

Map-based cloning of a salt tolerance gene *Ncl* and its utilization for improvement of salt tolerance in soybean

Soybean [*Glycine max* (L.) Merr.] is the world's primary crop source for protein and oil. Its cultivation is conducted in a wide range of environments and is exposed to many biotic and abiotic stresses that influence the sustainability of soybean production. Salt stress inhibits soybean growth and reduces grain yield. Genetic improvement of salt tolerance is essential for sustainable soybean production in saline areas. In this study, we isolated a quantitative trait locus (QTL) for salt tolerance using the map-based cloning strategy from a Brazilian soybean cultivar FT-Abyara to facilitate its use in soybean breeding.

To identify the gene that conditioned the salt tolerance QTL, we conducted fine mapping using 5,828 plants derived from F₉ residual heterozygous plants, and delimited the QTL to a 16.6-kb interval between markers SSR25.8 and CAPS42.4. Since only one predicted gene, *Glyma03g32900*, existed within the 16.6-kb region (Fig. 1), *Glyma03g32900* was thus determined as the causal gene underlying the salt tolerance QTL (*Ncl* locus). The salt-tolerant soybean lines showed higher expression of the salt tolerance gene *Ncl* in the root and resulted in lower accumulations of Na⁺, K⁺, and Cl⁻ in the shoot under salt stress. We transformed *Ncl* full-length cDNA driven with 35S promoter (*35S:Ncl*) into a Japanese soybean cultivar Kariyutaka using the *Agrobacterium*-mediated transformation method, and significantly enhanced its salt tolerance (Fig. 2). To determine the usefulness of *Ncl* in conventional soybean breeding, we conducted introgression of *Ncl* into a salt-sensitive variety Jackson through continuous backcross, followed by DNA marker-assisted selection (MAS) using primers Satt339, SSR222042, and SSR112166 in each generation, and finally produced an improved salt tolerance line (Fig. 3). This result demonstrated that the DNA markers around *Ncl* could be used for introgression of *Ncl* into a salt-sensitive cultivar for developing a soybean variety with high salt tolerance. To determine the effect of *Ncl* in salt stress field conditions, we evaluated the *Ncl* near isogenic lines (NILs) in a salt stress field condition. The field experiments, which were conducted over three years, showed that *Ncl* could increase soybean grain yield by 3.6–5.5 times in saline field conditions (Fig. 4).

Our study suggests that using *Ncl* in soybean breeding through transgenic or MAS would contribute to sustainable soybean production in saline-prone areas.

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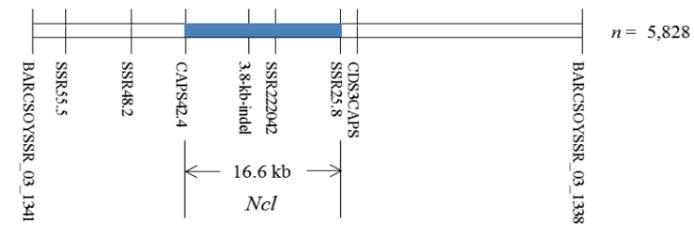


Fig. 1. Fine mapping ($n = 5,828$) delimits *Ncl* to a 16.6-kb region between markers SSR25.8 and CAPS42.4 on chromosome 3.

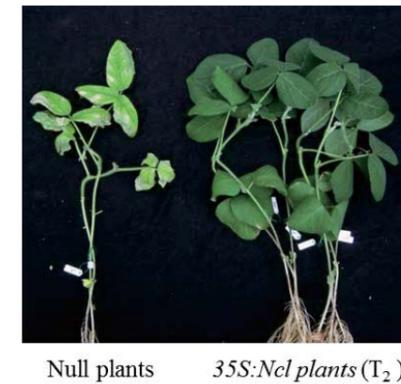


Fig. 2. Overexpression of *Ncl* in transgenic soybean lines enhanced its salt tolerance.



Fig. 3. Introgression of *Ncl* into a salt-sensitive variety "Jackson" by marker-assisted selection (MAS) produced an improved salt tolerance line.

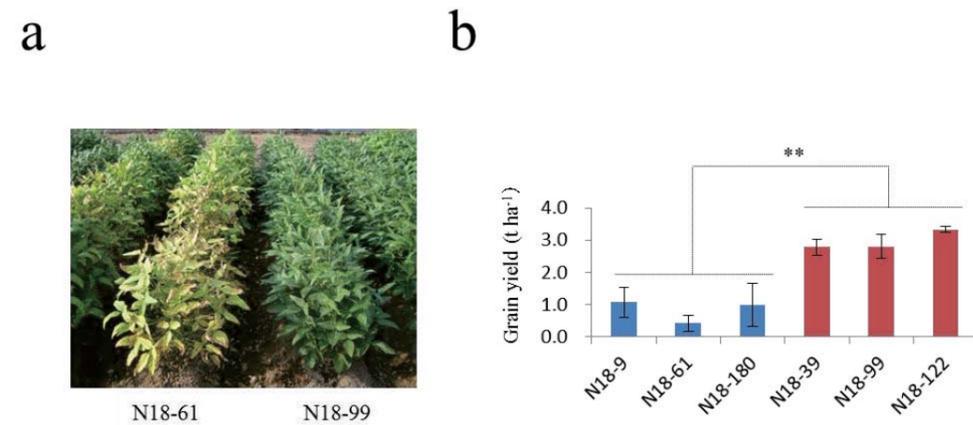


Fig. 4. Field performances of *Ncl* near isogenic lines (NILs) in a saline field condition in Miyagi Prefecture, Japan. (a) Top view of N18-61 and N18-99 grown in a salt stress field in 2012. (b) Grain yield results of the NILs in a salt stress field in 2012. N18-39, N18-99, and N18-122 are lines carrying the tolerant allele of *Ncl*, whereas N18-9, N18-61, N18-180 are lines that had the sensitive allele. Data are shown as mean \pm s.d. from three replicates. **: Significant difference ($P < 0.01$) based on ANOVA (Tukey's multiple comparison test).