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Mapping and validation of a dominant salt tolerance gene in the cultivated soybean (*Glycine max*) variety Tiefeng 8

*Rongxia Guan, Jiangang Chen, Jinghan Jiang, Guangyu Liu, Ying Liu, Lei Tian, Lili Yu, Ruzhen Chang, Li-juan Qiu**

The National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Key Laboratory of Germplasm & Biotechnology (MOA), Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

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ABSTRACT

Salt is an abiotic stress factor that strongly affects soybean growth and production. A single dominant gene has been shown to confer salt tolerance in the soybean cultivar Tiefeng 8. The objective of the present study was to genetically map the salt-tolerance gene in an $F_{2:3}$ population and a recombinant inbred line (RIL) population derived from a cross between two cultivated soybeans, Tiefeng 8 (tolerant) and 85-140 (sensitive). The $F_{2:3}$ families and RILs were treated with 200 mmol L^{-1} NaCl to evaluate salt tolerance. The $F_{2:3}$ population showed 1 (42 tolerant): 2 (132 segregating): 1 (65 sensitive) segregation, indicating a single dominant gene for salt tolerance in Tiefeng 8. A sequence-characterized amplified region (SCAR) marker from a previously identified random amplified polymorphic DNA (RAPD) marker and four insertion/deletion polymorphism (InDel) markers were developed within the mapping region. Using these markers along with SSR markers, the salt-tolerance gene was mapped within 209 kb flanked by SCAR marker QS08064 and SSR marker Barcsoyssr_3_1301 on chromosome 3. Three markers that cosegregated with the salt tolerance gene and SCAR marker QS08064 were used to genotype 35 tolerant and 23 sensitive soybean accessions. These markers showed selection efficiencies of 76.2% to 94.2%. The results indicate that these markers will be useful for marker-assisted breeding and facilitating map-based cloning of the salt tolerance gene in soybean.

1. Introduction

Soybean (*Glycine max* [L.] Merr.) is classified as a salt-sensitive glycophyte [1]. In soybean, salinity stress inhibits seed germination and seedling growth. Several salt-tolerant soybean accessions have been identified [2,3]. To characterize the inheritance of salt tolerance, Abel [4] performed crosses of parents differing in chloride accumulation tolerance. The F_2 population segregated in a 3:1 ratio of non-necrotic plants (very low in chloride) to necrotic plants (very high in chloride), and the segregation ratio from a test cross was 1:1. From these results, it was concluded that exclusion and inclusion of chloride in soybean leaves and stems were controlled by a single dominant gene *Ncl* and a recessive gene *ncl*,

* *Corresponding author*. Tel.: +86 10 82105843; fax: +86 10 82105840.
E-mail address: qiulijuan@caas.cn (L-J. Qiu).
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respectively. Lee et al. [5] evaluated the salt tolerance of a $F_{2:5}$ population derived from the cross of salt-tolerant parent S-100 with salt-sensitive parent Tokyo. Salt tolerance was scored from 0 (all plants dead) to 5 (plants with normal green leaves). As a result, a major quantitative trait locus (QTL) for salt tolerance was mapped near the simple sequence repeat (SSR) marker Sat_091 on soybean linkage group N (chromosome 3), accounting for 41%, 60%, and 79% variation of total genetic variation under three different conditions. The authors speculated that this QTL was the dominant gene *Ncl* reported by Abel [4]. The major QTL in this genomic region was confirmed in the cultivated soybeans FT-Abyra and Jindou No. 6 as well as a wild-type soybean, JWS156-1 [6,7]. Another salt tolerance-associated QTL was identified in a Chinese cultivar Nannong 1138-2 and mapped between SSR markers Sat_164 and Sat_358 on chromosome 18 [8]. Besides these QTLs, allelism testing showed that a wild soybean accession (*Glycine soja*) PI483463 carried a single dominant salt-tolerance gene, different from the one found in *G. max* S-100, but also mapping to soybean chromosome 3 [9,10]. Recently, an ion transporter gene *GmCHX1*, located on soybean chromosome 3, was cloned from wild soybean by a combination of genome sequencing and QTL mapping [11].

