RESEARCH ARTICLE

Salt tolerance in wild relatives of adzuki bean, *Vigna angularis* (Willd.) Ohwi et Ohashi

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Received: 11 December 2014 / Accepted: 26 May 2015 / Published online: 10 June 2015
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Abstract Salt stress is becoming a serious problem in food production field. To find sources of salt tolerance, we screened 74 accessions of adzuki bean (*Vigna angularis*) and 145 accessions of cross-compatible wild relatives (seven species). We performed the primary screening in soil culture and the secondary screening in hydroponic culture, and identified JP205833 of *V. riukiensis* (strain ‘Tojinbaka’) and JP107879 of *V. nakashimae* (strain ‘Ukushima’) as the valuable source of salt tolerance. We found these two strains had different salt tolerance mechanism, where

‘Ukushima’ prevented Na^+ accumulation in leaves by filtering Na^+ in roots and stems, while ‘Tojinbaka’ accumulated Na^+ throughout the whole plant body. We also found ‘Tojinbaka’ and ‘Ukushima’ could retain photosynthesis even under salt stress. In addition, ‘Ukushima’ and especially ‘Tojinbaka’ showed even better growth in a salt-damaged field in Fukushima, Japan where soybean cultivar ‘Tachinagaha’ could not survive. Since both salt tolerant strains are cross-compatible with adzuki bean, our results will facilitate developing salt tolerant cultivar by introducing two different mechanisms of salt tolerance.

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Electronic supplementary material The online version of this article (doi:[10.1007/s10722-015-0272-0](https://doi.org/10.1007/s10722-015-0272-0)) contains supplementary material, which is available to authorized users.

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Keywords Adzuki bean · Genetic resource · Genus *Vigna* · Legume · Salt tolerance · Wild crop relatives

Introduction

Soil salinity is a major constraint of food production because it limits crop yield and restricts uses of uncultivated land (Flowers and Yeo 1995). Currently, approximately 20 % of irrigated cropland in the world has already been salt-damaged, and another 30 % are considered salt-affected. In total, more than 800 Mha are salt-affected land in the world (FAO 2008). Given the rapidly growing world population, development of salt tolerant crop cultivars is inevitable to stabilize food production in cropland and to use currently unfavorable land.

The genus *Vigna* (Leguminosae) comprises six subgenera distributed in Africa, Asia, Australia and America (Verdcourt 1970; Maréchal et al. 1978). Most of the *Vigna* species in Asia belong to the subgenus *Ceratotropis*, known as the Asian *Vigna* (Tomooka et al. 2002). Of these, six species have been domesticated; adzuki bean [*V. angularis* var. *angularis* (Willd.) Ohwi and Ohashi], mung bean [*V. radiata* (L.) Wilczek], black gram [*V. mungo* (L.) Hepper], moth bean [*V. aconitifolia* (Jacq.) Maréchal], rice bean [*V. umbellata* (Thunb.) Ohwi et Ohashi] and creole bean [*V. reflexo-pilosa* Hayata var. *glabra* (Maréchal, Mascherpa et Stainier) N. Tomooka et Maxted]. Wild relatives possess greater genetic diversity than their related cultigens, and have been adapted to various environments including coastal and saline areas (Tomooka et al. 2009). Therefore, such genetic resources are expected to contain genetic factors for salt tolerance, which might be useful for cross breeding (Shannon 1997).

