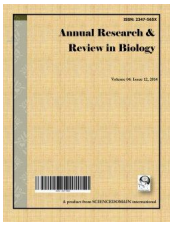

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Exploring Genetic Variability among Mutant Accessions of *Arabidopsis thaliana* L.

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Genetic diversity among 12 accessions of *Arabidopsis thaliana* possessing Pi transporter genes (*Pht*), compared to the related wild type, have been investigated using inter-simple sequence repeats (ISSR) and two biochemical marker systems; sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and isozymes. ISSR fingerprints clearly distinguished the 13 accessions and differentiate between the wild type and its mutant accessions. The six primers revealed a total of 79 fragments, ranging from 5 to 23 bands per primer (average 13.16 bands per primer). The polymorphism ranged between 44% for $(GA)_8C$ primer and 100% for the other primers. The cluster of ISSR patterns reflected high polymorphism (90.7%). AG repeat was the most common among the targeted units because it represented by the highest number of bands, whereas, AC and GA repeats gave the lowest number. No distinct polymorphism was observed in isozyme and SDS-PAGE patterns where ACP produced only one monomorphic band. Biochemical results could not characterize any variable behavior for mutant accessions compared to wild type even in the presence of three different members of Pht1 genes. In conclusion, ISSR markers are recommended for exploring genetic variability among different *Arabidopsis* mutants.

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1. INTRODUCTION

Arabidopsis thaliana is an annual and self-pollinating species that has a small nuclear genome. Its extensive distribution makes it resource model not only for molecular genetics but also for evolutionary and ecological studies [1,2].

Genetic diversity and phylogeny in *A. thaliana* was investigated using allozymes [3], RFLP [4] and AFLP markers [5]. Microsatellites (SSR) are the most considerable source of variability from repetitive sequences. ISSR are microsatellites which are plentiful, reproducible and variable in plant genomes [6,7]. Due to their simplicity of development and universality (no needs to sequence data), ISSR markers are useful for detecting species variants. Biochemical markers; SDS-PAGE and isozymes are also widely investigated to identify the genetic differences among plant taxa [8].

Pht1 family of Pi transporters genes were first identified in 1996. They play important roles in Pi uptake and translocation into the plant to maintain its growth and development [9]. Gene expression of *Pht* family changes in response to Pi conditions in soil [10]. It was, therefore, efficient to report the use of ISSR markers as an indicator of the number of SSR units among *A. thaliana* accessions and their benefit in assessing genetic variations among them compared to seed-protein and isozymes.

2. MATERIALS AND METHODS

A. thaliana, ecotype Columbia (Col-O) and T-DNA mutant seeds of the *Phtl* genes family including *Phtl:4*, *Phtl:2* and *Phtl:l* were supplied by Nottingham Arabidopsis Stock Centre (NASC, University of Nottingham, UK) (Table 1). Plants were grown at 22°C and the leaves were used for DNA and isozyme extractions.

Six primers for the PCR-based technique ISSR were used. Codes and sequences of these primers were listed in Table 2. DNA extraction was performed using protocols of Dellaporta et al. [11]. The conditions of the PCR amplification were: denaturation for 5 minutes at 94ºC followed by 35 cycles of 35 sec at 94ºC, 45 sec at 37ºC and 2 minutes at 72ºC, with a final extension step at 72ºC for 10 minutes. A volume of 15 µl of the ISSR products was electrophoresed in 1.2% agarose gel at 110 V for about 35 minutes.

Isozymes used wereacid phosphatase (ACP), alcohol dehydrogenase (ADH), α- and β-esterase (EST) and peroxidase (PRX). The separation of isozymes was performed in 10% Native-polyacrylamide gel electrophoresis [12]. The protocols of Wendel and Weeden [13] for ACP, Weeden and Wendel [14] for ADH, Scandalios [15] for α- and β- EST and Heldt [16] for PRX were used.

To extract seed proteins, 1 g of seeds of each accession was blended with 1 M Tris-HCl buffer, pH 8.8. Electrophoresis was carried out according to DISC SDS-PAGE method [17].

