotype: *MATa; his3 Δ1; leu2Δ0; met15Δ0; ura3Δ0*. This strain was provided by the European *Saccharomyces cerevisiae* Archives for Functional Analysis (EUROSCARF), Frankfurt, Germany.

2.3. Stress treatment of plants and RNA isolation

To give drought stress to the plants, the liquid medium (L½MS) in which the plants were grown during the acclimatization period in the culture room (described above) was replaced with fresh L½MS medium additionally containing 25% polyethylene glycol (PEG 8000, Sigma, St. Louis, USA). After 24 h, the plants were found to show clear signs of wilting (Fig. 2). In the case of the control plants, the L½MS medium in which plants were grown was replaced with fresh L½MS medium; the control plants were found to remain turgid even after 24 h. After 24 h of treatment, the whole plant was removed from the bottle, washed with distilled water and frozen in liquid nitrogen before being stored at −70 °C. Total RNA was isolated from these whole plant samples using the NucleoSpin RNA Plant (Macherey-Nagel, Düren, Germany) kit, following the

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