In Asian *Vigna* species, adzuki bean, mung bean and black gram are especially important as human food. As for mung bean and black gram, belonging to section *Ceratotropis*, many studies of salt tolerance have been reported and some tolerant wild and cultivated accessions have been identified (Mohamed and El Kramany 2005; Sehwat et al. 2013; Win et al. 2013). By contrast, for adzuki bean and other Asian wild species belonging to section *Angulares*, little has been studied (Yamauchi et al. 1989; Lee and Hong 2000). Adzuki bean is a traditional legume crop grown across East Asia and northern South Asia (Hanelt and IPK 2001; Zong et al. 2003). Since adzuki bean is cross-compatible with many wild species (*V. hirtella* Ridley, *V. minima* (Roxb.) Ohwi et Ohashi, *V. nakashimae* (Ohwi) Ohwi et Ohashi, *V. nepalensis* Tateishi et Maxted, *V. riukiuensis* (Ohwi) Ohwi et Ohashi, and *V. tenuicaulis* N. Tomooka et Maxted), (Doi et al. 2002) screening wild relatives for salt tolerance might be an efficient approach to develop salt tolerant cultivars via cross-breeding, and to isolate tolerance genes by linkage analysis.

Plant salt tolerance is considered to involve either avoidance (“excluder type”) or tolerance (“includer type”) mechanisms (Levitt 1980; Munns and Tester 2008). The “excluder type” plants are able to exclude toxic ions from internal plant tissue, while “includer type” plants take up Na^+ ions with a relatively lower toxicity (Johnson et al. 1991). In genus *Vigna*, only

“excluder type” has been reported so far, where salt tolerant accessions of mung bean and *V. unguiculata* (L.) Walp. (cowpea) can restrict Na^+ uptake from the root or prevent Na^+ migration from the root to the aerial part (Jacoby 1964; Lessani and Marschner 1978; Fernandes de Melo et al. 1994; West and Francois 1982; Bernardo et al. 2006). In adzuki bean, similar mechanisms were reported in response to salt stress while no salt tolerant cultivars were found (Yamauchi et al. 1989).

In this study, we screened adzuki bean and its wild relatives for salt tolerance. Some of the selected strains were further investigated for Na^+ accumulation and photosynthetic response, and tested for growth in a salt-damaged field.

Materials and methods

Plant materials

A total of 219 accessions consist of eight *Vigna* species (including 149 accessions of seven wild species) were provided from the genebank in NIAS (National Institute of Agrobiological Sciences) in Tsukuba, Japan. See details in Table 1 and Electronic Supplementary Materials (ESM) Table 1.

Salt tolerance screening in soil culture

Primary screening for salt tolerance was conducted at NIAS in Tsukuba, Japan (36°03'N, 140°10'E). The 219 accessions were grown in plastic pots (7 cm in diameter × 9 cm in height) under natural light condition in a greenhouse from June in 2009. The soil used in the pot was KUREHA-Engei-Baido (KUREHA Inc, Japan). Twenty-one days after sowing, an adequate amount of salt solutions was applied in the pool and then the pots were immersed for 2 weeks. NaCl concentration was set to 0, 50, 100, 150 or 200 mM. Two plants with two replications were tested in each treatment.

Tolerance was evaluated at 1 and 2 weeks after treatment started. Each plant was visually scored by percent of wilted leaves as follows; 1 = No leaves wilted, 2 = 1–25 % of leaves wilted, 3 = 26–50 % of leaves wilted, 4 = 51–75 % of leaves wilted, 5 = 76–99 % of leaves wilted and 6 = all leaves wilted (ESM Fig. 1).

Table 1 Plant materials used in this study

Species	No. of accessions	Origin (no. of accessions)
<i>V. angularis</i>		
var. <i>angularis</i>	69	Japan (49), Republic of Korea (18), Nepal (2)
var. <i>nipponensis</i>	5	Japan (3), Myanmar (2)
<i>V. hirtella</i>	31	Thailand (5), Myanmar (7), Laos (19)
<i>V. minima</i>	23	Thailand (11), Indonesia (1), Myanmar (3), Laos (8)
<i>V. nakashimae</i>	31	Republic of Korea (30), Japan (1)
<i>V. nepalensis</i>	1	Nepal (1)
<i>V. riukiensis</i>	28	Japan (27), Taiwan (1)
<i>V. tenuicaulis</i>	30	Thailand (4), Myanmar (24), Laos (2)
<i>V. umbellata</i>	1	Japan (1)
Total	219	

