GENETIC IMPROVEMENT OF SALT TOLERANCE IN SOYBEAN

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ABSTRACT

About 7% of the earth's land and 20% of the irrigated land is affected by salt stress. Salt-affected soils are generally classified into two main categories: saline and sodic (alkaline). Soybean (*Glycine max* (L.) Merr) is generally regarded as a salt sensitive crop compared with other crops such as wheat, rice, and cotton. Genetic improvement of salt tolerance is essential for maintaining sustainable production in areas where soybean growth is threatened by salt stresses. A series of studies consistently revealed a major quantitative trait locus (QTL) for saline tolerance located on chromosome 3 around the SSR markers Satt255 and Sat_091; other minor QTLs were also reported (1, 2, 3, 4). Map-based cloning and whole-genome-sequecing approches were employed for identification of the gene coffering saline tolerance at this locus. Field experiment results showed that the saline tolerance gene could greatly increasee soybean grain yield in a salinity field condition. Isolation and characterization of the saline tolerance gene might contribute to the sustainable of soybean production in saline area by introducing it into the local soybean varieties by either DNA marker-assisted slection or gene transformation methods. In the case of sodic tolerance, previous studies mainlu focused on iron deficiency caused by a high soil pH, and several QTLs associated with iron deficiency were identified (5, 6). A wild soybean (*Glycine soja* Sieb. & Zucc.) accession with high sodic tolerance was recently identified, and a significant QTL for sodic tolerance was detected on chromosome 17 (7, 8). These studies demonstrated that saline and sodic tolerances were controlled by different genes in soybean. DNA markers closely associated with these QTLs can be used for marker-assisted selection to pyramid tolerance genes in soybean for both saline and sodic stresses.

KEYWORDS

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Genetic improvement of salt tolerance in soybean

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About salt stress on soybean :

- 20% of the world's irrigated field is presently affected by salt stress.
- This figure is estimated to be 50% by 2050 due to drought and irrigation with low quality water.
- Salt stress is reported to inhibit soybean germination and plant growth (Abel and MacKenzie 1964, Wang and Shannon 1999), and nodule formation (Singleton and Bohlool 1984), resulting in decreased yield of soybean (Parker *et al.* 1983, Yang and Blanchar 1993).

Salt affected soybean field (2009, China)

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Contents

1. Screening of soybean germplasm with high salt tolerance using a salt-water flooding method

2. Map-based cloning of a salt tolerance gene in soybean

3. Genetic study on alkaline salt (sodic) tolerance in soybean

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1. Screening of soybean germplasm with high salt tolerance using a Salt-water flooding method

Evaluation of salt tolerance for more than 1,000 soybean accessions in greenhouse with a *Salt-water flooding method*
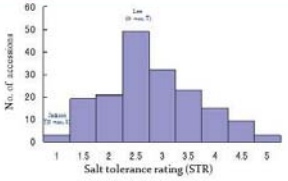
Before treatment

After treatment with 150 mM NaCl solution for three weeks

Salt-water flooding method (Tyuen et al (2010) Theor. Appl. Genet. 121: 229–236):
- Soybean were planted in a pot filled with upland field soil.
- After 7 days of emergence, the pots were transformed to a large plastic container containing 150 mM NaCl water solution for salt stress by introducing the salt solution into the pots through the small holes in the bottom of the pots.
- The salt solution was constantly circulated by an air bump to apply oxygen for soybean growing.

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Distribution frequency of salt tolerance rating (STR) for 174 wild soybean accessions from Japan. STR was scaled in 5 grades (1-5). Two cultivated soybean, Jackson and Lee were used as controls in this study.

Some wild accessions were identified to be high salt tolerance

Jackson   Lee   JWS573   JWS576   JWS582

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A wild soybean plant showed extreme high salt tolerance

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2. Map-based cloning of a salt tolerance gene in soybean

Plant materials for QTL analysis of salt tolerance

Four mapping populations

1. FT-Abyara (T, *G. max*) X C01 (S, *G. max*) F_7 RIL population (*n* = 99)

2. Jin dou No. 6 (T, *G. max*) X 0197 (S, *G. max*) F_6 RIL population (*n* = 81)
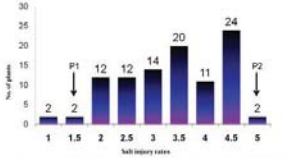
3. Jackson (S, *G. max*) X JWS061-1 (T, *G. soja*) F_6 RIL population (*n* = 132)

