Differential Screening of Randomly Amplified cDNAs Using RAPD Primers in Salt Tolerance and Sensitive Wheat

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Abstract

The identification of salinity-tolerance genes is a critical aspect of the new molecular technology. In this work cDNA-RAPD is used for the identification of genes expressed in salt tolerant but not in salt sensitive wheat. Two cultivars wheat, salt tolerance (Dijla) and sensitive (Tamooz2) were used for the preparation of RNA and cDNA synthesis. Eight primers were used for random amplification of cDNA constructed from RNA and three primers were differentially expressed in salt tolerant cultivars. Genes related to salt tolerant were predicted using NCBI blast for the three primers. The predicted genes were involved in salt tolerance of wheat and other plants as well. This indicates the suitability of the primers and the method for salt tolerance genes identification in wheat under study.

Key words: wheat, salt, RAPD-PCR

Introduction

Salinity is a major factor limiting plant growth and leads to lower agricultural production in arid and semi-arid regions [1]. Several technologies were used for the identification of differential expressed salt tolerance genes in wheat. Typically, closed systems such as microarrays and real-time polymerase chain reaction (PCR) have been extensively followed in gene expression analysis in plants [2]. In open systems, there is no need for previous knowledge of the genome or transcriptome of the organism. cDNA-AFLP (cDNA-Amplified fragment length polymorphism) have been successfully used to quantify transcript abundance and generate expression data across different types of tissue or developmental stages in wheat [3,4]. cDNA-RAPD PCR is a simple method established by other investigators [5] for the identification of differentially expressed genes in rice. This method was used to identify novel drought tolerance gene in *Gossypium hirsutum* [6], and evaluation of cellulolytic filamentous fungi phenotypes [7] and the identification of a novel Getah virus [8]. This study aimed to use this method to identify differentially expressed genes in salt tolerance and sensitive wheat cultivated in Iraq.

Materials and methods

Wheat genotypes cultivation

Two cultivars of wheat were used in this study, salt tolerance (Dijla) and sensitive (Tamooz2). Seed of both cultivars were washed with tap water for 30 min, immersed in 50% of sodium hypochlorite then treated with 2-3 drop of Tween 20 for 10 min, seeds were washed once with 70% ethanol and rinsed many times with sterile water. Five sterilized seeds from each plant were placed in culture bottle containing 15 ml of agar solidified, hormones-free MS medium [9] with each of 0 ds/m, 15 ds/m and 25 ds/m of (NaCl) salt concentrations. Each treatment for wheat was replicated three times. All cultures were kept in the light for 16h and dark for 8h at 25^{o}C. Data were recorded 15 days after treatment steps.

RNA isolation and cDNA synthesis

Total RNA was isolated by using Geneaid total RNA purification mini kit (Taiwan) according to the manufacturer's instructions. Isolated RNA was treated with RNase-free DNase I (Biobasic, Canada) for 20 min at 37°C, DNase I was inactivated at 65°C for 10 min. The integrity of the RNA was verified after separation by electrophoresis on a 1.5% agarose gel containing 0.5% (v/v) ethidium bromide. First-strand cDNA was synthesized from 500 ng of total RNA by using Reverse Transcription system (Bioneer, Korea) with an oligo-dT_{15} primer. The reaction solution was used as templates for reverse transcriptase polymerase chain reaction (RT-PCR) [3].

cDNA-RAPD

Eight primers (table1) were used for amplification of cDNA using optimized PCR protocols and master mixes. Polymerase chain reaction was initiated with hot start method by using the single strand cDNA template on Labnet Thermocycler (USA). The PCR reaction was carried out according to the program of 35 amplification cycles (94°C for 30 s, 61°C for 45 s and 72°C for 90 s). Ethidium bromide agarose gel electrophoresis (1%) is used for the analysis of PCR products. The generated bands were compared, the differential amplified bands were recorded and the sequences of these bands aligned to related sequences in NCBI blast database [3].