Salt tolerance screening in hydroponic culture

A total of 30 accessions from eight species (see Figs. 1, 2) was selected based on the result of primary screening (soil culture) and was sown in plastic pots with KUREHA-Engei-Baido supported and kept in green house for 21 days. Each accession was transplanted to a hydroponic culture in green house from August 2010. One culture pool (62.5 cm × 100 cm × 17 cm height) contained 64 plants (8 × 8 plants). The culture pool contained diluted nutrient solution of a 1:1 ratio of Otsuka house No. 1 (1.5 g/L): Otsuka house No. 2 (1 g/L) (Otsuka Chemical Co., Osaka, Japan: N, P, K, Ca and Mg = 18.6, 5.1, 8.6, 8.2 and 3.0 mEq/L, respectively). The nutrient stock solution was diluted to reach EC at 100 mS/m with water. Ten days after transplanting, salt stress was initiated. NaCl concentration in the hydroponic culture was 50 mM for the first week, 100 mM for the second week, and then 200 mM from the third week to the eighth week. Wilt scores were evaluated every week after the initiation of salt stress.

Measuring Na⁺ accumulation in plants

Four days after the NaCl concentration reached 200 mM, leaves, stems and roots were separately harvested from the three plants of *V. angularis* (JP233136, ‘Kyoto Dainagon’), *V. nakashimae* (JP107879, ‘Ukushima’) and *V. riukiensis* (JP235833, ‘Tojinbaka’). The harvested samples were dried at 62 °C for 24 h, ground into a powder and were reduced to ash for sodium extraction with 100 µL of 1 N HNO₃. The cation concentrations in the leaves, stems and roots were determined using an ion

chromatograph with a conductivity detector (Shimazu, Japan). Oxalic acid (3.3 mM) was used as the mobile phase. The mobile phase was degassed by degasser (DGU-12A) and pumped with liquid chromatograph pump (LC-9A) at speed 1 µL/min. This mobile phase was flown to the auto injector (SIL-6B) and mixed with 10 µL of ample solutions to be homogenized, which was controlled by the system controller (SCL-6B). The Na⁺ concentration was detected through the analytical column (IC-C3) in the column oven (CTO-10A) at 40 °C. The result was printed by a chromatopac (C-R 6A). The standard solution of the Na⁺ concentration (for 100 % was equated with 2 ppm) was measured for writing a standard calibration to calculate the correct ion concentrations of the sample solution.

Evaluation of photosynthetic activity and stomatal conductance

Seeds of *V. angularis* (JP233136, ‘Kyoto Dainagon’), *V. nakashimae* (JP107879, ‘Ukushima’) and *V. riukiensis* (JP235833, ‘Tojinbaka’) were germinated on Seramis (Effem GmbH, Verden, Germany) clay and hydroponically grown in a greenhouse for 4 weeks before measuring photosynthetic rate and stomatal conductance. To minimize the osmotic shock, 50 mM NaCl was added to the nutrient solution 3 days before the NaCl concentration was raised to 100 mM. The salt treated plants were grown for 7 days in 100 mM NaCl conditions. The control plants were grown in salt-free nutrient solution during the experiment. The photosynthetic rate and stomatal conductance of the topmost fully

Fig. 1 Heat maps of wilt scores in the primary screening using soil culture. The 219 accessions were cultivated in soil culture and exposed to 50, 100, 150, or 200 mM of NaCl for 1 week (left) and 2 weeks (right). Wilt score of 1 (no damage) is indicated as white whereas 6 (dead) is indicated as strong orange. Accessions selected for secondary screening are indicated by arrows. Strains ‘Ukushima’ (JP107879), ‘Tojinbaka’ (JP235833) and cultivar ‘Kyoto-Dainagon’ (JP233136) are also indicated by longer arrows. (Color figure online)

expanded leaves were measured using a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA). The measurements were obtained from 10:00 am–14:00 pm at 1 day before salt treatment, 3 days after 50 mM NaCl treatment, 3 and 7 days after 100 mM NaCl treatment. The measurement was done for 10 replications in each of salt treated and control plant under 1200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density, at 31.9 ± 3.2 °C leaf temperature, 38.8 ± 4.7 % relative humidity and 399.9 ± 0.2 ppm CO_2 concentration. The measurements were performed after approximately 3 min of light exposure when the CO_2 gas exchange rate reached a steady state.

