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ORIGINAL PAPER

QTL mapping for salt tolerance and domestication-related traits in *Vigna marina* subsp. *oblonga*, a halophytic species

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Abstract

Key message **QTL mapping in F_2 population [*V. luteola* × *V. marina* subsp. *oblonga*] revealed that the salt tolerance in *V. marina* subsp. *oblonga* is controlled by a single major QTL.**

Abstract The habitats of beach cowpea (*Vigna marina*) are sandy beaches in tropical and subtropical regions. As a species that grows closest to the sea, it has potential to be a gene source for breeding salt-tolerant crops. We reported here for the first time, quantitative trait loci (QTLs) mapping for salt tolerance in *V. marina.* A genetic linkage map was constructed from an F_2 population of 120 plants derived from an interspecific cross between *V. luteola* and *V. marina* subsp. *oblonga*. The map comprised 150 SSR markers. The markers were clustered into 11 linkage groups spanning 777.6 cM in length with a mean distance between the adjacent markers of 5.59 cM. The $F_{2:3}$ population was evaluated for salt tolerance under hydroponic conditions at the seedling and developmental stages. Segregation analysis indicated that salt tolerance in *V. marina* is controlled by a few genes. Multiple interval mapping consistently identified one major QTL which can explain about 50 % of phenotypic variance. The flanking markers may facilitate transfer of the salt tolerance allele from *V. marina* subsp. *oblonga* into related *Vigna* crops. The QTL for domestication-related traits from *V. marina* are also discussed.

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Introduction

Soil salinity, one of the most destructive abiotic stresses, is a global problem in crop production. About 800 million ha of global land are salt affected (FAO 2005). Soil salinity greatly affects plant growth and reduces crop productivity, especially in legume crops which are among the most sensitive plants to salinity (Rogers et al. 2005). Salts in the soil water cause osmotic effect and ion-excess effect that can inhibit plant growth or even kill the plant (Greenway and Munns 1980). Osmotic or water-deficit effect of salinity is a result of the presence of salt in the soil solution which reduces the ability of the plant to take up water, leading to reduction in growth rate. Ion-excess or salt-specific effect of salinity occurs when an excessive amount of salt enters the plants transpiration stream injuring cells in transpiring leaves. *Vigna* is a pan-tropical genus comprising 104 legume species distributed widely in tropical and subtropical regions (Lewis et al. 2005). It

is an important leguminous taxon for human food and animal feed. Up to nine *Vigna* species are domesticated or cultivated as food, feed and ground cover (Tomooka et al. 2002). Cowpea [*Vigna unguiculata* (L.) Walps], Bambara groundnut [*Vigna subterranea* (L.) Verdc.], mungbean [*Vigna radiata* (L.) Wilczek] and blackgram [*Vigna mungo* (L.) Hepper] are the most important crops of the genus *Vigna* and grown as a main component in cropping systems in several regions of the world. Seeds of these crops are a major inexpensive source of dietary proteins for poor people and vegetarians in the tropics and subtropics. In some areas, several wild *Vigna* species are harvested for young pods or mature seeds as food, or grown as forages or ground cover. Cultivated *Vigna* species are generally grown by poor farmers in marginal lands, including salt-affected soil. In Myanmar, a major producer and exporter of mungbean and blackgram, some of the major growing areas for these two crops in Ayeyawaddy and Bago contain saline soil caused by intrusion of seawater (Win et al. 2011). In Brazil, cowpea is mainly grown in agricultural areas with high salinity (Lobato et al. 2009). In the USA, growing cowpea in desert areas is limited by soil salinity (Wilson et al. 2009). Therefore, breeding for salt tolerance is among the major goals for *Vigna* crops in several countries. Although there are not many reports on molecular genetics of salt tolerance in legumes, quantitative trait locus (QTL) mapping for this trait has been reported in both model and crop species. Salt tolerance is controlled by several minor QTLs in *Medicago truncatula* (Arraouadi et al. 2012), single major QTL in soybean (*Glycine max* (L.) Merr.) (Lee et al. 2004; Tuyen et al. 2010), two minor QTLs in field pea (*Pisum sativum* L.) (Leonforte et al. 2013) and a minor QTL in chickpea (*Cicer arietinum* L.) (Samineni 2010). However, these studies used different treatments to promote salinity and measured different traits to declare the tolerance.