Results

Total RNA isolated from both cultivars and DNase treated in order to eliminate genomic DNA (figure1). Eight RAPD primers were used for the amplification of cDNAs generated

from both genotypes. Three primers produced differential bands between salt resistance and sensitive cultivars were 7, 13, and 20 (figure 2, 3).

To predict the differential expressed genes in salt resistant but not sensitive genotype, NCBI blast was performed with the three primers. The predicted genes were listed in table (2).

Discussion

Investigating the function of the predicted genes in published; revealed the role of these genes in salt tolerance in different ways in several plants. Of the predicted proteins are kinases and phosphatases which are major posttranslational regulators of numerous cellular processes. These enzymes regulate metabolic pathways and are intimately involved in cellular signaling networks as shown in many studies which have involvement in salt tolerance [10]. Cytokinin oxidase dehydrogenase involved in cytokinins synthesis which showed they are involved in stress responses [11,12]. Thioredoxin is involved in the stress response through the regulation of the apoplastic reactive oxygen species in rice [13]. These genes need to be verifies its contribution in salt tolerance by other techniques such as real time PCR.

References

1-Bai, R. ; Zhang, Z.; Hu, Y.; Fan. M. and Schmidhalter, U. (2011). Improving the salt tolerance of Chinese spring wheat through an evaluation of genotype genetic variation. *Aust J Crop Sci.* 5: 1173-1178.

2-Monroy, A. ; Dryanova, A.; Malette, B.; Oren, D.; Ridha Farajalla, M. and Liu, W. (2007) Regulatory gene candidates and gene expression analysis of cold acclimation in winter and spring wheat. *Plant Mol. Biol.* 64:409-423.

3-Chen, G.; Ma, W.; Huang, Z. ; Xu, T.; Xue, Y.; and Shen, Y. (2003) Isolation and characterization of TaGSK1 involved in wheat salt tolerance. *Plant Sci.* 165: 1369–1375.

4-McIntosh, S. L.; Watson, P. ; Bundock, A.; Crawford, J.; White, G.; Cordeiro, D.; Barbary, L.; Rooke and Henry, R. (2007) SAGE of the developing wheat caryopsis. *Plant Biotechnol*. J. 5:69-83.

5-Yoshida, K.; Naito, S. and Takeda, G. (1994) cDNA cloning of regeneration-specific genes in rice by differential screening of randomly amplified cDNAs using RAPD primers. *Plant Cell Physiol.* 35(7):1003-9.

6-Selvam, J.; Kumaravadivel, N.; Gopikrishnan, A.; Kumar, B.; Ravikesavan, R. and Boopathi, M. (2009) Identification of a novel drought tolerance gene in *Gossypium hirsutum* L. cv KC3. *Commun. Biometry Crop Sci.* 4: 9–13.

7 – Oliveira, E.; Barros, N.; Ferreira, T.; Oliveira, T.; Terzi, S. and Damaso, M. (2010). Evaluation of cellulolytic filamentous fungi phenotypes using randomly amplified cDNA with RAPD primers. 32nd symposium on biotechnology for fuel and chemicals, USA.

8-Hu, T.; Zheng, Y.; Zhang, Y.; Li, G.; Qiu, W.; Yu, J.; Cui, Q.; Wang, Y.; Zhang, C.; Zhou, X.; Feng, Z.; Zhou, W. and Fan, Q. (2012) Identification of a novel Getah virus by Virus-Discovery-cDNA random amplified polymorphic DNA (RAPD). *BMC Microbiol*. 12:305.

9- Murashige, T. and Skoog, F.A. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15, 473-497.

10-Wang, H.; Chevalier, D.; Larue, C.; Cho, S. and Walkera, J. (2007). The Protein Phosphatases and Protein Kinases of *Arabidopsis thaliana*. The Arabidopsis Book. American Society of Plant Biologists First.

11-Tran, L.S.; Urao, T.; Qin, F.; Maruyama, K.; Kakimoto, T.; Shinozaki, K. and Yamaguchi-Shinozaki, K. (2007). Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. *Proc. Natl. Acad. Sci.* USA 104: 20623–20628.