Growth test in a salt-damaged field

We borrowed a salt-damaged field and a de-salted field from a farmer in Soma-city, Fukushima prefecture, Japan, where tsunami covered his field by the East Japan Earthquake on March 11, 2011. We sowed seeds of *V. nakashimae* (JP107879, ‘Ukushima’) and *V. riukiensis* (JP235833, ‘Tojinbaka’) and soybean (*Glycine max*) (JP67666, cv. ‘Tachinagaha’) on June 11th, 2013, and harvested the whole plant shoot above ground on October 31th, 2013. We cultivated 32 plants for each strain/cultivar in a salt damaged and a de-salted field, and measured dry weight of each plant to evaluate plant growth.

Fig. 2 Heat maps of wilt scores in the secondary screening using hydroponic culture. The 30 accessions selected from the primary screening were cultivated in a hydroponic culture with NaCl. Wilt score of 1 (no damage) is indicated as white whereas

6 (dead) is indicated as strong orange. Times of onset (50 mM) and increase of NaCl concentration (100 mM and 200 mM) are indicated at the top. (Color figure online)

Results

Evaluation of salt tolerance in soil culture

A total of 219 accessions of eight species (Table 1, ESM Table 1) were evaluated for salt tolerance in soil culture (ESM Fig. 1; ESM Table 2). Levels of damage under 50, 100, 150 or 200 mM of NaCl for 1 and 2 weeks were visualized as heat maps based on the wilt score (Fig. 1). The results showed most

accessions of *V. nakashimae* and *V. riukiensis* were highly tolerant and survived 2 weeks in 150 mM NaCl. In this screening *V. nakashimae* showed better performance, since in 200 mM NaCl condition, 19 of 31 accessions of *V. nakashimae* survived 2 weeks, while only 3 of 28 accessions of *V. riukiensis* could survive. Especially, JP212341, JP212342 and JP107879 (strain ‘Ukushima’) of *V. nakashimae* showed no visual damage symptoms in 150 mM NaCl for 2 weeks and in 200 mM for 1 week (ESM

Table 2). Large intraspecific variations were observed in *V. minima*, *V. tenuicaulis* and *V. angularis*, while no tolerant accessions were found in *V. hirtella* and *V. napelensis*. In cultivated accessions, one of the *V. angularis* accession (JP81144) was the most tolerant but leaf wilt was already observed at 2 weeks of 100 mM treatment (ESM Table 2).

Evaluation of salt tolerance in hydroponic culture

According to soil culture screening, we selected 20 tolerant and 10 susceptible accessions and re-evaluated in hydroponic culture (Figs. 1, 2; ESM Table 3). To attenuate osmotic shock, we initiated salt stress with 50 mM NaCl for 1 week, 100 mM for another 1 week, and finally set it to 200 mM. We evaluated wilt scores every week (ESM Fig. 1). The result was visualized as heat maps (Fig. 2). Almost all accessions were severely damaged at 1 week after NaCl concentration reached 200 mM (Fig. 2; ESM Table 3). However, some accessions of *V. nakashimae* and *V. riukiuensis* exhibited no or little damage at this time point and survived for 2 weeks or longer in 200 mM NaCl condition. In this experiment, JP235833, strain ‘Tojinbaka’ of *V. riukiuensis*, showed the highest tolerance and survived up to 6 weeks in 200 mM NaCl (week 8 in Fig. 2). Although *V. nakashimae* accessions scored the higher tolerance in soil culture screening, none of them could survive more than 3 weeks in 200 mM NaCl (week 6 in Fig. 2). Compared to soil culture, phenotypic variation in *V. angularis* and *V. hirtella* was smaller in hydroponic culture while that in *V. nakashimae* was greater.