Thousands of Chinese soybean accessions have been screened for salt tolerance, and several Chinese cultivated soybeans cultivars have been identified as sources of salt tolerance at seedling and adult stages [3,12]. Genetic analysis using F_1 hybrids and F_2 and $F_{2:3}$ populations constructed from crosses between tolerant varieties Wenfeng 7, Teifeng 8, and Jindou 33 and sensitive cultivars Union, Hark, and Zaoshu 6 revealed that salt tolerance in soybean at the seedling stage was controlled by a single dominant gene [13]. Using the same crosses as those used by Shao and Chang [13], Guo et al. [14] found a codominant RAPD marker (a 600 bp product in the sensitive parent and a 700 bp product in the tolerant parent) closely linked to the salt-tolerance gene, but its location was not determined. In the present study, we mapped the dominant salt-tolerance gene on chromosome 3 and used salt tolerance-linked markers for determining the salt tolerance of soybean accessions.

2. Materials and methods

2.1. Development of mapping populations

Soybean variety Tiefeng 8 had been determined to be salt-tolerant, whereas 85-140 was relatively sensitive [12,13,15]. A segregating F_2 population (n = 392) was developed from the cross 85-140 × Tiefeng 8 in 2005. The F_1 plant was verified using SSR markers polymorphic between the parents [16], and a $F_{2:3}$ family (n = 239) with sufficient seed was used for mapping the salt tolerance gene. To confirm the gene detected in the $F_{2:3}$ population, an $F_{5:6}$ RIL mapping population (n = 230) developed by single-seed descent from the $F_{2:3}$ families was employed.

2.2. Evaluation of salt tolerance

Twelve seeds of each $F_{2:3}$ line were planted in a 100 × 80 × 80-mm pot filled with vermiculite and watered with tap water from the bottom every three days. The experiment was conducted under a rainout shelter under ambient weather conditions at the Chinese Academy of Agricultural Sciences in July 2009. The daily air temperature ranged from 33 (daily highest) to 21 °C (night lowest) with an average daily temperature of approximately 25 °C. Each of these experiments was conducted with two replications, and each pot had eight to ten plants. Twenty-two $F_{2:3}$ lines and the two parents were placed in 600 × 400 × 80-mm tanks. Ten days after planting, when the true leaves of plants were fully expanded, 2 L of 200 mmol L^{-1} NaCl was added twice within six days to each tank. Thereafter, 2 L of tap water was added to each tank every three days. When the salt-sensitive parent 85-140 appeared salt-toxicity symptoms, about 2 weeks after treatment, the genotype of each family was scored for salt tolerance. Lines with no obvious salt toxicity were considered tolerant, whereas lines with obvious leaf scorch were considered sensitive. Lines appearing both symptomatic and asymptomatic were considered heterzygous.

Salt-tolerance evaluation of the $F_{5:6}$ RIL population and 58 cultivated soybean accessions was performed in July of both 2011 and 2012, respectively, in two replications as described for $F_{2:3}$ families. After treatment for 2 weeks, the RILs (n = 8–10) were scored for salt tolerance. A salt-tolerance rating for each of the 58 soybean accessions was assigned according to the level of leaf chlorosis (Table S1). The salt-tolerance ratings ranged on a scale from 1 to 5, as follows: 1) plants with normal green leaves; 2) less than 25% leaf area showing chlorosis; 3) less than 50% leaf area showing chlorosis; 4) more than 50% leaf area showing chlorosis, but plants not completely dead; 5) all plants dead. The salt-tolerance evaluations of the RIL population and the 58 soybean accessions were both performed twice.

2.3. DNA isolation, RAPD analysis, and SCAR marker development

Leaf samples from all F_2 and F_5 individuals and the parents were collected and frozen in liquid nitrogen. For the germplasm used for marker-assisted selection and two parents, leaf samples were collected from ten plants of each accession. DNA was isolated with a Genomic DNA Purification kit (Fermentas, Burlington, Canada) and diluted to a final concentration of 20 ng μL^{-1}. PCR was conducted in a 20-μL reaction mixture containing 40 ng DNA and 1 U Ex *Taq* (TaKaRa, Japan) using RAPD primer 5′-GGCACGTAAG-3′ under the following conditions: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 37 °C for 30 s, and 72 °C for 1 min. The PCR products were separated on 2% agarose gel with 1× TAE buffer at 40 V for 3 h and stained with ethidium bromide.

The RAPD polymorphic fragments present in the salt-tolerant and -sensitive parents were excised and purified from the agarose gel and then cloned into the pEASY-T1 Cloning Vector (TransGen Biotech, Beijing, China). The positive (white) colonies were verified by PCR and the insert was sequenced on an ABI 3730XL Capillary DNA Sequencer using the fluorescent dye terminator method. A sequence-characterized amplified region (SCAR) marker was designed from the sequence information of the two fragments. The SCAR marker (QS08064), amplified by using forward 5′-ACGTAAGTGG