

Chapter 2

Preliminary Study on Salt Resistance Seedling Trait in Maize by SRAP Molecular Markers

Chunyang Xiang, Jin Du, Peipei Zhang, Gaoyi Cao and Dan Wang

Abstract In this study, different genotypes of maize salt tolerance inbred line and salt sensitive inbred line were used as the parent hybrid combinations to obtain F₂ populations. Two salt tolerance extreme types of DNA pools were established, where BSA method was used to select polymorphic SRAP markers. The result showed that 48 pair primers can be amplified and clear and stable bands can be obtained by parental, tolerant, and sensitive gene pools. Six pair primers of M2E1, M2E7, M6E15, M7E7, M11E4, and M14E6 showed polymorphism between two parents and between tolerant and sensitive bulks. The six SRAP molecular markers closely linked to salt tolerance were determined. The best maize SRAP-PCR reaction system was established. This research will accelerate maize marker-assisted selection breeding and lay the foundation for salt-tolerant gene cloning.

Keywords Maize · Salt tolerance · SRAP molecular marker

In China, salt-affected soils are found in large areas [1]. Saline soil is one type of middle and low yield soil. It not only affects the growth of plants, but also limits the yield and quality of crops [2]. Improving and reusing salinized soil have played an important part in increasing agricultural production. Maize is an important food and feed crop in China, however, the salt tolerance of maize is relatively poor. Some researchers have proposed extreme salinity at 0.017 mol/L NaCl [3]. Therefore, cultivation of resistant maize salt has been attracting considerable attention.

Sequence-related amplified polymorphism (SRAP) is a kind of newly developed molecular marker system with advantages of stabilization, simplicity, high co-dominance, moderate throughput ratio, and easily obtainable sequence of selected bands. Especially, it can be amplified by PCR without any sequence information [4]. SRAP markers were mainly studied in domestic maize that revealed the genetic diversity and heterotic grouping of maize germplasm using SRAP markers [5]. Although studies on SRAP markers of salt tolerance in maize have been few for a

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long time, some researchers have studied it in other plants. Seven SRAP molecular markers closely linked to salt tolerance were obtained in zoysiagrasses [6]. Its research can not only provide theoretical guidance for breeding of salt tolerant maize, but also benefit the selection and cultivation of new varieties of salt-tolerant maize. Salt resistance seedling traits of maize that were preliminarily studied by SRAP molecular markers in this research provide the scientific basis for gene mapping studies of salt tolerance in maize.

2.1 Materials and Methods

2.1.1 Experimental Materials

The F₂ segregated population originating from the selfing of F₁ hybrids, and F₁ from a cross between maize inbred line N1 (A060233, salt tolerant) and M2 (A06148, salt sensitive).

2.1.2 Experimental Methods

2.1.2.1 DNA Extraction and DNA Pools Construction

Total DNA in F₂ generations seedlings of maize was extracted with improved CTAB.

The two relative DNA pools (salt-tolerant DNA pool and susceptible DNA pool) which come from the F₂ populations were made according to the method of BSA (bulked segregant analysis).

2.1.2.2 SRAP Analysis

SRAP Primer

SRAP primer was designed with reference to the literature [4, 7, 8], and synthesized by Sangon biotech Shanghai Co. Ltd. as shown in Table 2.1.

PCR Amplification System

The total volume of the reaction system was 20 μ L, and concentration of the components was according to Table 2.2.

Table 2.1 Primers used in this study

Code	Forward primers	Code	Reverse primers
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTGTC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTGTA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGTAA	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGTCC	Em7	GACTGCGTACGAATTCAA
Me8	TGAGTCCAAACCGGTGC	Em8	GACTGCGTACGAATTCTG
Me9	TGAGTCCAAACCGGACG	Em9	GACTGCGTACGAATTCTGA
Me10	TGAGTCCAAACCGGACT	Em10	GACTGCGTACGAATTCAG
Me11	TGAGTCCAAACCGGAGG	Em11	GACTGCGTACGAATTTCCA
Me12	TTCAGGGTGGCCGGATG	Em12	GACACCGTACGAATTTGTC
Me13	TGGGGACAACCCGGCTT	Em13	GACACCGTACGAATTGAC
Me14	GTAGCACAAAGCCGAAG	Em14	GACACCGTACGAATTTGA
Me15	CGAATCTTAGCCGGATA	Em15	CGCACGTCCGTAATTAAC

