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Identification of Abiotic Stress-Induced Differentially Expressed Genes of Rye Leaves

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Abstract

In the present study, we investigated abiotic stress-responsible differentially expressed genes (DEGs) in rye leaves using reverse transcription-polymerase chain reaction (RT-PCR) technique based on the annealing control primer (ACP)-based differential display method.

Using 120 ACPs, a total of 18 genes were identified to be up- or down-regulated under abiotic stress such as drought, salt, and cold treatments and followed by sequencing.

The identification of novel genes involved in abiotic stresses provides new insights for a better understanding of the molecular basis of plant response in rye plants to different environmental stresses, especially drought, salt, and cold stresses.

Keywords: abiotic stresses, GeneFishing, rye

1. Introduction

Environmental stresses such as drought, salt, and cold stresses have adverse effects on plant growth and productivity (URANO & al. [1]) and they are the most damaging factors to agricultural productivity worldwide (MITTLER [2]). Under severe conditions, these adverse environmental stresses can cause death of plants (ZHOU & al. [3]).

In order to survive such stresses, plants respond and adapt to a continuously changing environment by appropriate physiological mechanisms, rapid regulation of altered gene expression, and change in biochemical regulation.

Genetics, in particular, molecular genetic studies have determined that many gene products contribute to drought, salt, and cold temperature tolerance (PRIEST & al. [4], RABBANI & al. [5]).

At the molecular level, plants have developed mechanisms to overcome environmental stress by up- or down-regulating a number of proteins, transcription factors (TFs), enzymes, and molecular chaperones, which are believed to have a role in different defense mechanisms (CUSHMAN & BOHNERT [6], HU & al. [7]).

Annealing control primer (ACP)-based PCR method using the GeneFishing™ system has been extensively used in gene expression analysis in plants. This screening method has been used in plant systems (LEE & al. [8], LEE & al. [9], LEE & al. [10]) for identifying differentially expressed genes from two RNA samples. Comparative GeneFishing™ analysis has been used to study the differences of gene expression in various tissues, and to identify candidate genes implicated in a specific stress-tolerance pathway.

Therefore, the identification of genes involved in abiotic stress responses is a fundamental step in understanding the molecular mechanisms of stress responses and developing transgenic plants with enhanced tolerance to stress (LEE & al. [10]).

In this study, we identified genes that were up- or down-regulated, as a response to three abiotic stresses including drought, salt, and cold temperatures in rye. These genes were identified by sequencing.

The main goal of this study was to identify novel genes that are differentially expressed upon exposure to major environmental stresses, such as drought, salt, and cold, and thus to provide new insight into the development of forage crops with enhanced tolerance to environmental stress conditions.

2. Materials and methods

Rye (*Secale cereale* L. cv. Paldang homil) seeds were obtained from the National Institute of Animal Science, Rural Development Administration, South Korea. Surface-sterilized seeds were germinated in a plastic tray for 14 d in a controlled growth chamber at 24±1°C with a 12 h photoperiod (day/night), under an irradiance of 350 μmol $m^{-2}s^{-1}$ and relative humidity of 60%-70%.

Drought, salt, and cold treatments were conducted as described by LEE & al. [10]. Total RNAs that were extracted from leaf tissues of rye were used for synthesis of first-strand cDNAs by reverse transcriptase according to LEE & al. [9].

First-strand cDNAs were diluted by the addition of 80 μL of ultra-purified water for the GeneFishing™Polymerase chain reaction (PCR), and stored at -20°C until analyzed. Differentially expressed genes (DEGs) were screened by the annealing control primer (ACP)-based PCR method [9] using GeneFishing™ DEG premix kit (Seegene, South Korea).

The amplified PCR products were separated in 2% agarose gel stained with ethidium bromide and DEGs were selected visually. Selected DEGs were extracted from the gel using the GENECLEAN® II Kit (Qbiogene, Inc., CA, USA) and directly cloned into a TOPO TA cloning vector (Invitrogen™, CA, USA) according to the manufacturer's instructions.