Beach cowpea [*Vigna marina* (Burm.) Merrill] is a wild leguminous plant belonging to subgenus *Vigna* together with cowpea. It is found throughout the tropics and subtropics (Verdcourt 1970) and ranked among the most widely distributed flowering plants. *Vigna marina* is often confused with hairy pod cowpea [*Vigna luteola* (Jacq.) Benth.] because of their similar morphology and cross-fertility with each other (Palmer et al. 2002). However, their habitats are very different. *Vigna marina* grows along seashores, while *V. luteola* grows along riverbanks or lake shores. Padulosi and Ng (1993) recognized two subspecies of *V. marina*, viz. ssp. *marina* and ssp. *oblonga*. Since *V. marina* thrives along sandy beaches, it is a potential gene source for drought and saline soil tolerance. Seeds of *V. marina* remain viable even after being immersed in seawater for years (Lawn and Cottrell 1988), while young plants of *V. marina* ssp. *marina* can survive for at least 1 month submerged under a solution of 400 mM NaCl (Tomooka et al. 2011). Based on its habitats and salt tolerance ability, *V. marina* is considered a halophytic species. Although *V. marina* shows phenotypes of wild legume, seed size of accessions collected from the Indian Ocean coasts are the largest among wild *Vigna* species (Padulosi and Ng 1993). Non-shattering pods and large seed size of *V. marina* suggest that humans might have attempted to domesticate this legume (Smartt 1978). Mehra and Ibrahim (1989) reported that *V. marina* is cultivated as human food and cover crop in islands of the Maldives. In Australia, the Aborigines use *V. marina* root as food (Lawn and Cottrell 1988). It is also used to re-vegetate and stabilize coastal sand dunes in Australia.

In the genus *Vigna*, QTL mapping for domestication-related traits has been reported for azuki bean (*Vigna angularis* (willd.) Ohwi and Ohashi) (Isemura et al. 2007; Kaga et al. 2008), cowpea/yardlong bean (*Vigna unguiculata* (L.) Walp.) (Kongjaimun et al. 2012a, b), mungbean (*Vigna radiata* (L.) Wilczek) (Isemura et al. 2012) and rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi) (Isemura et al. 2010). These studies have revealed that several major QTLs for domestication-related traits in *Vigna* species are conserved.

In this study we developed a genetic linkage map for a population derived from an interspecific cross between *V. luteola* and *V. marina* ssp. *oblonga* using SSR and EST-SSR markers and reported a mapping of quantitative trait loci (QTLs) conditioning salt tolerance and domestication-related traits in *V. marina*.

Materials and methods

Mapping population and DNA extraction

An F_2 mapping population was developed from interspecific cross between *V. luteola* accession JP233389 and *V. marina* subsp. *oblonga* accession JP235855. JP233389 is salt susceptible and originated from Brazil, while JP235855 is salt tolerant and originated from Benin. *Vigna marina* was used as male parent and crossed onto *V. luteola* to obtain F_1 seeds. An F_1 plant was self-pollinated to develop an F_2 population comprising 120 individuals. The F_2 and the parental plants were grown in a field of Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand from December 2011 to March 2012. The F_2 population was self-pollinated to produce F_3 seeds.

Total genomic DNA of the parents and the F_2 population was extracted from fresh leaf tissue using the method described by Lodhi et al. (1994) with a slight modification. The DNA was quantified against a lambda DNA on 1.0 % agarose gel stained with ethidium bromide and diluted to 5 ng/μL for SSR marker analysis.

Measurement of domestication-related traits

Pod length and seed size were determined in the F_2 population grown in Thailand as described above. The length in centimeters of ten dry pods harvested from individual F_2 plants were measured. To determine seed size, 100 F_3 seeds from individual F_2 plants were weighed in grams.

Evaluation for salt tolerance

All experiments for evaluation of salt tolerance were conducted under greenhouse conditions at the National Institute of Agrobiological Sciences, Tsukuba, Japan from August to October 2012.

Seedling stage test

F_3 seeds produced from each F_2 plant were sown in trays with clay granule (SERAMIS®) and kept in a growth chamber at 25–27 °C for 10 days. For evaluation, 60 plants of *V. luteola*, 63 plants of *V. marina* and 8 plants of each $F_{2:3}$ line were tested. Seedlings at the first-leaf stage were initially irrigated with salt water having concentration of 50 mM NaCl. The concentration level was increased daily from 50, 100, 150, 200, 250 to 300 mM NaCl. Prior to changing NaCl concentrations, the clay granules were leached with 2 L distilled water. Two weeks after applying the highest concentration of 300 mM NaCl solution, the number of plants that survived in each $F_{2:3}$ line was recorded and the percentage calculated.