Table 2.2 PCR reaction optimization

Reaction components	Adding quantity (μL)	Final concentration
ddH ₂ O	10.42	–
10 × Buffer	2	1.5 mM
dNTP	0.4	0.2 mM each
Taq E	0.18	0.04 U/μL
Forward and reverse primers (1 μM)	5	0.25 μM each
DNA(20 ng/μL)	2	3 ng/μL
Total	20	

2.1.2.3 Screening of SRAP Primers

Salt tolerance and salt sensitive DNA pool were used in this study, which was developed from a cross between N1 and M2. The material of this study included F2 generation of salt tolerance and salt sensitive, and their parental lines. A total of 225 pairs of SRAP primers were composed of 15 forward primers and 15 reverse primers, which were used to amplify the mapping population. The amplified products were checked by polyacrylamide gel electrophoresis (PAGE) electrophoresis.

2.1.2.4 SRAP Primers Authentication

SRAP primers were verified using 20 individuals in salt tolerance and sensitive DNA pools. Identification selection criteria of primers with salinity-tolerance is that

differential DNA bands in initial screening primers was of good consistency with 10 individuals of salt tolerance or salt sensitive.

2.2 Results and Analysis

2.2.1 Quality Analysis of Total DNA

The genomic DNA samples of maize were isolated by improved CTAB method, which based on optimizing extraction, deposition, and dissociation. The purity and quantity of DNA was evaluated by agarose gel electrophoresis and SRAP analysis. The results are as shown in Figs. 2.1 and 2.2. The concentration range of genomic DNA was among 20–23 ng/μL. Figure 2.1 shows that DNA bands identified by SRAP-PCR were all clear, and the brightness of bands was the same during parent N1, parent M2, salt tolerant DNA pool, and susceptible DNA pool. Figure 2.2 shows that brightness of DNA bands was the same in 20 individual plants that identify SRAP markers lined in the salt tolerance gene.

2.2.2 Preliminary Screening of SRAP Markers

225 SRAP primer combinations were screened by patent parent N1 and parent M2 from salt-tolerant gene pool and susceptible gene pool. 48 pair primers could be amplified and clear and stable bands were obtained. There were six pair primers with high polymorphism fragments (Fig. 2.3).

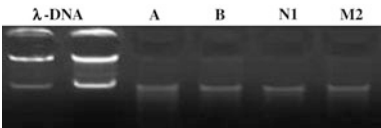


Fig. 2.1 Results of genomic DNA after diluted

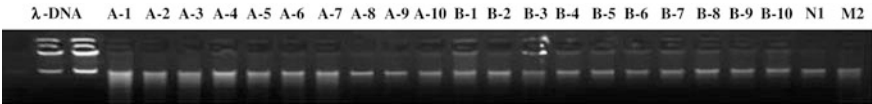


Fig. 2.2 Results of genomic DNA after diluted

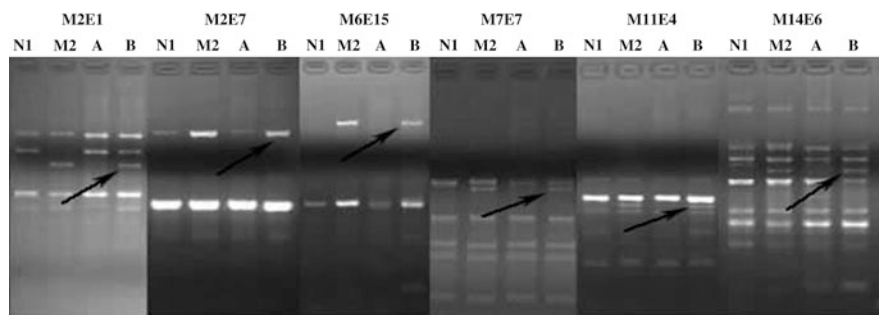


Fig. 2.3 Electrophoresis figure of SRAP primer screening in N1 × M2 populations

2.2.3 Verification of SRAP Markers

The present experiment screened six pair of SRAP primers, which could distinguish the two pools; then, these diversity pairs were used to amplify the individual of F2 plants that used built gene pools. M2E1 primer could amplify five bands clearly and stably. There is a specific band at 700 bp (Fig. 2.4). M2E7 primer could amplify two bands clearly and stably. There is a specific band at 1,500 bp (Fig. 2.5). M6E15 primer could amplify two bands clearly and stably. There is a specific band at

