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SEMI-QUANTITATIVE RT-PCR ANALYSIS OF TWO SILICON TRANSPORTER GENES IN SILICON UPTAKE AND STEM BORER RESISTANCE IN SOME RICE GENOTYPES

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ABSTRACT

Relative gene expression of two Si transporter genes (Lsi1 and Lsi2) was examined in the roots of seven tested rice genotypes grown in solution culture (9 days old) using semi-quantitative RT-PCR technique. The Si concentration in roots, stems and shoots of these rice accessions (30 days old grown in solution culture) was measured. Moreover, stem diameter, white head percentage and plant Si content were studied at the heading stage for the tested rice genotypes grown in field in order to insure stem borer resistance trait in the higher Si content and lower stem diameter rice plants. The results of this study indicate the direct association of the level of gene expression of Si transporter genes and plant Si content and its associated characters with stem borer resistance trait in rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals in the world. The rice stem borer, *Chilo agamemnon* Bles. is a serious pest of rice causing considerable damage to the plant from seedling to maturity, thus accounts for a large share of crop losses (Assas, 2005). Rice stem borers at the larval stages causing, in many cases, severe crop loss. Rice requires high silicon levels for healthy growth and stable and high productivity (Ma and Takahashi, 2002). Rice is a typical silicon accumulating plant (up to 10% of dry weight). High accumulation of silicon in rice has been attributed to the ability of the roots to take up silicon (Richmond and Sussman, 2003). Silicon plays an important role in reducing plant susceptibility against a variety of different biotic and abiotic stresses (Ma, 2004). Saeb and Oskou (2004) reported that higher silica level resulted in significantly lower stem diameter and with the decrease in stem diameter the infection to stem borer was decreased and consequently decreasing of white head percentage. Two silicon transporter genes, control silicon accumulation, were identified in rice. *Lsi1* (low silicon rice) is a transporter gene responsible for Si transport from the external solution to the root epidermis cells (Ma *et al*. 2006) and Lsi2 is another transporter gene involved in Si transport from the root epidermis cells to the xylem parenchyma (Ma *et al*. 2007). It is important to develop rice cultivars with adequate levels of resistance to the striped stem borer. Conventional breeding plays an essential role in rice cultivar innovation, but the progress is slow owing to several barriers, such as the time consuming selection process and the quantitative nature of most agronomic traits. Molecular breeding tools are very useful for rice breeding, and have been

used to identify new germplasms and elite rice cultivars. (Wang *et al*. 2005). Some SSR marker associated with stem borer resistance trait were previously studied in some Egyptian rice genotypes (Eldenary *et al*. 2015). This work aimed to study the gene expression of two rice Si transporter genes, (i.e. *Lsi1* and *Lsi2*) in seven Egyptian cultivated rice genotypes using the semi-quantitative RT-PCR method and to distinguish the association of the genotypic differences in gene expression level and Si uptake with the Si concentration in the tested rice genotypes. Finally, to investigate the correlation between the high accumulated Si, stem diameter and stem bore resistant.

MATERIALS AND METHODS

Plant material:

Seven selected rice genotypes for this study were supplemented by Dr. Abo Youssef Mahmoud at Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh, Egypt. Rice genotypes, origin, resistance against rice stem borer are listed in Table (1).

Table 1. Rice genotypes, origin, and resistance against rice stem borer

No.	Genotypes	Origin	Resistance to stem borer
1	Sakha101	Egypt	Resistant
2	Sakha104	Egypt	Resistant
3	Giza177	Egypt	Moderate Resistance
4	Sakha102	Egypt	Moderate Resistance
5	Giza178	Egypt	Susceptible
6	Egyptian Yasmine	Egypt	Susceptible
7	08FAN6	China	Susceptible

Plant cultivation:

Rice seeds were soaked in tap water overnight at 28°C in the dark. Seeds were then transferred onto a net floated on nutrient solution supplemented with Si in a plastic container according to Fleck *et al.* (2011). Silicon was added to the nutrient solution as 0.5 mM K_2SiO_3. The nutrient solution was changed every seven days. Plant samples for total RNA extraction were taken after 9 days, while plants were harvested after 30 days for Si measurement. Plants were grown at 28°C and 14/10 h light/dark condition.