Hydroponic solution test

Evaluation for salt tolerance under hydroponic condition was carried out using salt water flooding method (Tuyen et al. 2010) with some modification. F_3 seeds from each F_2 plant were sown in trays with clay granules and kept in a growth chamber at 25–27 °C for 10 days. For each $F_{2:3}$ line, eight seedlings at the first-leaf stage were washed off the clay granules and transplanted to a hydroponic culture. The culture contained diluted nutrient solution of a 1:1 ratio of Otsuka house 1: Otsuka house 2 (Otsuka Chemical Co., Osaka, Japan). Otsuka house 1 contains 11 % total N, 8 % P_2O_5, 7 % K_2O, 0.1 % MgO, 0.1 % B_2O_3 and 0.2 % F; while Otsuka house 2 contains 11 % total N and 23 % CaO. The final solution was adjusted with water to an EC of 100 mS/m. One week after transplanting, NaCl was applied to the nutrient solution at 2 day intervals with 50 mM increasing concentration starting from 50 mM until a final concentration of 300 mM, after which the NaCl level was constantly maintained at this concentration.

Two traits related to salt tolerance were recorded in each plant, leaf wilt and plant recovery. Leaf wilt of each plant was visually scored after being maintained at 300 mM for 30 days using scales of 1–9; where 1 = normal healthy leaves, 3 = 1–25 % of leaves wilted, 5 = 26–50 % of leaves wilted, 7 = 51–75 % of leaves wilted, and 9 = 76–100 % of leaves wilted or plant completely dead (supplementary Fig. S1). One month after scoring the leaf wilt, plant recovery was scored as 1 or 3, where 1 = completely dead plant and 3 = leaf or bud re-growth after leaf defoliation. In each trait, the average score of each line was used for statistical and QTL analyses.

SSR marker analysis

One thousand three hundred and thirty-six SSR primer pairs from azuki bean (Wang et al. 2004; Chankaew et al. in preparation), cowpea (Li et al. 2001; Kongjaimun et al. 2012a) and common bean (*Phaseolus vulgaris*) (Yu et al. 2000; Gaitán-Solís et al. 2002; Blair et al. 2003) were used to screen for polymorphism between the parents. Each PCR was performed with 5 μL of PCR reaction mixture containing 5 ng of genomic DNA, 1× QIAGEN Multiplex PCR Master Mix (QIAGEN) and 5 pmol of the forward and reverse primers. The 5′-end of the reverse primer was fluorescent labeled with one of the four following fluorescent dyes: 6-FAM (blue), HEX (green), NED (yellow) and ROX (red) (Applied Biosystems). PCR amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems). The PCR thermal cycling was programmed as follows: 95 °C for 15 min followed by 40 cycles of 94 °C for 30 s, 55 °C for 90 s, 72 °C for 60 s, and a final cycle at 72 °C for 10 min. After amplification, 1 μL of ten times diluted PCR product was mixed with 8.5 μL of Hi-Di formamide and 0.125 μL of LIZ size standard (Applied Biosystems). The mixer was denatured at 95 °C for 5 min and run on an ABI Prism 3100 and 3130xl Genetic Analyzer (Applied Biosystems). Allele size for the highest stutter peak with the height ranging between 500 and 10,000 RFU was recorded and used to create bins for automatic assignment of genotypes. The genotyping was performed using the GeneMapper 3.0 software (Applied Biosystems) with default settings. After marker screening, six or seven primers with different labels and product sizes were put into a single PCR reaction mixture and amplified as a multiplex PCR using the same procedures described above.

Linkage and QTL analyses

A genetic linkage map of the F_2 population was constructed with JoinMap 4.0 (Van Ooijen 2006). The calculation was set with a minimum logarithm of the odds (LOD) of 3.0 and a maximum recombination frequency (r) of 0.25. Kosambi mapping function (Kosambi 1944) was used to calculate the distance between SSR loci. Linkage groups

were named following azuki bean linkage map (Han et al. 2005) to enable comparative linkage and QTL analyses.