Na⁺ accumulation in plant body

According to the primary screening in soil culture and the secondary screening in hydroponic culture, we selected JP107879 of *V. nakashimae* (strain ‘Ukushima’) and JP235833 of *V. riukiuensis* (strain ‘Tojinbaka’). To understand the mechanisms of salt tolerance in these strains, we measured the amount of Na⁺ absorbed into the plants under salt stress. For comparison, we also tested JP233136 of *V. angularis* (cv. ‘Kyoto-Dainagon’). During this experiment, we observed some chlorosis in the leaves of ‘Ukushima’, while most of the leaves of ‘Tojinbaka’ stayed green and maintained some growth (Fig. 3). In cv. ‘Kyoto-Dainagon’ and strain ‘Ukushima’, Na⁺ accumulation

differed by tissues, where Na⁺ amount was the least in the leaves and the highest in the stems (‘Kyoto-Dainagon’, *V. angularis*) or in the roots (‘Ukushima’, *V. nakashimae*) (Fig. 4). However, in ‘Tojinbaka’ of *V. riukiuensis*, Na⁺ accumulation was nearly the same between the tissues and thus the amount of Na⁺ accumulated in leaves was higher compared to cv. ‘Kyoto-Dainagon’ and strain ‘Ukushima’.

Photosynthetic rate and stomatal conductance under salt stress

Vigna angularis cv. ‘Kyoto-Dainagon’ and *V. nakashimae* strain ‘Ukushima’ decreased Photosynthetic rate (*Pn*) rapidly after salt treatment (Fig. 5a). In contrast, in *V. riukiuensis* strain ‘Tojinbaka’, *Pn* did not decline under the condition of 50 mM NaCl (Fig. 5a). In 100 mM NaCl, it started to decline but the extent of reduction was much smaller than that of cv. ‘Kyoto-Dainagon’ and strain ‘Ukushima’. *Pn* value of ‘Tojinbaka’ at 13 days after salt stress onset was more than five times and two times

Fig. 3 Photos of adzuki bean cultivar ‘Kyoto-Dainagon’ (JP233136), strain ‘Ukushima’ (JP107879) of *V. nakashimae* and strain ‘Tojinbaka’ (JP235833) of *V. riukiuensis* grown in hydroponic culture under control (left) and salt-stressed conditions (right) for 16 days (50 mM NaCl for a week, 100 mM for another week and then 200 mM)

Fig. 4 Na^+ concentrations in different plant tissues adzuki bean cultivar 'Kyoto-Dainagon' (JP233136), strain 'Ukushima' (JP107879) of *V. nakashimae* and strain 'Tojinbaka' (JP235833) of *V. riukiensis*. Samples were collected 4 days after 200 mM

NaCl treatment (16 days after salt treatment). Leaf samples were collected including fallen leaves. Error bars indicate standard deviations ($n = 3$)

higher than that of 'Kyoto-Dainagon' and strain 'Ukushima', respectively.

Stomatal conductance (gs) of 'Kyoto-Dainagon', 'Ukushima' and 'Tojinbaka' under salt stress for 13 days or control condition was compared (Fig. 5b). The result showed that gs fell down to almost zero in 'Kyoto-Dainagon' and 'Ukushima' by salt stress, whereas 'Tojinbaka' still maintained gs approximately 25 % of that in control condition.

Growth test in a salt-damaged field

Finally, we cultivated strain 'Ukushima' of *V. nakashimae* and strain 'Tojinbaka' of *V. riukiensis* in a salt-damaged and de-salted farmer's field in Fukushima prefecture to evaluate salt tolerance in a field condition (Fig. 6a). For comparison, we cultivated soybean cv. 'Tachinagaha', which is one of the most popular soybean cultivars in this region. As a result, almost all the soybean plants were dead in the salt-damaged field, whereas 'Ukushima' of *V. nakashimae* showed comparable growth to that in the de-salted field, and, surprisingly, 'Tojinbaka' of *V. riukiensis* showed even increased growth in the salt-damaged field (Fig. 6b).