12-Argueso, C.T.; Ferreira, F.J. and Kieber, J.J. (2009) Environmental perception avenues: the interaction of cytokinin and environmental response pathways. *Plant Cell Environ.* 32: 1147–1160.
13-Zhang, C.; Zhao, B.; Ge, W.; Zhang, Y.; Song, Y.; Sun, D. and Guo, Y. (2011) An apoplastic H-type thioredoxin is involved in the stress response through regulation of the apoplastic reactive oxygen species in rice. *Plant Physiol*. 157: 1884–1899.

Table (1): RAPD primers used.

Primers	Sequence
Maj-OPA-07	5'-AAGTCCGCTC-3'
Maj-OPA-09	5'-GGGTAACGCC-3'
Maj-OPC-08	5'-TGGCGGTG-3'
Maj-OPN-16	5'-CAAGGTGGGT-3'
Maj-OPC-12	5'-TGTCATCCCC-3'
Maj-OPA-11	5'-CAATCGCCGT-3'
Maj-OPA-13	5'CAGCACCCAC-3'
Maj-OPD-20	5'-TGTCATCCCC-3'

Table (2): NCBI Blast- predicted expressed genes and their Genbank accession numbers

Primer	Predicted gene	Genbank accession number
Primer No. 7	purple acid phosphatase	JX501672.1
	vacuolar proton inorganic pyrophosphatsae mRNA	AY296911.1
	thioredoxin	AJ005840.1
Primer No. 13	Galactosyltransferase B3	JN165358.1
	Galactosyltransferase B2	JN165357
	Galactosyltransferase B1	jn165356
	Galactosyltransferase A	JN165355
	vaculor proton inorganic pyrophosphatase	JQ180506.1
	Cytokinin oxidase dehydrogenase (Ckx2.4)	JN381555.1
	Galactosyltransferase	GQ 231955.1
	ABA binding protien1	HQ166718.1
Primer No. 20	Low affinity nitrate transporter	HF544988.1
	Cytokinin oxidase/dehydrogenase salt tolerant protein	EF415486.1
	Storage protein activator	FM242575.1
	Plastid acetulye coA carboxylase	EU660902.1
	Lon1 protease	AY494984.1
	Serine therionine kinase	AY036609.1

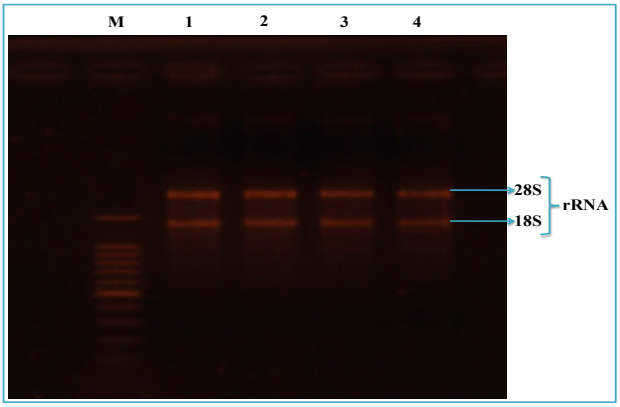

Figure (1): ethidium bromide stained agarose gel electrophoresis (1%), M: 100bp DNA ladder, Lanes 1 to 4: DNase- treated total RNA preparations.