4. Jackson (S, *G. max*) X JWS156-1 (T, *G. soja*) F_2 population (*n* = 225)

QTL: Quantitative trait loci
RIL: Recombinant inbreed line
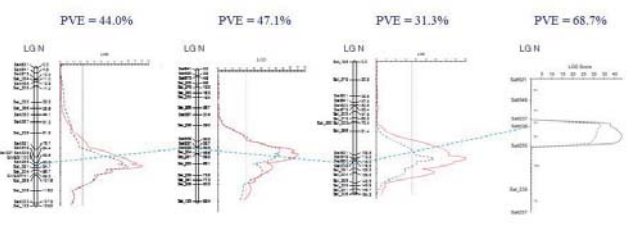
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The visual salt tolerance rating

Frequency distribution of salt injury rating values of the 99 RILs and their parents FT-Abyara (P1) and C01 (P2)

Linkage map of the F7 99 RILs derived from a cross between FT-Abyara and C01
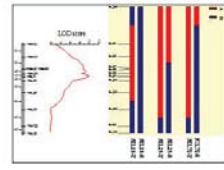
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PVE = 44.0%   PVE = 47.1%   PVE = 31.3%   PVE = 68.7%

FT-Abyara X C01
RIL population
(*G. max* X *G. max*)

Jin dou No. 6 X 0197
RIL population
(*G. max* X *G. max*)

Jackson X JWS061-1
RIL population
(*G. max* X *G. soja*)

Jackson X JWS156-1
F2 population
(*G. max* X *G. soja*)
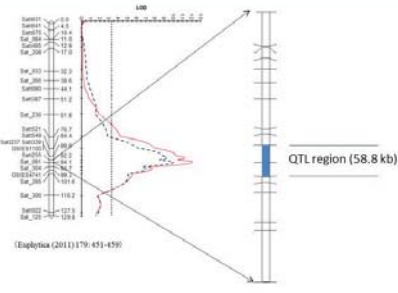
PVE: Percentage of variance explained by the QTL

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Development of near-isogenic lines (NILs) for salt tolerance by selfing of residual heterozygous lines (F_7) selected from the FT-Abyara X C01 RIL population

Table. Comparison of salt tolerance rating (STR) and leaf chlorophyll content (SPAD) between the salt-tolerant line (-T) and the sensitive line (-S) for each NILs set (NILs18, NILs25, and NILs72) as well as their original parental cultivar FT-Abyara and C01 after treatment with hydroponic culture solution containing 0, 100, and 120 mM NaCl

	Control (0 mM NaCl)		100 mM NaCl		120 mM NaCl	
	STR ± SD	SPAD ± SD	STR ± SD	SPAD ± SD	STR ± SD	SPAD ± SD
NILs18-T	5.0 ± 0.0	36.0 ± 3.2	5.0 ± 0.0**	38.6 ± 2.6**	4.3 ± 0.8**	33.7 ± 3.0
NILs18-S	5.0 ± 0.0	39.0 ± 2.3	1.3 ± 0.5	16.4 ± 6.3	1.0 ± 0.0	7.0 ± 7.5
NILs25-T	5.0 ± 0.0	38.7 ± 2.2	5.0 ± 0.0**	43.5 ± 2.4**	5.0 ± 0.0**	37.6 ± 9.7**
NILs25-S	5.0 ± 0.0	41.6 ± 3.3	3.9 ± 0.4	26.3 ± 2.3	1.8 ± 0.7	19.5 ± 4.8
NILs72-T	5.0 ± 0.0	38.0 ± 4.1	5.0 ± 0.0**	43.5 ± 2.7**	5.0 ± 0.0**	41.4 ± 3.2**
NILs72-S	5.0 ± 0.0	39.4 ± 3.1	1.6 ± 0.7	15.4 ± 5.7	1.0 ± 0.0	3.3 ± 2.9
FT-Abyara	5.0 ± 0.0	35.7 ± 2.3	5.0 ± 0.0**	42.7 ± 2.6**	4.7 ± 0.6**	43.4 ± 1.2**
C01	5.0 ± 0.0	39.8 ± 2.7	1.0 ± 0.0	6.5 ± 6.8	1.0 ± 0.0	2.9 ± 2.9

**: Significant difference at 0.01 probability level between the tolerant (-T) and sensitive (-S) lines for each NILs set, or between the soybean cultivars FT-Abyara and C01. SD: Standard deviation

