Inheritance of Salt Tolerance in Wild Soybean (*Glycine soja* Sieb. and Zucc.) Accession PI483463

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Tolerant soybean (*Glycine max* [L.] Merr.) cultivars aid in reducing salt damage in problem fields. New genes are important to reduce losses from salt injury. Objectives of this study were to determine inheritance of salt tolerance in wild soybean (*Glycine soja* Sieb. and Zucc.) PI483463 and to test allelism of tolerance genes from genotypes PI483463 and S-100, a common ancestor of southern US cultivars. Tolerant (T) PI483463 was crossed to sensitive (S) cultivar Hutcheson to study inheritance. PI483463 (T) was crossed with S-100 (T) to test for allelism. Parents, F1 plants, F2 populations, and F2:3 lines were assayed in a 100 mM salt solution to determine tolerance. F2 from T × S cross segregated 3 (T):1 (S) and the F2:3 lines responded 1 (T):2 (segregating):1 (S) indicating different genes from the 2 sources. Results showed that *G. soja* line PI483463 had a single dominant gene for salt tolerance, which was different than the gene in *G. max* line S-100. The symbol, *Ncl2*, was designated for this new salt tolerance allele.

**Key words:** abiotic stress, salt tolerance, wild soybean

Salinity in soybean (*Glycine max* [L.] Merr.) is a problem in coastal areas where tides from hurricanes inundate farm land, in irrigated fields where irrigation water is high in salt content, and in overfertilized fields or fields naturally high in salt (Shannon and Carter 2003; Pathan et al. 2007). The total saline area including sodic areas is 15.8 for North America, and 129.0 million hectares for South America (Rengasamy 2006). More than 80% of world’s soybean are produced in North and South America (www.soystats.com). Thus, it is important to develop soybean cultivars with increased salt tolerance for stable production under saline conditions.

Soybean is considered a salt-sensitive species (Lauchli 1984). Soybean yield of sensitive cultivars is decreased dramatically under salt stress. Soybean yield was 80% at 4.0 dS m⁻¹ and 44% at 6.7 dS m⁻¹ versus 100% at 0.8 dS m⁻¹ (Katerji et al. 2003). Salt damage in soybean results from accumulation of chloride in stems and leaves of plants and is exhibited as leaf scorch (Able and MacKenzie 1964; Parker et al. 1983; Yang and Blanchar 1993; Panneerselvam et al. 1998; Wang and Shannon 1999; Essa 2002; An et al. 2002). However, genotypes differed in level of injury indicating genetic variability for salt tolerance.

Abel (1969) reported that a single dominant gene controlled salt tolerance in the soybean cultivar Lee (Johnson 1958) which is derived from S-100 × CNS with S-100 being the source of the tolerance gene. Lee et al. (2004) detected a major quantitative trait locus (QTL) for tolerance on linkage group (LG) N from S-100. Pedigree tracking using flanking simple sequence repeat markers for the salt tolerance QTL revealed that many cultivars with salt tolerance trace to S-100 and have the same gene (Lee et al. 2004).

Genetic diversity is important to make genetic progress in crop breeding. Wild soybean (*Glycine soja* Sieb. and Zucc.) is known as the ancestor of cultivated soybean and has been shown to have more genetic diversity than cultivated soybean (Hyten et al. 2006; Lee, Yu, et al. 2008). Luo et al. (2005) reported that the salt tolerance mechanism of wild soybean was different from cultivated soybean. Salt tolerance in *G. max* was mainly due to prevention of Cl⁻ ion transport from the roots to the upper portion of the plant preventing toxic accumulation in stems and leaves. In contrast, leaves of salt-tolerant *G. soja* strains or wild soybeans were not as susceptible as *G. max* to Cl⁻ toxicity but were more susceptible to Na⁺ accumulation. Salt tolerance in *G. soja* was primarily from exclusion of sodium ions preventing accumulation at toxic concentrations in stems and leaves. This indicates that interspecific crosses between *G. max* and *G. soja* offer the possibility of improving salt tolerance in soybean cultivars.