Sequences were confirmed with the GenBank database through the BlastX program of NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results and discussion

In order to identify the abiotic stress-responsive genes in rye, we analyzed the annealing control primer (ACP)-based GeneFishing technology coupled with PCR under various stresses, including drought, salt, and cold treatments. A total of 120 arbitrary GeneFishing primers (GPs) were analyzed. Of those, 18 GPs showed differentially expressed DNA bands under at least one stress (Fig. 1). All bands, except GP116, were up- or down regulated compared to the control in the drought treatment. Among the DEGs, GP2, 17, 22, 32, 33, 35, 63, 69, 74, 79, 96, and 112 were up-regulated, whereas GP44, 52, 79, 108, and 114 were down-regulated in the drought treatment. In salt treatment, 4 GPs (63, 69, 112, and 116) were up-regulated on DNA bands, whereas GP79 was down-regulated. In the cold treatment, 7 GPs (2, 63, 69, 79, 96, and 112) had the same expression pattern as that in the salt treatment.

The differentially expressed DNA bands under various stress conditions were purified from agarose gels and cloned into the TOPO TA cloning vector for sequence analysis. The sequence similarities of these DEGs are summarized in Table 1. Along with ferritin, we detected ribulose bisphosphate carboxylase (Rubisco) small subunit and galactinol synthase, all of which were previously identified under drought, cold, or heat treatments in genomics or proteomics analyses (SEKI & al. [11], FOWLER & THOMASHOW [12], LEE & al. [13]).

DEG9, identified to encode for chloroplast ATP synthase delta chain precursor, was considerably down-regulated under drought stress. ATP synthase is well-known for its physiological role in the energy production pathway. In this study, we identified the delta chain of chloroplast ATP synthase.

This result indicates that drought stress has stronger effect on ATP synthase capability than the salt and cold treatments. Four DEGs (DEG13, 14, 16, and 23) had equal expression pattern across all stress treatments. These DEGs seem to be abiotic stress biomarkers specific to drought, salt, and cold. Additional studies, such as gain/loss of function analyses, are required for the validation of this hypothesis. A total of 6 DEGs (DEG5, 8, 14, 17, 23, and 25) were identified as a hypothetical or predicted protein, or were without specific function. These genes were up-regulated under at least one treatment.

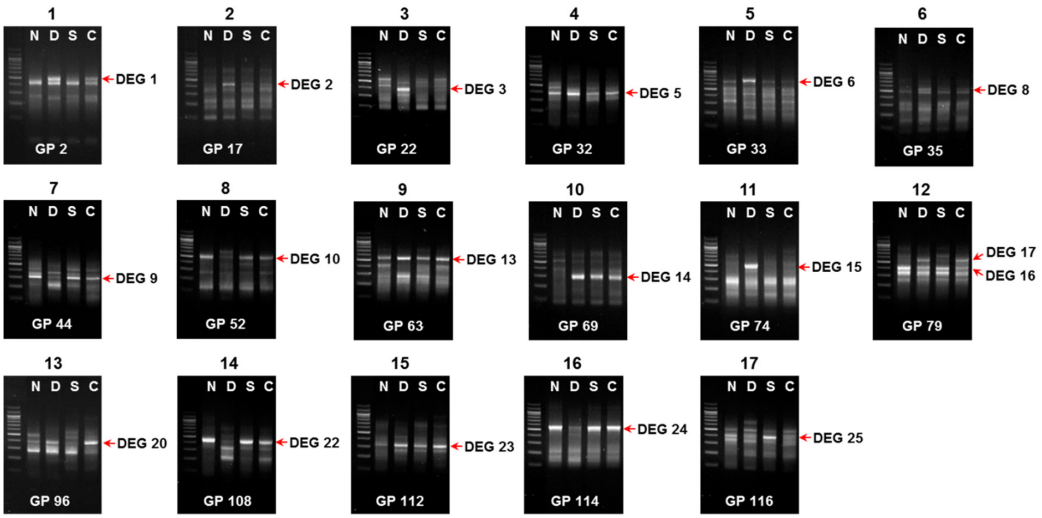

Figure 1. Agarose gel electrophoresis shows results of annealing control primer system coupled with reverse transcriptase PCR for identification of differentially expressed genes (DEGs) in response to drought, salt, and cold treatments. Arrows indicate DEGs under abiotic stresses compared to the control. N, non-treatment; D, drought treatment; S, salt treatment; C, cold treatment.