SSR markers associated with all traits were determined by single regression analysis (Kearsey and Pooni 1996) using WinQTL Cartographer 2.5 (Wang et al. 2007). Only markers showing significance at $P < 0.01$ were considered.

QTL analysis was conducted using the software package MultiQTL ver. 2.6 according to the procedures described by Kaga et al. (2008). Initially, the entire genome was scanned for QTLs using general interval mapping with the following approach. First, a single QTL model was fitted for each trait–LG combination. LG-wise statistical significance thresholds ($P = 0.005$) for QTL were determined by 10,000 runs of a permutation test (Churchill and Doerge 1994), and parameters (position, additive effect and percentage of explained variance) for the significant QTL were obtained. Multiple interval mapping (MIM) (Kao et al. 1999) was then conducted to reduce the background variation by taking into account QTL effects from the other LG. On the basis of parameters defined for each putative QTL above, the LGs were included or removed iteratively into/from the MIM model at a more stringent level of significance ($P = 0.005$) than default. The stepwise selection of LGs based on significance using a permutation test was repeated until the process converged when no QTL on the remaining LGs was found. The QTL effects were re-evaluated by fitting all positive QTL in the order of their power and by a global permutation test (10,000 runs) to obtain more precise estimates of significance.

Results

Pod length and seed size in parents and F_2 population

The pod length and 100-seed weight of ten dry pods harvested from 120 individual F_2 plants were measured. The mean pod length of *V. luteola* and *V. marina* was 3.82 and 7.7 cm, respectively. Pod length among F_2 individuals ranged from 3.86 to 6.9 cm with a mean of 5.49 cm. One hundred-seed weight of *V. luteola* and *V. marina* was 1.99 and 3.87 g, respectively. In the F_2 population, 100-seed weight varied between 2.04 and 3.74 g with an average of 2.8 g. The frequency distribution of both traits showed continuous variation (Fig. 1) suggesting quantitative inheritance of these traits.

The F_2 plants had pods that were neither longer than *V. luteola* nor shorter than *V. marina* and 100-seed weight varied between the parents, showing no transgressive segregation for these traits.

Salt tolerance in parents and $F_{2:3}$ populations

One hundred and twenty $F_{2:3}$ lines (8 F_3 plants from each F_2 plant) were evaluated for salt tolerance under hydroponic condition using three traits at two growth stages, viz. plant survival at the seedling stage, and leaf wilt and plant recovery at the vegetative stage.

The survival rate of each $F_{2:3}$ lines was recorded 2 weeks after applying 300 mM NaCl. The survival rates of *V. luteola* and *V. marina* were 1.67 (1 out of 60) and 98.41 % (62 out of 63), respectively. Among the F_2 lines, the percentage of survival of $F_{2:3}$ plants varied from 0.00 to 100.00 % with a mean of 52.35 %. The F_2 population showed continuous distribution of percentage of surviving seedlings (Fig. 2a), suggesting a quantitative inheritance of this trait.

Leaf wilt at the vegetative stage was scored 30 days after applying 300 mM NaCl using 1 (tolerant) to 9 (susceptible) scales and the *V. luteola* was 7.13, while that of *V. marina* was 4.65. In the F_2 population, $F_{2:3}$ lines had leaf wilt scores between 3.75 and 8.50 with a mean of 5.96. The population showed continuous variation for this trait (Fig. 2b). This implies that leaf wilt score is a quantitative trait. Transgressive segregation was observed beyond both *V. luteola* and *V. marina*.