Measurement of silicon content:

Plant materials were dried at 60°C for three days and ground, then 100 mg samples were weighed from each genotype for digestion and silicon content measurement. The determination of silicon content was measured using the rapid method according to Dai Wei-min *et al*. (2005**)**.

Stem diameter (mm):

Stem diameter (SD) was measured using micrometer at 40 cm above the ground from five good tillers of five plants and average was taken according to Mallikarjuna *et al*., (2011). Stem diameter and white head

percentage measurements were carried out in the farm of the Rice Research and Training Center at Sakha, Kafr EL-Sheikh, season 2015.

RNA extraction:

Total RNA was extracted from the roots of rice plants using the Simply P total RNA extraction kit (BioFlux, China), according to the manufacturer's instructions. The total RNA was extracted from each rice genotype roots after nine days of germination. The quality and integrity of the extracted RNA were assessed (as a nondegraded band) using 1.5% agarose gel. RNA purity (A260/280 ratio was determined) and the concentration was measured by spectrophotometer. RNA concentration was adjusted to 100 ng/µl and stored at -80°C. Total extracted RNA of the tested rice genotypes is shown in Fig. 1.

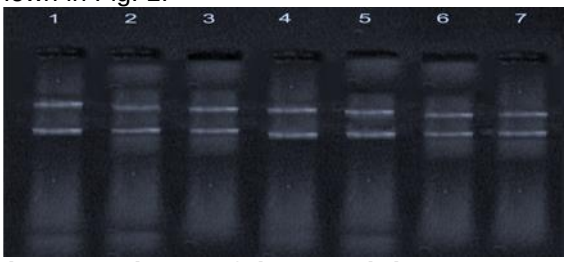

Fig. 1 The total extracted RNA of the tested rice genotype, nondegraded bands (large RNA molecules) and smeared bands (different small/large). Lanes 1-7 are for the rice genotypes Sakha101, Sakha104, Giza177, Sakha102, Giza178, Egyptian Yasmine and 08FAN6 respectively.

Semi-quantitative RT-PCR analysis:

Using the Semi-quantitative RT-PCR method, the expression level of both *Lsi1* and *Lsi2* genes was studied in the different rice genotypes in the presence of Si in the nutrition media. Actin gene expression was determined as an internal control.

Reverse transcriptase PCR was performed to study the expression level of *Lsi1*, *Lsi2* and actin genes. RT-PCR reaction was achieved in one step using AccessQuick™ RT-PCR System (one step kit), Promega, USA according to the manufacturer's instructions. The RT-PCR mixture contained: 5 µl of the extracted total RNA (about 500 ng), 25 µl 2X AccessQuick™ Master Mix, 1 µl (5 U) AMV reverse transcriptase, 1 µl (1µM) F-primer, 1 µl R (1µM)-primer and nuclease-free H_2O up to 50 µl were mixed in each sterile PCR tube. The AccessQuick™ Master Mix contains *Tfl* DNA polymerase, dNTPs, oligo dT, magnesium sulfate and reaction buffer. The reactions were initially incubated at 43°C for 40 min. for reverse transcription, then the PCR cycling was proceeded as fellow; initial denaturation 2 min at 95°C followed by 35 cycles of 1 min. at 95°C, 30 sec 54-60°C (5°C below the calculated melting temperature of the primers) and 30 sec. at 72°C and final extension of 5 min. at 72°C. Finally the reactions were hold at 4°C. The PCR plateau was adjusted (manually) at the cycle No 25. PCR products and 100 bp DNA ladder (Larova GmbH- Germany) were then separated on 1.5% agarose gel

supplemented with ethidium bromide for 90 min. at 70 volt, visualized via UV transilluminator and then photographed. Molecular size of the amplified fragments separated on gels was measured by gel images with Gel Analyzer software and band density was analyzed by ImageJ programe. The used specific primers are listed in Table 2.

Table 2. Primers, name, sequence and annealing temperature

Primer name	Primer sequence		Ann. Temp.
	F 5¯→ 3¯	**R** 5¯→ 3¯	
Actin	GACTCTGGTGATGGTGTCAGC	GGCTGGAAGAAGACCTCAGG	60 C°
Lsi1	CGGTGGATGTGATCGGAACCA	CGTCGAACTTGTTGCTCGCCA	60 C°
Lsi2	ATCTGGGACTTCATGGCCC	ACGTTTGATGCGGGTTGG	54 C°

These primers were chosen referring to Ma *et al*., 2006 and Ma *et al*., 2007.