Bands were scored as (1) or (0) for presence and absence of ISSR markers using Gel-pro analyzer (ver. 3). Dice coefficient was used to calculate pairwise comparisons [18]. The phenogram was generated by values of similarity using the unweighted pair group method analysis (UPGMA). NTSYSpc (ver. 2.0) performed the previous analyses [19].

3. RESULTS AND DISCUSSION

Among the fifteen tested ISSR primers, six primers produced considerable polymorphic bands. Fig. 1 shows the the amplified bands of three ISSR primers that yielded highly informative patterns. The six primers generated a total of 79 fragments, ranging from 5 to 23 bands per primer (average 13.16 bands per primer) (Table 2). Seventy-four fragments were polymorphic with a mean polymorphism percentage 90.7%. The polymorphism ranged between 44% for $(GA)_8$ C primer and 100% for other primers. Sixteen markers obtained by primers ISSR-5, ISSR-11, ISSR-14 and RAMP-TAG were specific to the accessionsSALK_098784, SALK_098939 and SALK_136421 (Table 2). The cluster of ISSR patterns reflected the high polymorphism level (90.7%). The 13 accessions were divided into different groups (Fig. 1). As shown in Fig. 1, primer 5 was the most efficient in discriminating the 12 SALK lines from the wild parental one where no band was recorded for the wild line. Meanwhile, the 12 accessions were discriminated into two main clusters; cluster contained accessions 3, 7 and 12 and the second contained the other 9 accessions. Primer 6 discriminated accessions 3, 4 and 12 from the

other SALK and wild ones. Primer 11 indicated the highest similarity among all accessions without definite discrimination between the wld and SALK accessions. The results conflict those of Barth et al. [20] and Bornet et al. [21] that ISSR markers were inappropriate for the genetic diversity in *A. thaliana*. Whereas, they were in agreement with Gupta and Varshney [22] that the SSRs have high abundance in genomes of plant species. Also He et al. [1] detected high polymorphism level (95.93 %) in *A. thaliana*.

Three SSR units were selected as targets. AG repeat represented by the highest number of bands, whereas, AC and GA repeats gave the lowest number (Table 2). GA and AG repeats of ISSR markers were cost-effective and informative in studying the genetic relationships among plant accessions [23], breeding programs [24], the fingerprinting [25] and gene regulation [26].

Isozyme variability of 13 accessions of *A. thaliana* was studied using native PAGE. No distinct polymorphism was observed in isozyme systems where ACP produced only one monomorphic band (Fig. 2). In this concern, isozyme diversity had been investigated to study the genetic structure of *A. thaliana* populations and ACP and EST were the most valuable for differentiation and genetic diversity estimations [27,28]. Fig. 3 shows denatured polypeptides of *Arabidopsis* accessions separated by SDS-PAGE. A total number of 38 protein bands ranged from 245 to 10.6 kDa, were detected without any polymorphism percentage. Biochemical results could not help in characterizing any variable behavior for mutant accessions compared to wild type reflecting the symmetry of gene expression in *A. thaliana* in spite of the existence of three different members of *Pht1* genes.

Table 1. Accessions of *Arabidopsis thaliana* investigated in this study

No.	Name	Gene	Homozygosis
1	Wild Type	-	-
2	SALK_103881	*Pht I:4*	Homozygous
3	SALK_110194	*Pht I:2*	Homozygous
4	SALK_151938	*Pht I:1*	Homozygous
5	SALK_126481	*Pht I:1*	Homozygous
6	SALK_136420	*Pht I:1*	Homozygous
7	SALK_098684	*Pht I:1*	Unknown
8	SALK_098784	*Pht I:1*	Unknown
9	SALK_098939	*Pht I:1*	Unknown
10	SALK_136421	*Pht I:1*	Unknown
11	SALK_126488	*Pht I:1*	Unknown
12	SALK_126489	*Pht I:1*	Unknown
13	SALK_103880	*Pht I:4*	Unknown