We determined that *G. soja* accession PI483463 was salt tolerant from earlier screening trials (Pathan et al. 2007). The objectives of this study were to determine the inheritance of salt tolerance in PI483463 and to determine the allelic relationship of the salt tolerance genes in wild genotype PI483463 and cultivated genotype S-100.
Materials and Methods

Tolerance of Parents and Population Development

PI483463, a wild soybean accession collected from Shanxi, China (GRIN, http://www.ars-grin.gov/npgs/searchgrin.html), was determined to have salt tolerance comparable to salt-tolerant G. max S-100 (Lee et al. 2004) from greenhouse screening trials during the winter of 2005–2006. After screening, 3 PI483463 plants in the V4 growth stage (Fehr et al. 1971) were rescued and transplanted into 15-l plastic pots filled with soil. At flowering, PI483463 was crossed with salt-sensitive soybean Hutcheson (Lee et al. 1983; Yang and Blanchar 1993; Pantalone et al. 1997; Lee, Smothers, et al. 1983; Yang and Blanchar 1993; Pantalone et al. 1997; Lee, Smothers, et al. 2008). F1 plants were planted at the University of Missouri–Delta Research Center Lee Farm during the summer of 2006.

Following harvest in November 2006, about 400 F2 seeds from the PI483463 × Hutcheson cross were planted in a greenhouse in 15-l plastic pots at 3 seeds per pot. F2 seedlings were individually harvested from 308 F2 plants in spring, 2007.

In summer 2006, PI483463 was also crossed with S-100. F1 seeds of the S-100 × PI483463 cross were planted in the off-season soybean nursery in Costa Rica and advanced to the F2 during the summer of 2006.

Determination of Salt Reaction

To determine the salt response for parents, F1, F2, and F2:3 generations, plants were exposed to NaCl (99.1% table salt; Morton International, Inc., Chicago, IL) in the greenhouse using the plastic cone-tainer method reported by Lee, Smothers, et al. (2008). In short, soybean genotypes were randomly planted in 21-cm tall Ray Leach cone-tainers (Stuewe & Sons, Inc., Corvallis, OR) filled with a sandy soil and placed in cone-tainer racks. At the V2–V3 growth stage (Fehr et al. 1971), racks of cone-tainers with seedlings of parents, F1, F2, and F2:3 generations were placed in 39-l racks of cone-tainers with seedlings of F2:3 families were assayed to confirm the segregation ratio observed in F2. Eleven to fifteen (average 14) seedlings of each F2:3 family, 10 seedlings of each parent, and S-100 (T) per replication were exposed to the salt solution in a randomized complete block design with 2 replications. Therefore, 1128 seedlings were tested for the 40 F2:3 families.

Genotypes with average leaf scorch scores of 1–2 were considered as tolerant (T), and genotypes with scores of 3–5 were rated as sensitive (S).

To determine the segregation ratios of F2 progeny from the PI483463 × Hutcheson cross, 10 seedlings of each parent, 8 F1 seedlings, and 116 F2 seedlings were assayed in a 100 mM NaCl solution described above. For confirmation of F2 segregation of the first test, the assay was repeated by screening 10 seedlings of each parent, 7 F1 seedlings, and 100 F2 seedlings for salt tolerance. Forty randomly selected F2:3 families were assayed to determine the goodness-of-fit of observed to expected genetic ratios from data combined from the 2 tests each in the F2 and F2:3 generations.

Results and Discussion

Plant reaction of parents to salt treatment showed that salt-sensitive cultivar Hutcheson had severe leaf scorch. However, leaves of PI483463 showed little leaf scorch or salt injury (data not shown). Previous studies reported that leaf scorch after salt treatment was significantly correlated with increased chloride content in soybean leaves (Parker et al. 1983; Yang and Blanchard 1993; Pantalone et al. 1997; Lee, Smothers, et al. 2008). Lee et al. (2004) used leaf scorch score as a parameter for determination of salt tolerance or salt sensitivity among soybean genotypes to map a salt tolerance QTL on LG N from cultivar S-100. Since then, leaf scorch score has been commonly utilized as a reliable variable to differentiate salt tolerance and sensitivity among parents or segregating populations derived from crosses of salt-tolerant and salt-sensitive soybean genotypes.

Roots of parents, F1, and 216 F2 progenies from the PI483483 × Hutcheson cross were tested in 100 mM salt solution to determine their response to salt stress. Leaves of F1 plants showed no injury or were similar in reaction to the tolerant parent, PI483436. Reaction of 216 F2 plants to salt stress showed segregation with a statistically significant fit to a ratio of 3 (Tolerant or T):1(Sensitive or S) Table 1. When F2:3 families were subsequently evaluated for reaction to salt solution, they segregated in a 1(all T):2 (3T:1S):1 (all S) ratio. Also, F3 plants showed an acceptable fit to a ratio of 3T:1S (Table 1).