RESULTS AND DISCUSSION

In this study, the relative gene expression of two Si transporter genes (Lsi1 and Lsi2), Si concentration in roots, stems and shoots and stem diameter in addition to white head percentage were studied to analyze the genotypic efficiency of Si uptake and stem borer resistance in seven rice genotypes.

Relative gene expression:

Expression of two Si transporter genes (Lsi1 and Lsi2) was examined in the roots of the seven chosen rice genotypes grown in solution culture (9 days old) using semi-quantitative RT-PCR technique. Only one concentration of Si (0.5 mM K_2SiO_3) was used and the genotype Sakha101 was used as a positive control (stem borer resistant) and 08FAN6 as a negative control (stem bore susceptible). Data shown in Fig's 2 and 3 demonstrated the stable level of actin gene expression as an internal control in the different tested rice accessions. At the level of gene expression; Sakha101 genotype (positive control) showed the highest level of both Lsi1 and Lsi2, while 08FAN6 genotype elucidated the lowest level. These data was clearly agreed with Si uptake and stem borer resistance.

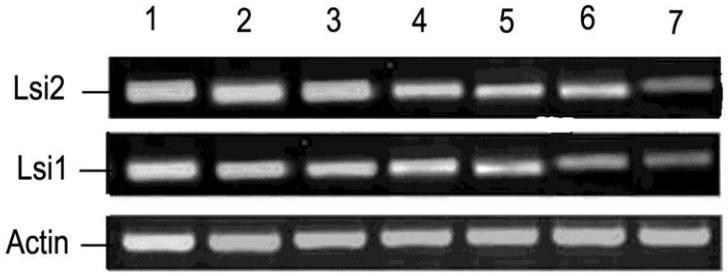

Fig. 2 Relative expression of Lsi1, Lsi2 and actin (as an internal control) genes amplified by semi-qRT-PCR. Lanes 1-7 are for the rice genotypes as mentioned in Fig.1.

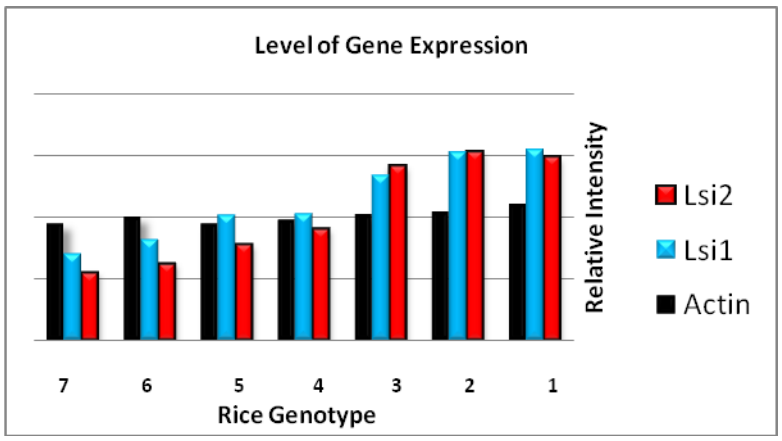

Fig. 3 Graphic shows gel analysis for band intensity indicating the level of gene expression for the tested genes; actin gene (as an internal control), Lsi1 and Lsi2 genes. Number 1-7 are the rice genotypes as mentioned in Fig. 2.

Genotypic difference in Si uptake:

Results in Table 3 show the differences in the uptaken silicon in the tested plants (30 days old) either in leavs, stems or roots. This variability referred to the efficiency of roots to transfer Si from soil/nutrient solution to plant according to the genetic backgrounds. Although the Si uptake occurred in roots, but it is accumulated with larger amounts in stems and leaves. Data shown in Table 3 indicate that Si content in the tested rice genotype at 30 days old agree with the level of gene expression and with the end Si concentration at the mature stage. These results insure that Si content was affected by the efficiency of the two studied transporter genes and differ according the genotype. These results agree with those of Mitani and Ma (2005), Ma *et al*., (2006) and Ma *et al*., (2007).