4. Conclusions

Few studies have investigated the response to environmental stresses in rye. In the present study, we used a newly developed ACP-based RT-PCR method with differential display to identify abiotic stress-responsible genes in rye leaves. The stresses included in this study were drought, salt, and cold. We identified some novel genes, including ornithine-oxo-acid aminotransferase (DEG2), acid beta-fructofuranoside precursor (DGE3), abscisic stress ripening protein 2 (DEG6), and nitrous oxide reductase (DEG16). These genes are considered to be candidates for the stress adaptation factors in rye leaves. Our study contributes to the knowledge of the molecular basis of the abiotic stress response in plants. However, further comparative analysis should be conducted in order to better understand the stress defense mechanism in plants.

Table 1. Differentially expressed genes (DEGs) identified in rye leaves using annealing-control-primer-based differential display reverse transcriptase-PCR analysis.

Gel No.	GP[1]	DEG No.	D[2]	S[3]	C[4]	Acc. No.[5]	Annotation [Species]	Score	E Value
1	2	DEG 1	↑		↑	ACJ05649.1	Ferritin 1B [*Triticum aestivum*]	152	1.00E-35
2	17	DEG 2	↑			ABP38411.1	Ornithine-oxo-acid aminotransferase [*Saccharum officinarum*]	112	1.00E-23
3	22	DEG 3	↑			CAG25609.1	Acid beta-fructofuranosidase precursor [*Triticum aestivum*]	138	1.00E-31
4	32	DEG 5	↑			EAZ17510.1	Hypothetical protein OsJ_031719 [*Oryza sativa* (japonica cultivar-group)]	112	1.00E-23
5	33	DEG 6	↑			ABR25748.1	Abscisic stress ripening protein 2 [*Oryza sativa* (indica group)]	77	1.00E-12
6	35	DEG 8	↑			EEC84129.1	Hypothetical protein OsI_30469 [*Oryza sativa* (indica group]	157	4.00E-37
7	44	DEG 9	↓			ABR26187.1	Chloroplast ATP synthase delta chain precursor [*Oryza sativa* (indica cultivar-group)	100	5.00E-20
8	52	DEG 10	↓			P07398.1	Ribulose bisphosphate carboxylase small chain clone [*Triticum aestivum*]	176	6.00E-43
9	63	DEG 13	↑	↑	↑	CAA57340.1	Putative tumor suppressor [*Oryza sativa* (indica group)]	197	4.00E-49
10	69	DEG 14	↑	↑	↑	XP_001947368.1	similar to predicted protein [*Acyrthosiphon pisum*]	33.5	5.9
11	74	DEG 15	↑			BAF51566.1	Galactinol synthase [*Triticum aestivum*]	81.3	2.00E-14
12	79	DEG 16	↓	↓	↓	ABF83472.1	Nitrous oxide reductase [*Marinobacter hydrocarbonoclasticus*]	36.2	0.93
13	79	DEG 17	↑		↑	EEC74243.1	Hypothetical protein OsI_09446 [*Oryza sativa* (indica group)]	162	1.00E-38
14	96	DEG 20	↑		↑	YP_001839293.1	Gamma-glutamyltranspeptidase [*Leptospira biflexa serovar Patoc* strain 'Patoc 1 (Paris)']	33.9	4.5
15	108	DEG 22	↓			CAG25595.1	Putative rubisco small subunit [*Triticum turgidum* subsp. *durum*]	119	8.00E-26
16	112	DEG 23	↑	↑	↑	NP_001054651.1	Os05g0148700 [*Oryza sativa* (japonica cultivar-group)]	83.6	5.00E-15
17	114	DEG 24	↓			P07398.1	Ribulose bisphosphate carboxylase small chain clone [*Triticum aestivum*]	221	2.00E-56
18	116	DEG 25		↑		EEC77300.1	Hypothetical protein OsI_15948 [*Oryza sativa* (indica group]	96.7	6.00E-19

[1],GeneFishing primer identifying number; [2], drought treatment; [3], salt treatment; [4], cold treatment; ↑, up-regulated; ↓, down-regulated

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