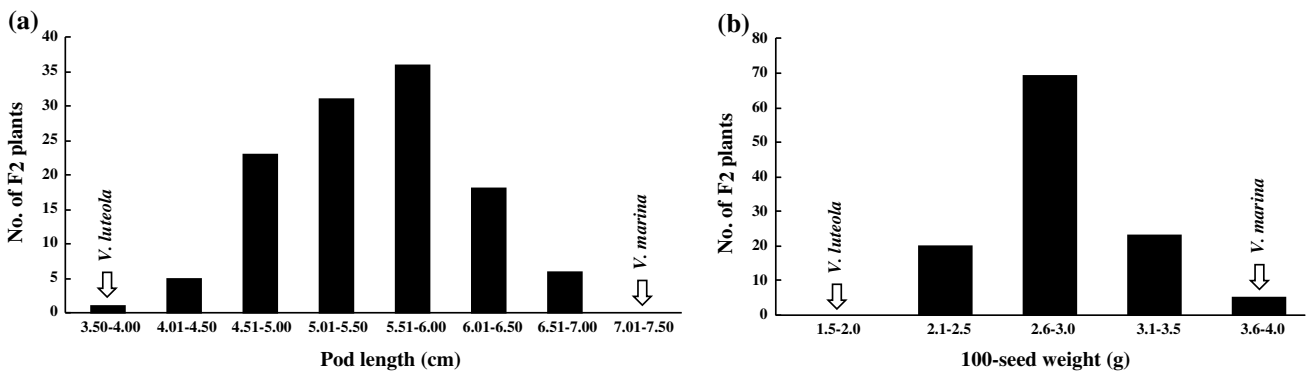

Fig. 1 Frequency distribution of pod length **a** and 100-seed weight **b** of 120 F_2 plants derived from the cross between *V. luteola* and *V. marina* subsp. *oblonga*

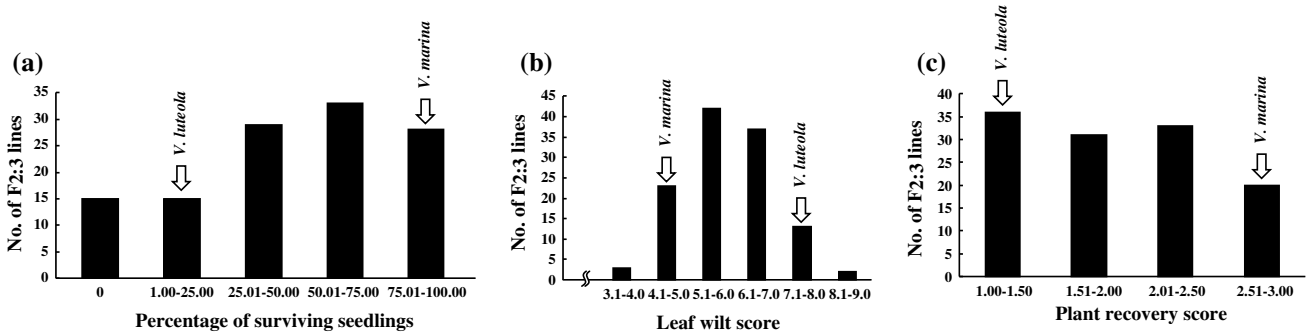

Fig. 2 Frequency distribution of traits for response to salt in the $F_{2:3}$ population (*V. luteola* × *V. marina* subsp. *oblonga*); percentage of surviving seedlings **a**, leaf wilt score at vegetative stage **b**, and plant recovery score **c**

Plant recovering ability was scored 2 months after applying 300 mM NaCl; all plants of *V. marina* recovered after leaf defoliation (score 3), while all plants of *V. luteola* completely died after leaf defoliation (score 1). In the $F_{2:3}$ population, the scores for plant recovery varied between 1.00 and 3.00 with an average of 2.10. Frequency distribution of plant recovering ability was also continuous (Fig. 2c).

Amplification and polymorphism of SSR markers

For genotyping the population, SSR primers developed from azuki bean, cowpea and common bean were used to screen for polymorphism between the parents. The genomes of *V. marina* and *V. luteola* were rather similar. Although 561 out of 1336 (42 %) SSR primers amplified single locus of the two genotypes (Table 1), only 150 (11.2 %) of the amplifiable markers showed polymorphism and were successfully used in LG construction. Of those SSRs, 7 were from common bean SSR, 64 from azuki bean SSR, 37 from azuki bean EST-SSR and 42 from cowpea SSR (Table 1). Fifteen primers amplified a fragment in only one of the parents; four (VES0061, VES0021, cp00451 and cp10667) amplified a fragment of *V. luteola*, while 11 (VES0793, cp00680, CEDG152, CEDG245, cp00347, cp10757, cp03602, cp04540, PV-ggc001, CEDG136 and VES0099) amplified a fragment of *V. marina*. These loci were also included in our linkage construction.