Table 3. Measured Si mg/g in leaf, stem and root of 30 days tested rice genotypes

Genotype	Mean Si (mg/g) 30 days		
	leaf	Stem	Root
Sakha101	62.15	48.08	19.01
Sakha104	51.24	42.12	16.34
Giza177	42.20	36.31	11.05
Sakha102	46.13	39.20	11.92
Giza178	29.10	18.11	6.89
Egyptian yasmine	29.04	20.23	7.12
08FAN6	25.20	19.00	6.10

The increase of stem borer resistance of the tested rice genotypes was closely associated with the increase of Si content. In all tested rice genotypes, Lsi1 and Lsi2 Si transporter genes were expressed, but with different levels. Naturally; there are other genes involved in Si transport and transform, but the tested two genes played direct important role in Si transport and in the end result of plants Si content. The susceptible genotypes 08FAN6 and Egyptian yasmine contained the lowest Si content (23.18 and 26.11 mg/g respectively), while the resistant two genotypes Sakha101and Sakha104 accumulated the largest Si amount (78.10 and 66.20 mg/g respectively). The moderate Genotypes Giza177 and Sakha102 showed intermediate amounts of Si under the same conditions (56.80 and 51.00 mg/g respectively) as shown in Table 4.

Table 4. Stem diameter, white head percentage and Si content mg/g of the tested rice genotypes at mature stage

Genotype	Stem diameter (mm)	White head %	Silica content (mg/g)
Sakha101	2.97	1.16	78.10
Sakha104	2.86	2.26	66.20
Giza177	3.27	4.94	56.80
Sakha102	3.03	5.23	51.00
Giza178	3.97	7.96	33.12
Egyptian yasmine	4.81	7.28	26.11
08FAN6	4.96	14.43	23.18

Si content, stem diameter and white head percentage:

Different levels of Si uptake according the genotypic differences brought clear differences in stem diameter (Table 4). The decrease of white head percentage indicated that infection with stem borer was decreased. These results agree with those of Khan and Saxena (1986) who reported that rice genotypes with lower plant height, higher tiller and thinner stem diameter were tolerant against stem borer. The results of this study are also agree with the results of Saeb and Oskou (2004) and Hosseini *et al*. (2011) who found that high .silica content effectively reduces the susceptibility of plants to pests and that the increase of silica in fertilizers can be used for making the plants partially resistant to the larvae of striped stem borer. The tested rice morphological traits (Si content and stem diameter) are associated with the level of gene expression of the studied two transporter genes and agree with those obtained by (Ma and Yamaji, 2006).

CONCLUSION

According to standard evaluation of the white head percentage (at the mature stage); rice genotypes were classified into susceptible, moderate resistant and resistant to stem borer. Using the molecular techniques, such as marker assisted selection and based on levels of expression of Si

transporter genes, in this study , and the morphological traits; Si concentration and stem diameter, rice accessions could be classify into susceptible and resistant, at earlier stage in the lab.

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التحليل نصف الكمى لإثنين من جينات نقل السيليكون باستخدام تقنية RT-PCR وذلك للتحصيل النبات للسيليكون ومقاومة ساقبة الساق فى بعض التراكيب الوراثية فى الأرز

مدحت عراقى الدينارى
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تم اختبارالتعبير الجينى النسبى لإثنين من جينات نقل السيليكون (Lsi1 و Lsi2) فى جذور عمرها تسعة أيام لسبع تراكيب وراثية من الأرز تم تنميتها على بيئة سائلة وذلك باتباع تقنية البلمرة متعددة السلاسل النصف كمية Semi-quantitative RT-PCR. تم تقدير تركيز السيليكون فى جذور وسوق والمجموع الخضرى (لعينات نباتية عمرها 30 يوم) لهذه الأصناف المنزرعة من الأرز.

بالاضافة الى ذلك تم دراسة نصف قطر الساق و النسبة المئوية للرؤس البيضاء و محتوى النبات من السليكون عند طور طرد السنابل وذلك للأصناف المنزرعة التى تم اختيارها للدراسة وزراعتها فى الحقل وذلك للتأكد من وجود صفة المقاومة لثاقبات السوق بالأصناف المنزرعة ذات المحتوى العالى من السيليكون والأقل قطرا لسيقانها. النتائج المتحصل عليها دلت وجود ارتباط مباشر بين مستوى التعبير الجينى لجينات نقل السيليكون ومحتوى النبات من السيليكون وصفة مقاومة ثاقبات السوق فى الأرز.