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QTL region (58.8 kb)

(Euphytica (2011) 179: 451-459)
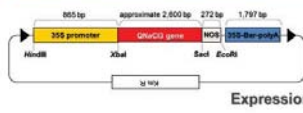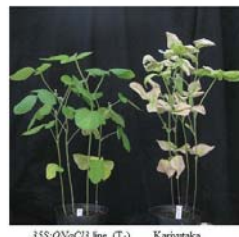
The QTL was narrowed down to a 58.8 kb genomic region based on fine mapping analysis (*n* = 1,053). In this region, a gene, *QNaCl3*, was determined as the causal gene underlying the salt tolerance QTL.

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Gene transformation for the salt tolerant gene *QNaCl3* with a *Agrobacterium*-mediated method

Expression vector

Overexpression of *QNaCl3* resulted in improved salt tolerance in transgenic soybean. Kariyutaka is the wild-type soybean cultivar. Soybean plants were treated with 120 mM NaCl.

35S:QNaCl3 line (T4)   Kariyutaka

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Introgression of *QNaCl3* into the salt-sensitive cultivar Jackson by DNA marker-assisted selection (MAS)

Jackson (Recipient) x JWS156-1 (Donor)
F₁
⊗
F₂ x Jackson
↓ MAS
BC₁F₁ x Jackson
↓ MAS
BC₂F₁ x Jackson
↓ MAS
BC₃F₁ x Jackson
↓ MAS
BC₄F₁
↓ ⊗ MAS
BC₄F₂
↓ ⊗ MAS
BC₄F₃ –T, BC₄F₃ –S

Scheme for development of *QNaCl3* Introgression lines

BC₄F₃ –S   BC₄F₃ –T

Performances of the BC₄F₃ lines developed by MAS for introducing the tolerance allele of *QNaCl3* from a wild soybean accession JWS156-1 into Jackson after treatment with 100 mM NaCl for about three weeks in hydroponic condition.

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Slide 14

Testing the effect of *QNaCl3* on soybean grain yield in saline field conditions

Evaluation of the salt tolerant NILs in a salt affected field in Miyagi prefecture, Japan

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Performances of lines carrying *QNaCl3* in a saline field condition in Miyagi Prefecture, Japan. *QNaCl3* could increase soybean grain yield by 3–5 times in saline field conditions.

(Grain weight (g/10 plant)) — Tachiyutaka, NIL18-S, NIL25-S, NIL72-S | Salt sensitive lines; NIL18-T, NIL25-T, NIL72-T | Salt tolerant lines

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➢ A gene *QNaCl3* conferring salt tolerance was isolated by map-based cloning strategy.

➢ The isolated gene has potential for improving soybean salt tolerance by marker assisted selection or gene transformation.

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3. Genetic analysis for alkaline salt (sodic) tolerance in soybean

Table: World regional distribution of salt-affected soils (million ha) (FAO, AGL, 2000)

Regions	Total area	Saline soils	percent	Sodic soils	percent
Africa	1899.1	38.7	2.0	33.5	1.8
Asia and the Pacific and Australia	3107.2	195.1	6.3	248.6	8.0
Europe	2010.8	6.7	0.3	72.7	3.6
Latin America	2038.6	60.5	3.0	50.9	2.5
Near East	1801.9	91.5	5.1	14.1	0.8
North America	1923.7	4.6	0.2	14.5	0.8
Total	12781.3	397.1	3.1%	434.3	3.4%

FT-Abyara   Jackson   JWS156-1
(*G. max*)   (*G. max*)   (*G. soja*)

A wild soybean (*Glycine soja*), JWS156-1, was identified to be tolerance to alkaline salt stress (NaHCO₃).

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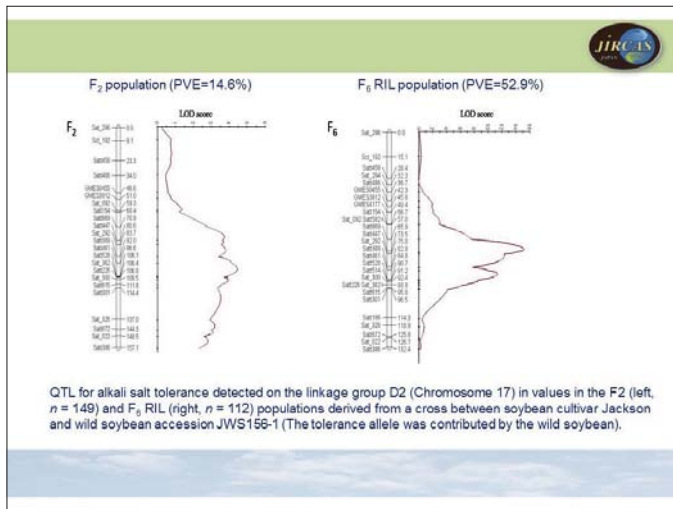QTL study on alkali (sodic) salt tolerance in soybean