These data support that G. soja accession PI483463 had a single dominant gene responsible for salt tolerance. Similarly, Able (1969) reported a single dominant gene that
governed salt tolerance in cultivar Lee, which was traced back to one of its original parents, S-100. A symbol Ncl was assigned for the dominant salt tolerance gene in S-100. The Ncl locus was mapped on LG N by Lee et al. (2004). The authors also suggested that this allele was found in several salt-tolerant soybean cultivars grown in the United States of America and that additional salt tolerance alleles may exist in other soybean germplasm.

The cross of S-100 and PI483463 was made to determine whether tolerant genes from these 2 sources were the same or different. F2 plants showed an acceptable fit to a ratio of 15T:1S (Table 2). The allelism analysis showed that wild soybean PI483463 had a single, nonallelic gene different from the salt tolerance gene in cultivated soybean, S-100. Mapping studies are necessary to determine the LG where the tolerance gene from PI483463 is located.

Different responses among Glycine species to salt stress have been studied. Generally, perennial Glycine accessions were more tolerant than G. soja and G. max (Pantalone et al. 1997; Kao et al. 2006). Luo et al. (2005) reported that the salt tolerance mechanism of G. max was different from G. soja. Leaves of salt-tolerant G. soja strains or wild soybeans were not as susceptible as G. max to Cl− toxicity but were more susceptible to Na+ accumulation. Salt tolerance in G. soja was primarily from exclusion of sodium ions from the roots preventing accumulation at toxic concentrations in stems and leaves. The descendants of a cross between tolerant G. soja and G. max were more tolerant to salt stress from Cl− salts than those from a cross between tolerant G. max cultivars (Luo et al. 2005). In this study, we did not analyze ions, Na+, Cl−, and SO4−, related to salt injury (Chinnusamy et al. 2005) to compare toxic ion or cation accumulation in parents or progeny from various crosses. Further studies are needed to determine the physiology of the salt tolerance mechanism in G. soja accession PI483463.

In summary, the data of the current study indicate that a single dominant gene, different from the gene in S-100 controls salt tolerance in wild soybean PI483463. The symbol, Ncl2, was approved by the Soybean Genetics Committee for this new salt tolerance allele. To our knowledge, this is the first report identifying a gene for salt tolerance from a G. soja accession from the USDA soybean germplasm collection.

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### Table 1. Segregation ratio of F1, F2, F2:3 families and F3 plants derived from cross of salt-tolerant wild (Glycine soja) soybean PI483463 × salt-sensitive Hutcheson cultivated (Glycine max) soybean to salt stress

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Observed T</th>
<th>Seg</th>
<th>S</th>
<th>Expected T</th>
<th>Seg</th>
<th>S</th>
<th>χ² (15T:1S)</th>
<th>P</th>
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<tbody>
<tr>
<td>PI483463 × Hutcheson</td>
<td>PI486463 (n = 20)</td>
<td>20</td>
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<td>Hutcheson (n = 20)</td>
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<td>F1 (n = 15)</td>
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<td>F2 (n = 216)</td>
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<td>PI486463 (n = 20)</td>
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<td>Hutcheson (n = 20)</td>
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<td>S-100 (n = 20)</td>
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<td></td>
<td>F2:3 families (n = 40)</td>
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<td></td>
<td>F3 plants (n = 1128)</td>
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</table>

T, Tolerant; Seg, Segregating; and S, Sensitive.

* Number in parenthesis indicates number of tested plants or families.

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### Table 2. Segregation ratio of F2 plants derived from a cross of salt-tolerant S-100 (Glycine max) soybean × salt-tolerant PI483463 (Glycine soja) soybean

<table>
<thead>
<tr>
<th>Cross</th>
<th>Progeny</th>
<th>Observed T</th>
<th>Sensitive (S)</th>
<th>Expected</th>
<th>χ² (15T:1S)</th>
<th>P</th>
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<tbody>
<tr>
<td>S-100 × PI483463</td>
<td>S-100 (n = 20)*</td>
<td>20</td>
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<td></td>
<td>PI483463 (n = 20)</td>
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<td>Hutcheson (n = 20)</td>
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<td></td>
<td>F2 (n = 214)</td>
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<td>200.6</td>
<td>13.3</td>
<td>3.59</td>
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</table>

T, Tolerant; S, Sensitive.

* Number in parenthesis indicates number of tested plants.
Acknowledgments

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References


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