Table 1 Amplification and polymorphism of SSR and EST-SSR markers from azuki bean, cowpea and common bean in *V. luteola* and *V. marina* subsp. *oblonga*

Marker sources	No. screened	No. amplified (%)[a]	No. successfully used in LG construction (%)[b]
Cowpea SSRs	487	106 (21.8)	42 (8.6)
Azuki bean SSRs	329	214 (65.0)	64 (19.5)
Common bean SSRs	40	33 (82.5)	7 (17.5)
Azuki bean EST-SSRs	480	208 (43.3)	37 (7.7)
Total	1,336	561 (42.0)	150 (11.2)

[a] (No. of SSRs that amplified single locus in both parents/No. of total SSRs screened) × 100

[b] (No. of SSRs successfully used in multiplexed PCR/No. of total SSRs screened) × 100

χ^2 analysis for the goodness of fit of marker segregation revealed that 130 (86.67 %) markers segregated into the expected 1:2:1 ratio, while 20 (13.33 %) showed significant segregation distortion (SD) at $P \leq 0.05$.

Genetic linkage map

The genetic linkage map was constructed with JoinMap 4.0 program. All the 113 polymorphic SSR marker loci (42 cowpea, 64 azuki bean and 7 common bean) and 37 EST-SSR marker loci from azuki bean (Table 1) could be assigned to 11 LGs, which corresponds to the haploid chromosome number of *Vigna* species (Fig. 3). The LGs spanned 777.6 cM in total length, with a mean distance between the adjacent markers of 5.59 cM. The length of the LGs ranged from 40.4 cM (LG5) to 122.9 cM (LG01). The number of marker loci per LG varied from 10 (LG2 and LG9) to 20 (LG8). All the LGs, except LG5 and LG6, had a length of 50 cM or longer (Fig. 3). Most of the segregation-distorted markers were found on LG1 and LG4, especially on LG4 that the markers showed high level of distortion ($P \leq 0.001$). Six linkage groups including LGs 3, 5, 6, 9, 10 and 11 did not contain distorted markers. There were three gaps where the distance between two adjacent markers was greater than 20 cM: one each on LG4 (cp10271-AY1), LG5 (cp08729-CEDG145) and LG11 (VES0196-CEDG042) (Fig. 3). The largest gap was 27.4 cM on the LG4.

Genetic linkage maps of mungbean (*V. radiata*) and yardlong bean (*V. unguiculata*) were previously constructed (Isemura et al. 2012; Kongjaimun et al. 2012a). We used these maps as representatives of Asian and African *Vigna*,