Discussions

In this study, we have screened accessions of adzuki bean and the six related wild species for breeding salt

tolerant cultivars or isolating tolerance genes. Our results revealed salt tolerance in adzuki bean was limited, but was prominent in several accessions of the two wild species, *V. riukiensis* and *V. nakashimae* (Figs. 1, 2; ESM Tables 2, 3). Of these, we identified two strains, JP235833 of *V. riukiensis* ('Tojinbaka') and JP107879 of *V. nakashimae* ('Ukushima') as highly valuable genetic source of salt tolerance (Figs. 1, 2, 3, 4, 5, 6). The amount of accumulated Na^+ in plant body revealed that the strain 'Ukushima' filtered Na^+ by roots and stems to prevent Na^+ uptake into leaves while 'Tojinbaka' accumulated similar amount of Na^+ throughout the whole plant body (Fig. 4). In addition, both strains have capacity to retain photosynthetic activity and growth under salt stress compared with that of adzuki bean cv. 'Kyoto-Dainagon' or soybean cv. 'Tachinagaha', although this capacity is limited in 'Ukushima' (Figs. 5, 6).

Salt tolerance and other features in the selected strains

The different characteristics of Na^+ accumulation observed in strains 'Ukushima' and 'Tojinbaka' indicated different mechanisms of salt tolerance. Since in 'Ukushima' the amount of Na^+ uptake was highest in the roots and the lowest in the leaves, this strain is considered to have "excluder type" mechanism where Na^+ is excluded from internal tissue, or accumulated in apoplastic compartments (Levitt 1980, Fig. 4). In contrast, Na^+ was accumulated throughout

Fig. 5 **a** Photosynthetic rate (P_n) of the fully expanded leaves of control plants and salt stressed plants. P_n was measured 1 day before stress onset and 3, 6 and 13 days after NaCl treatment. The control plants were grown in salt-free nutrient solution during the experiment. NaCl treatment was started at 50 mM

NaCl and raised to 100 mM on the third day. Error bars indicate standard deviations ($n = 10$). **b** Stomatal conductance (g_s) of fully expanded leaves of control plants and salt stressed plants on 13th day after salt stress treatment. Error bars indicate standard deviations ($n = 10$)

the whole plant body in 'Tojinbaka', suggesting this strain has an "includer type" mechanism where Na^+ is partly detoxified or isolated into vacuoles (Levitt 1980). Perhaps the former mechanism was also present in adzuki bean cv. 'Kyoto-Dainagon' but was not strong enough to prevent Na^+ uptake into aerial parts, since Na^+ uptake into the leaves were still lower compared to that into the stems (Fig. 4).

Since both 'Ukushima' and 'Tojinbaka' are cross compatible with adzuki bean, they could be of great

value for breeding to improve salt tolerance of this domesticated species. In addition, it would be interesting to introduce both "excluder type" and "includer type" mechanisms into one adzuki bean cultivar. Further, crossing these two strains to adzuki bean would also facilitate linkage analysis to isolate genes of different salt tolerance mechanisms. Such genes, if identified, will be very useful to improve salt tolerance of crops through transgenic techniques, or genome editing. Indeed, we already developed F_2 plants

Fig. 6 Field test of soybean (cv. ‘Tachinagaha’), *V. nakashimae* (strain ‘Ukushima’) and *V. riukiensis* (strain ‘Tojinbaka’) in a salt-damaged field. **a** Location of salt-damaged field, Soma-city. **b** Dry weight of the plant shoots grown in a de-salted field and a salt-damaged field

derived from ‘Ukushima’ X ‘Tojinbaka’ and observed a transgressive segregation of salt tolerance (unpublished data). This fact suggested that salt tolerance in these two strains is genetically different and additive to each other.