Plant materials for QTL analysis

1. F₂ population (*n* = 149) Jackson (*G. max*) X JWS156-1 (*G. soja*)
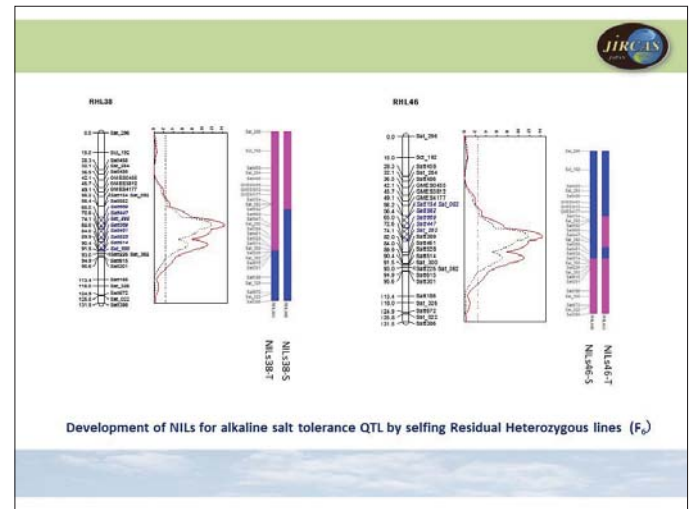2. F₇ RIL population (*n* = 112) Jackson (*G. max*) X JWS156-1 (*G. soja*)

Evaluation method: Salt-water flooding method

- 6 plants/pot (RIL)
- 2 replications (RIL)
- 180 mM NaHCO₃ (pH=9.0, EC=1.04 S/m)
- 3-4 weeks treatment

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QTL for alkali salt tolerance detected on the linkage group D2 (Chromosome 17) in values in the F2 (left, n = 149) and F6 RIL (right, n = 112) populations derived from a cross between soybean cultivar Jackson and wild soybean accession JWS156-1 (The tolerance allele was contributed by the wild soybean).

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Development of NILs for alkaline salt tolerance QTL by selfing Residual Heterozygous lines (F6)

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Fine mapping of a major QTL for alkaline salt tolerance

Genetic map and QTL LOD score plots for salt tolerance rating (STR) in a fine mapping population (n = 1,109)

Fine mapping analysis identify the alkaline salt tolerance QTL in a 3.3 cM region between markers GM703907-Satt447

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◆ A major QTL for alkali salt tolerance was identified on linkage group D2 (Chr. 17).

◆ The QTL for NaCl tolerance and the QTL for alkali salt tolerance located on different genomic regions.

◆ The alkaline salt tolerance QTL was in a 3.3 cM region between markers GM703907-Satt447 based on fine-mapping result analysis.

◆ The DNA markers closely associated with the QTLs might be used for marker assisted selection (MAS) to pyramid tolerant genes for both kinds of salt stresses.

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Thank you!

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Chair Shono: Let me introduce third speaker Dr. Xu.   He holds a doctorate degree in Agriculture from Nanjing Agricultural University in China and now he is a senior researcher of the Biological Resources and Post-harvest Division, JIRCAS in Japan.   His expertise is in the area of genetic studies of environmental stress tolerance in crops.   Today's his presentation title is "Genetic Improvement of Salt Tolerance in Soybean."

Dr. Donghe Xu: Thank you, Chairman, for your very kind introduction.   Good afternoon ladies and gentlemen. First of all, I'd like to express my sincere thanks to the organizer, the committee of this symposium for providing such a chance for introducing our work.

Today, what I'd like to talk about with you is "Genetic Improvement of Salt Tolerance in Soybean."

So why salt tolerance?   Currently, about 20% of the world's irrigated field is affected by salt stress.   This figure is estimated to be 50% by 2050 due to drought and irrigation with low quality water.   Why soybean? Because salt stress inhibits soybean germination and plant growth, and nodule formation; and finally, decreases yield of soybean.   This is a picture of salt-affected soybean field in China.   As you can see, soybean is inhibited by high salt concentration in the field.