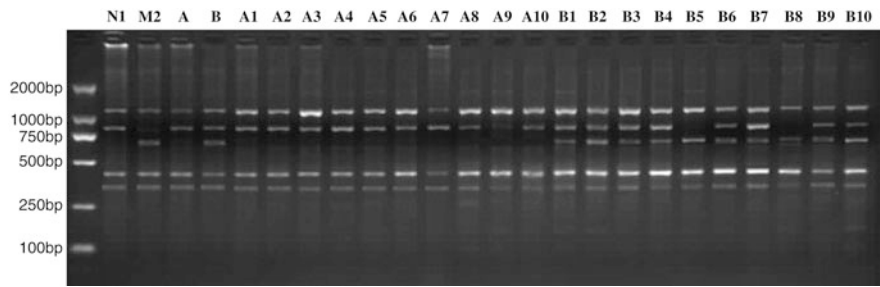


Fig. 2.4 The amplified result of M2E1primer among salt tolerance maize individuals

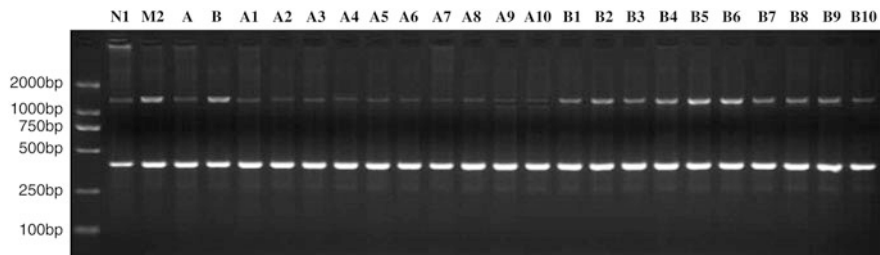


Fig. 2.5 The amplified result of M2E7 primer among salt tolerance maize individuals

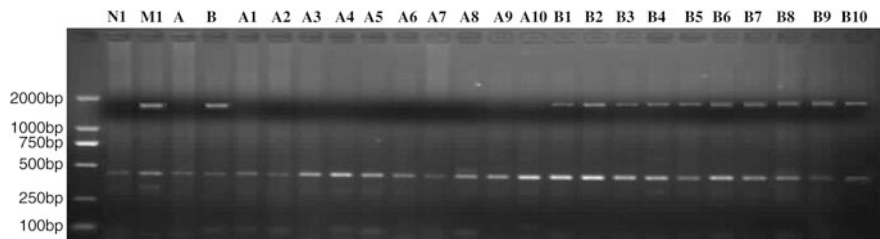


Fig. 2.6 The amplified result of M6E15 primer among salt tolerance maize individuals

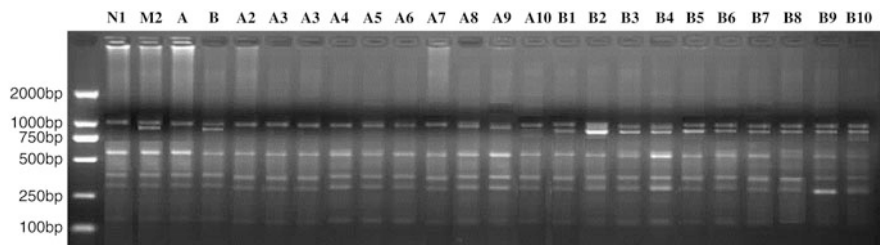


Fig. 2.7 The amplified result of M7E7 primer among salt tolerance maize individuals

1,800 bp (Fig. 2.6). M7E7 primer could amplify five bands clearly and stably. There is a specific band at 950 bp (Fig. 2.7). M11E4 primer could amplify seven bands clearly and stably. There is a specific band at 625 bp (Fig. 2.8). M14E6 primer could amplify eight bands clearly and stably. There is a specific band at 625 bp (Fig. 2.9).