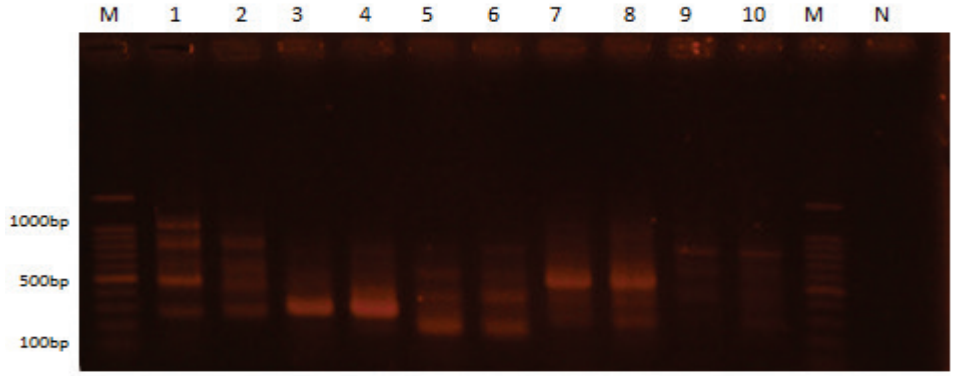

Figure (2): Ethidium bromide stained agarose gel electrophoresis (1%) of PCR products. M: 100bp DNA ladder. Lanes (1-2): PCR products of primer number (07), lanes (3-4): PCR products of primer number (08), lanes (5-6): PCR products of primer number (09), lanes (7,8): PCR products of primer number (13), lanes (9-10): PCR products of primer number(11), N: negative control.

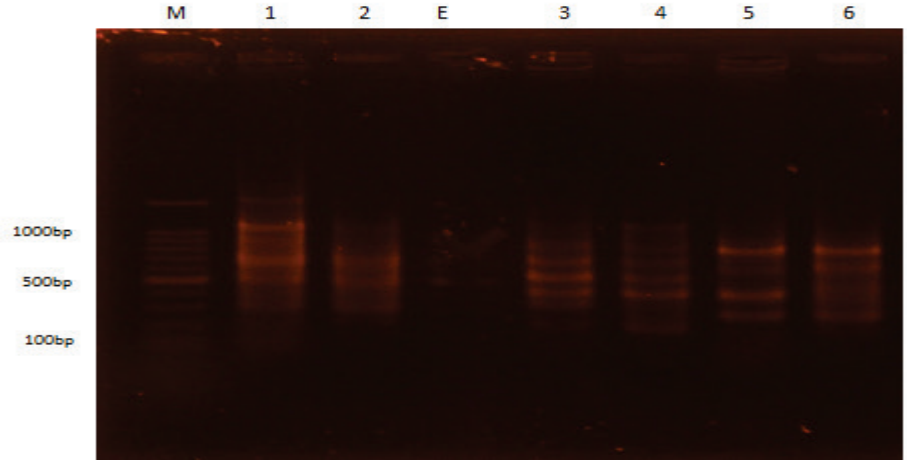

Figure (3): Ethidium bromide stained agarose gel electrophoresis (1%) of PCR products. M: 100bp DNA ladder. Lanes (1-2): PCR products of primer number (20), lanes: PCR products of primer number (12), lanes (5-6): PCR products of primer number (16), E: Empty lane.

المسح التفريقي لقطع ال cDNA المضخمة باستخدام بادئات عشوائية في الحنطة المتحملة والحساسة للملوحة

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الخلاصة

استخدمت تقنية cDNA-RAPD PCR لتحديد الجينات التي يعبر عنها اصناف الحنطة المتحملة للملوحة ولايعبر عنها في الاصناف الحساسة. استعملا صنفا الحنطة المتحملة للملوحة (دجلة) والحساسة (تموز2) لإعداد الحمض النووي الريبي وتصنيع ال cDNA. استعملت ثماني بادئات للتضخيم العشوائي من cDNA مصنعة من الحمض النووي الريبي. وقد أظهرت ثلاث بادئات تعبير تفاضلي في الصنف المتحمل للملوحة. تم توقع الجينات المتعلقة بتحمل الملوحة باستعمال NCBI blast للبادئات الثلاثة. تبين أن الجينات التي تم توقعها ودورها بتحمل الملوحة في الحنطة أو غيرها من النباتات تشير إلى اهمية هذه البادئات والطريقة المستخدمة في تحديد الجينات التي لها علاقة بتحمل الملوحة في الحنطة تحت الدراسة.

الكلمات المفتاحية: الحنطة، الملوحة، cDNA-RAPD PCR