There are three topics at this presentation.   First is about screening of soybean germplasm with high salt tolerance using a salt-water flooding method.   The second topic concerns map-based cloning of a salt tolerance gene in soybean.   The third one, I'll talk about the genetic study on alkaline salt or sodic salt tolerance in soybean.

The first topic: screening of soybean germplasm.   For studying the salt tolerance a reliable and simple evaluation method is very, very important.   Actually, this is true not only for salt tolerance but also for any trait.   Several years ago, we established a method.   This method can be used to evaluate a larger number of soybean germplasm.   So, we called salt-water flooding method.   Because of time limitation, I couldn't give you the details.   So if you're interested, you can access this paper.   This is before treatment.   So after the treatment with this method, as you can see, the soybean germplasm show different reaction to salt stresses.   By using this method, we have evaluated more than 1,000 soybean accessions from around the world and we do find some accessions with high tolerance to salt stress.

This slide just shows you as an example. This is the result of salt tolerance evaluation for 174 wild soybean accessions from Japan.   As you can see, there are variations for salt tolerance in this soybean collection, some are tolerant and some are sensitive and we do find some accessions with high salt tolerance.

We find one accession or one plant with very strong salt tolerance, as you can see from this picture.   And these soybean plants were treated by salt stress for more than 2 months.   As you can see, all other plants died only this one survived.   You can imagine how strong it is.

To obtain to get the germplasm is not our purpose.   Our purpose is how to use this germplasm to increase the soybean salt tolerance in the breeding practice.   For this purpose, we must know the gene which controls the salt tolerance, the number of the gene, the position of the gene and the effect of the genes, namely QTL analysis, quantitative trait loci analysis.   For the QTL analysis, we employed, we used four mapping populations.   The first one and the second one are derived from crosses between cultivated and cultivated soybeans; and the third one and the fourth one are derived from crosses between cultivated and wild soybeans.   And the first three populations are F6 or F7 generation populations and the last one is F2 population.

This slide shows QTL analysis for the mapping population of FT-Abyara X C01, and just show you as example. After the treatment or evaluation, the lines shows different performance to salt tolerance.   Most of the lines located between the two parents, showed continuous distribution.   As to the linkage map, we made the linkage map by using SSR markers because in soybean there are several thousand SSR markers are available.

In these populations, we detected one major QTL on linkage group N or chromosome 3.   The most interesting result is that the QTL was detected in all the four different populations and in the same position. You can see, it is in a same position.   This QTL is the major QTL and the PVE, the percentage of variance explained by the QTL, is very high.   It ranged from 31% to 68.7% is the major gene – major QTL.

Based on the QTL analysis results, we developed a near-isogenic line for the salt tolerance. Near-isogenic lines mean two lines have the similar genetic backgrounds, but they are different in the QTL regions. So using these lines, we can only focus on the target region and can rule out the influence caused by other factors. We developed three sets of near-isogenic lines. As you can see, the right plant has the gene and the left doesn't have the gene, and the same, has the gene and doesn't have the gene, has the gene and doesn't have the gene. And so they show very clear difference for salt tolerance.

Next we performed fine mapping analysis to narrow down the gene in small region. So, we used a large population. The population size is more than 1000, it is a large population. With this population, we narrowed down the gene, the QTL in a 58.8-kilobase region. So in this region, we identified a gene that controls salt tolerance. This means the gene behind the salt tolerance QTL was obtained, we got the gene.

In order to confirm the effect of the gene, we transformed this gene into a commercial soybean variety Kariyutaka. And this is the expression vector. As you can see, the transgenic lines show higher expression of this gene; and as expected, it showed very high salt tolerance. This gene enhanced the salt tolerance of the Kariyutaka soybean variety and the effect of the gene was confirmed by the gene transformation experiment.

We also introduced this gene from a salt tolerance wild soybean accession into a sensitive soybean variety Jackson. We made a cross between these two accessions and then followed continuous backcross. And in each generation, we performed the MAS, marker-assisted selection. In the BC4F3 generation, we found the lines with the gene showed significant higher salt tolerance compared with the line without the gene.

We also confirmed the effect of this gene on yield. We performed this experiment in a salt-affected field in Miyagi prefecture, Japan. The experiment was done in the paddy field and irrigated with diluted sea water.