Measuring photosynthetic response of ‘Ukushima’ and ‘Tojinbaka’ also revealed different features of these two strains. Even 50 mM of NaCl decreased photosynthetic rate in adzuki bean cv. ‘Kyoto-Dainagon’ (JP233136), but not at all in ‘Tojinbaka’ (Fig. 5a). In the 100 mM NaCl condition, cv. ‘Kyoto-Dainagon’ almost lost stomatal conductance and arrested photosynthesis while ‘Tojinbaka’ still retained 50–60 % photosynthetic rate with relatively lower stomatal conductance (Fig. 5a, b). In ‘Ukushima’, the photosynthetic response to salt stress was similar to cv. ‘Kyoto-Dainagon’ but less severe. Thus,

at least in hydroponic culture, the strain ‘Tojinbaka’ of *V. riukiensis* is the most tolerant to salt stress. Interestingly, without salt stress, the strain ‘Ukushima’ exhibited higher photosynthetic rate than cv. ‘Kyoto-Dainagon’, while ‘Tojinbaka’ achieved similar rate of photosynthesis with lower stomatal conductance (Fig. 5a, b). Thus, these two strains could also be useful in breeding for higher productivity (photosynthetic rate), drought tolerance or efficient water use (comparable photosynthetic rate under lower stomatal conductance).

In addition to the two strains described above, we also identified other tolerant accessions from *V. nakashimae* and *V. riukiensis*. The tolerant accessions of these species are often found in coastal areas and thus are considered to adapt to salt affected environment (Tomooka et al. 1992, 2002). Our results

also demonstrated the importance of detailed geographical information of genetic resources when screening for tolerance against environmental stresses.

Performance of selected strains under salt-damaged field

The tsunami that followed the East Japan Earthquake on March 11, 2011 left 24,000 ha of salt-damaged farmland (Endo and Kang 2015). In the salt-damaged field, it is still impossible to cultivate crops including soybean (Fig. 6). However, both of the selected strains of *V. nakashimae* and *V. riukiensis*, ‘Ukushima’ and ‘Tojinbaka’, respectively, showed similar or even better growth in the salt-damaged field. Therefore, these strains with different salt tolerance mechanism selected by soil pot and hydroponic culture experiments are considered to be practically useful genetic resources for breeding of salt tolerant adzuki bean cultivars.

Difference of salt tolerance in soil culture and hydroponic culture

Although several strains of *V. nakashimae* and *V. riukiensis* repeatedly exhibited salt tolerance, we found some differences between the results of soil culture and hydroponic culture (Figs. 1, 2, 6; ESM Tables 2, 3). In general, strains of *V. nakashimae* were revealed to be more salt tolerant than those of *V. riukiensis* in soil culture, whereas the tendency was opposite in hydroponic culture. Such differences might be because in soil culture the salt water in the pool gradually reached the root system of the plants, while in hydroponic culture plant roots were directly exposed to salt-containing solution. The effect of symbiotic arbuscular mycorrhizal fungi (Evelin et al. 2009) or other soil micro-organisms is also possible, which would make evaluation of salt tolerance more complicated. Thus, the screening in hydroponic culture might provide more direct evaluation of salt tolerance in genetic resources.

Conclusions

We would demonstrate that wild relatives of domesticated species are genetic resources of great value for tolerance against salt, and probably against other

environmental stresses. The wild species of genus *Vigna* could be a good example according to its huge diversity of environmental habitations. Although we screened only ~200 wild accessions belonging to section *Angulares*, which is one of the sections of subgenus *Ceratotropis* of genus *Vigna*, we successfully obtained highly valuable source of salt tolerance. Since more diversity is found in other subgenera including subgenus *Plectotropis* and subgenus *Vigna*, we expect we can find more valuable source of stress tolerance. Finding adaptive traits and genes from the wild species will facilitate development of stress tolerant crops and contribute the issue of global food security.

Acknowledgments We appreciate the National Institute of Agrobiological Sciences (NIAS) genebank project for financial support and also supplying the seeds of accessions.

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