Table 2. Number and types of the ISSR bands as well as the total polymorphism percentages generated in 13 accessions of *Arabidopsis thaliana*

Primer code	Sequence	Monomorphic band	Polymorphic band		Total band	Polymorphism (%)
			Unique	Shared		
HB-5	$(AG)_8T$	0	3	15	18	100
HB-6	$(GA)_8C$	5	0	4	9	44
HB-11	$(AG)_8GT$	0	6	17	23	100
HB-14	$(AC)_8GG$	0	4	8	12	100
RAMP-GAC	$G(AC)_9$	0	0	5	5	100
RAMP-TAG	$T(AG)_9$	0	3	9	12	100
Total		**5**	**16**	**58**	**79**	**(Average) 90.7**

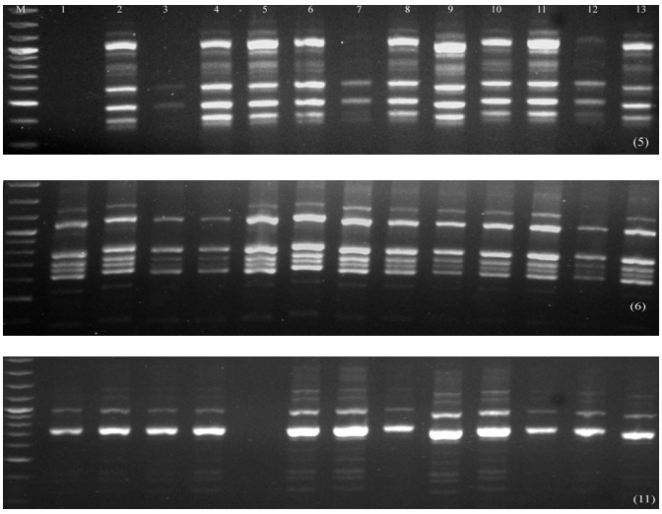

Fig. 1. ISSR profiles for 13 accessions of *Arabidopsis* using primers 5, 6 and 11. M, DNA marker

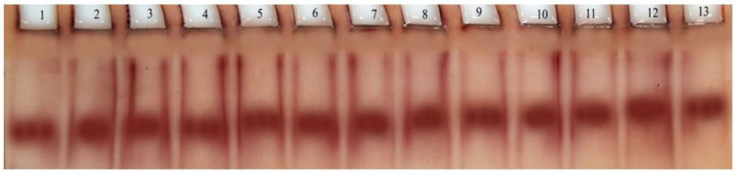

Fig. 2. Acid phosphatase profile for 13 accessions of *Arabidopsis*

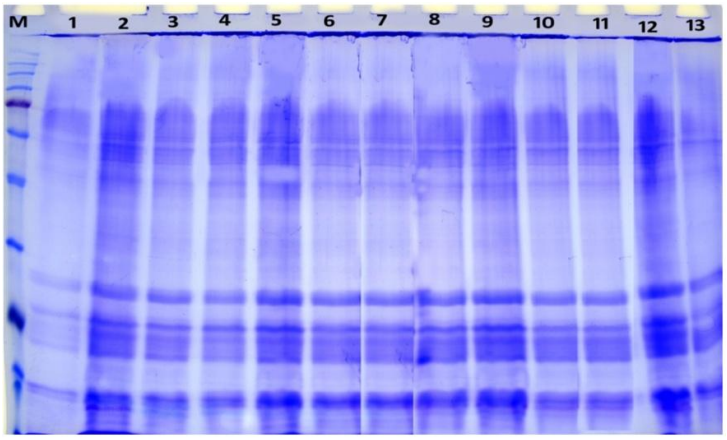

Fig. 3. SDS-PAGE profile for 13 accessions of *Arabidopsis*

4. CONCLUSION

In conclusion, compared to the biochemical markers, issr markers (due to its neutrality and evolution) give high levels of polymorphism and their employment for estimation of genetic diversity within genus *arabidopsis* could be recommended due to their highly irregular genomic distribution.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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