So as you can see, similar to the experiment in the greenhouse, the line which has the gene shows significant higher salt tolerance. In terms of the yield, this gene can increase the soybean yield by three to five times in saline field conditions. The effect of this gene on yield was confirmed.

By using the map-based cloning strategy, we successfully identified and isolated a gene conferring on salt tolerance. And the isolated gene has potential for improving soybean salt tolerance by marker-assisted selection or gene transformation.

Last topic, very briefly. This table is used by my previous speaker, Dr. Sharma. The data are somewhat old, but I don't think there are big changees. If you look at the two figures – two numbers, you will realize that the sodic and the saline areas in the world are almost the same. So, this means that sodic is also a serious problem in the world.

Based on the evaluation, we found one wild soybean accession showed high tolerance to alkaline salt tolerance. Using the same strategy, the same method, as we did for identification of the gene for salt tolerance, we performed QTL analysis using two mapping populations.

We detected a QTL on linkage group D2 or Chromosome 17, a major QTL control, for alkaline salt tolerance. Using the same method, the same strategy, we developed near-isogenic lines. Also, we performed the fine mapping. By now, we have already narrowed down this QTL in the region of 3.3 centimorgan between these two markers. Of course, this work is still ongoing.

Based on the QTL analysis for alkaline salt tolerance, a major QTL for alkaline salt tolerance was identified on Chromosome 17. The QTL for NaCl tolerance and the QTL for alkaline salt tolerance located on different chromosome regions. This means that these two kinds of salt tolerances were controlled by different gene systems. The alkaline salt tolerance QTL was mapped in a 3.3 centimorgan regions between the two markers. The markers closely associated with these two kinds of QTLs might be used for combine these two kinds of genes to tolerant both kinds of salt stresses.
That's all. Thank you very much.

Chair Shono: Now we have the information from Dr. Xu how to use QTL analysis map-based cloning approach for identification of QNaCl3 gene which is related to salt tolerance of soybean. So is there any question and comments from the hall? Yes, please there.

Dr. Dinesh Sharma: Thank you for the presentation. I have two points to discuss. One is about the QNaCl3 gene. So what is the phenotypic character is salt tolerance score is the phenotypic character. What is the character name? I mean scoring is the character. You've used for find the QTL.

Dr. Donghe Xu: One is chlorophyll content, leaf chlorophyll content, and one is the salt tolerance score. We also measured the ion contents in leaves and stems. We used different characters to evaluate the salt tolerance.

Dr. Dinesh Sharma: Thank you.

Dr. Donghe Xu: Of course, also the biomass, the yield.

Dr. Dinesh Sharma: Yeah, thank you. So second one is about do you have the idea? I mean find the gene function, what kind of function this gene is really doing is a kind of sodium exclusion, because in wheat HKT gene all those things are already. I am just wondering. I mean, of course, maybe you well-established the marker-assisted selection and already well-performed in the field. Still I am just wondering in the molecular side. I mean already the function is known. Of course, soybean already genome sequence is there.

Dr. Donghe Xu: Yeah, we have data. Based on the homology blast search, this gene belongs to the antiporter family.

Dr. Dinesh Sharma: Thank you.

Chair Shono: Is there any question or comments? Yes, there please.

Male Questioner: Thank you very much. Any rhizospheric effect or any root systems effect? How to say. You found the gene, but its effect was rhizospheric effect? Yes, surface of the root.

Dr. Donghe Xu: You mean the…

Male Questioner: At root system.

Dr. Donghe Xu: At the root, does the gene have any effect on the root?

Male Questioner: Yes.

Dr. Donghe Xu: We found the gene had effect on the growth of the leaves and stems. This gene is specifically expressed in root system.

Male Questioner: Thank you very much.

Chair Shono: If there is no question, we'd like to close the second – Session 2. Thank you very much for impressive presentation from three speakers for the Session 2. Many technologies for crop production under the salt stress, both in sodic and salinic soils in India were introduced from Dr. Sharma, and salinity problem observed in check-dam farmland in China and mechanism of salinization was shown from Dr. Shimizu. In way of genetic improvement, salt tolerance, QTL analysis, map-based cloning and so on, these approaches were introduced from Dr. Xu. This include important topic for solve the salinity problem both point of view from soil and plants. So, I think it was very interesting topic from these three speakers.

Chair Suenaga: Okay. So, we close this session. Thank you very much.