Amplification bands of each pair primers are consistent among salt tolerance maize individuals in parent N1, parent M2, salt-tolerant DNA pool, and susceptible DNA pool. This indicated that six SRAP molecular markers were closely linked to salt tolerance.

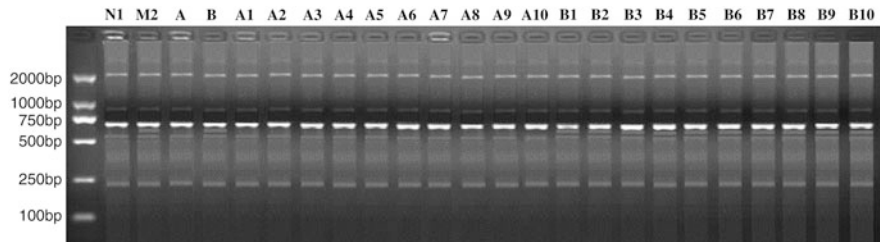


Fig. 2.8 The amplified result of M11E4 primer among salt tolerance maize individuals

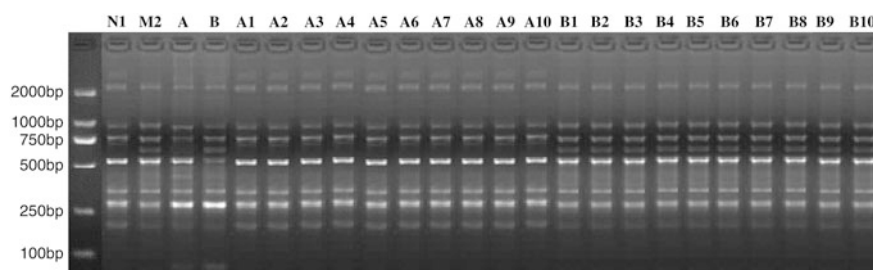


Fig. 2.9 The amplified result of M14E6 primer among salt tolerance maize individuals

2.3 Discussion

Salt-tolerant characteristics of plants that belong to complex quantitative traits are controlled by multiple genes, and are easily affected by the environment. Some researches have shown that salt tolerance can be controlled by the main gene. Exploring and screening SRAP markers associated with main salt tolerance gene have important practical significance for cultivation of salt-tolerant crops. The near isogenic lines of high and low salt tolerance maize were used as experiment materials. DNA was extracted from the seedlings to develop high and low salt tolerance DNA gene pools, respectively. SRAP and BSA were the most efficient strategies for looking for molecular markers related to salt-tolerant genes in maize.

Currently, there are four major DNA-based molecular mapping methods: restriction fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR). These technologies are being widely applied in the aspect of plants' salt tolerance [9–13], and these methods exist with their own advantages and defects. For example, AFLP marker has high polymorphism, but high false-positive and complex operation. RAPD marker has instability. The SSR marker is of high cost. SRAP, which possesses advantages over other molecular markers, possesses extraordinary characters such as simple design of primers, low cost, stable amplification, and high polymorphism. It is a simple, economic, effective, and reliable molecular marker. In order to screen molecular markers of salt-tolerant genes, 225 pair primers, which were designed, were mined using genomic DNA of maize. The 48 pairs of primers could amplify stable and clear bands. Six pairs of primers could amplify polymorphic PCR products among parent N1, parent M2, salt tolerant DNA pool, and susceptible DNA pool. And the six primers produced clear, stable, and reproducible polymorphic patterns. Therefore, SRAP could be used for screening marker which is associated with the salt-tolerant gene. However, the little tested population, incomplete genetic population, and less primer may have some influence on the results, and which need expanded population and increased primers for further exploration and study in future experiments.

In this paper, BSA was used to identify SRAP molecular markers linked to salt tolerance in maize using two salt-tolerant, extreme types of DNA pools for the first time. Six SRAP molecular markers closely linked to salt tolerance were obtained. This suggests that the salt tolerance of maize was determined by one incomplete dominant gene. The molecular markers obtained can be applied in salt-tolerant identification of the maize germplasm resources, and markers-assisted selection in salt-tolerant breeding of maize. This research will accelerate maize marker-assisted selection breeding and lay the foundation for salt-tolerant gene cloning.

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