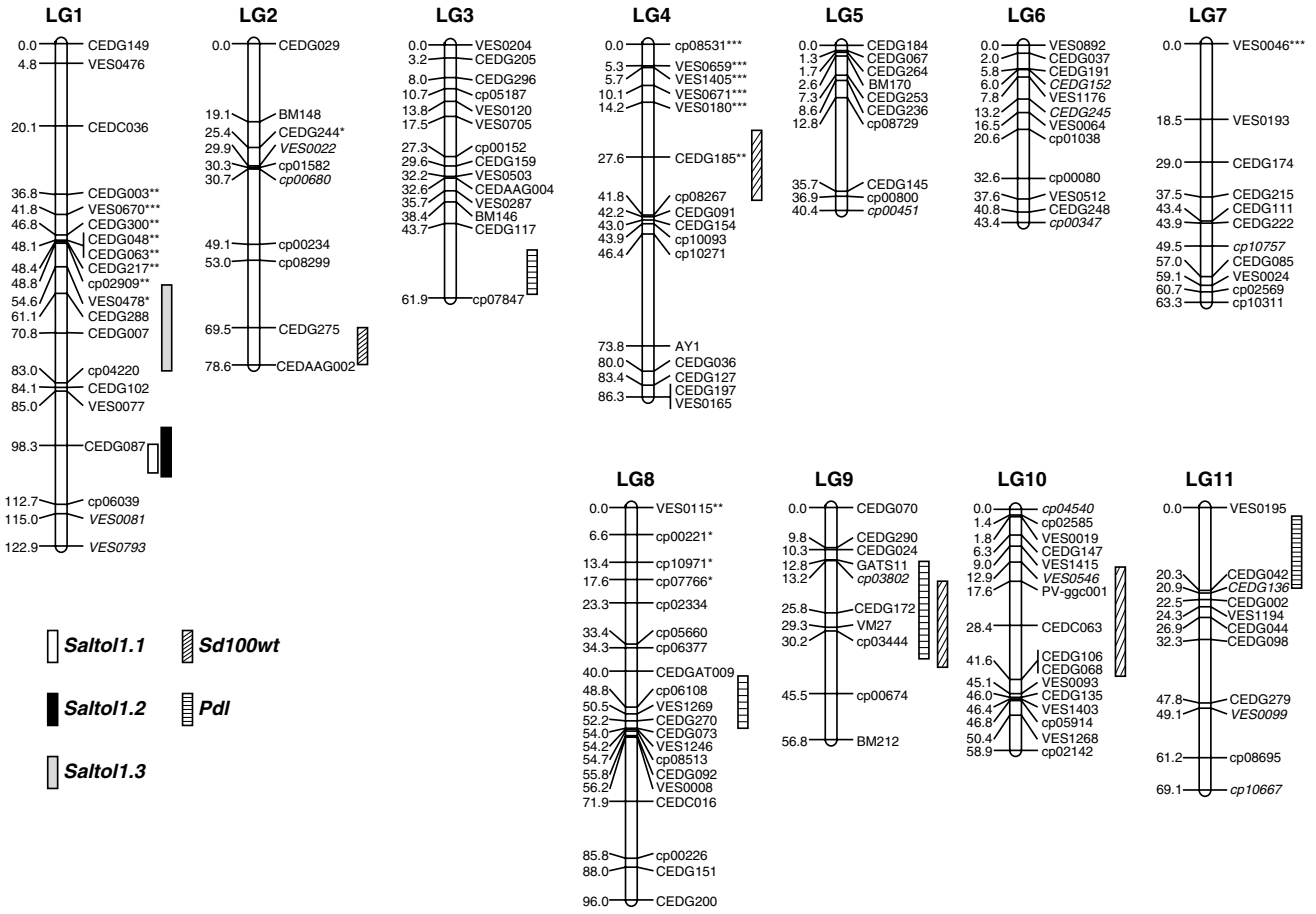

Fig. 3 A genetic linkage map of interspecific cross (*Vigna luteola* × *V. marina* subsp. *oblonga*) constructed from an F_2 population. Map distances and marker names are shown on the left and right sides of the linkage groups, respectively. Marker names in *italic* indicate dominant loci. Markers showing significant deviation from the expected segregation ratio at the 0.05, 0.01 and 0.001 levels are indicated with *, ** and ***, respectively. SSR markers with prefix CED are derived from azuki bean. Markers with prefixes cp and VM are derived from cowpea. Markers with prefixes BM, AY1, GATS11 and PV-ggc001 are derived from common bean. EST-SSR markers with prefix VES are derived from azuki bean. Bars on the left side of the linkage groups indicate the positions of putative QTLs

respectively, and compared them to the linkage map of *V. luteola* and *V. marina*. Based on common SSR markers, the main structure of the linkage maps was highly conserved between each other. However, a reciprocal translocation was found between yardlong bean and *V. marina* or *V. luteola*, while such structural alteration was not detected between mungbean and the two species (Fig. 4).

QTL for salt tolerance-related traits

Single regression analysis was carried out to identify SSR markers associated with salt tolerance in the $F_{2:3}$ population. The results revealed that seven markers from LG1 were associated with the percentage of seedling survival ($P < 0.001$) (Table 2). The phenotypic variance explained (PVE) of these markers ranged from 9.6 % (VES0793) to 35.9 % (CEDG087). Eight markers from LG1 and one marker from LG11 associated with leaf wilt score at the vegetative stage ($P < 0.001$) with the PVE between 9.9 (VES0099) and 32.3 % (CEDG087) (Table 2). Five markers from LG1 associated with plant recovery score ($P < 0.0001$). The PVE of these markers ranged from 8.4 % (CEDG288) to 17.1 % (CEDG007 and cp04220) (Table 2).

Multiple interval mapping was performed to locate QTLs conferring salt tolerance onto the linkage map. One QTL each was detected for surviving seedlings, leaf wilt at vegetative stage and plant recovering ability (Fig. 3; Table 3). All the QTLs were located on LG1. The QTL for percentage of surviving seedlings, *Saltol1.1*, was located at 101.6 cM, between markers CEDG087 and cp06039 with an LOD score of 13.62. *Saltol1.1* accounted for 50.7 % of the trait variation and had additive and dominant effects of 20.71 and 0.41 % in survival rate, respectively. QTL for leaf wilt score at the vegetative stage was located at 98.8 cM, between the markers CEDG087 and cp06039 and designated as *Saltol1.2* with an LOD score of 11.5 and explained

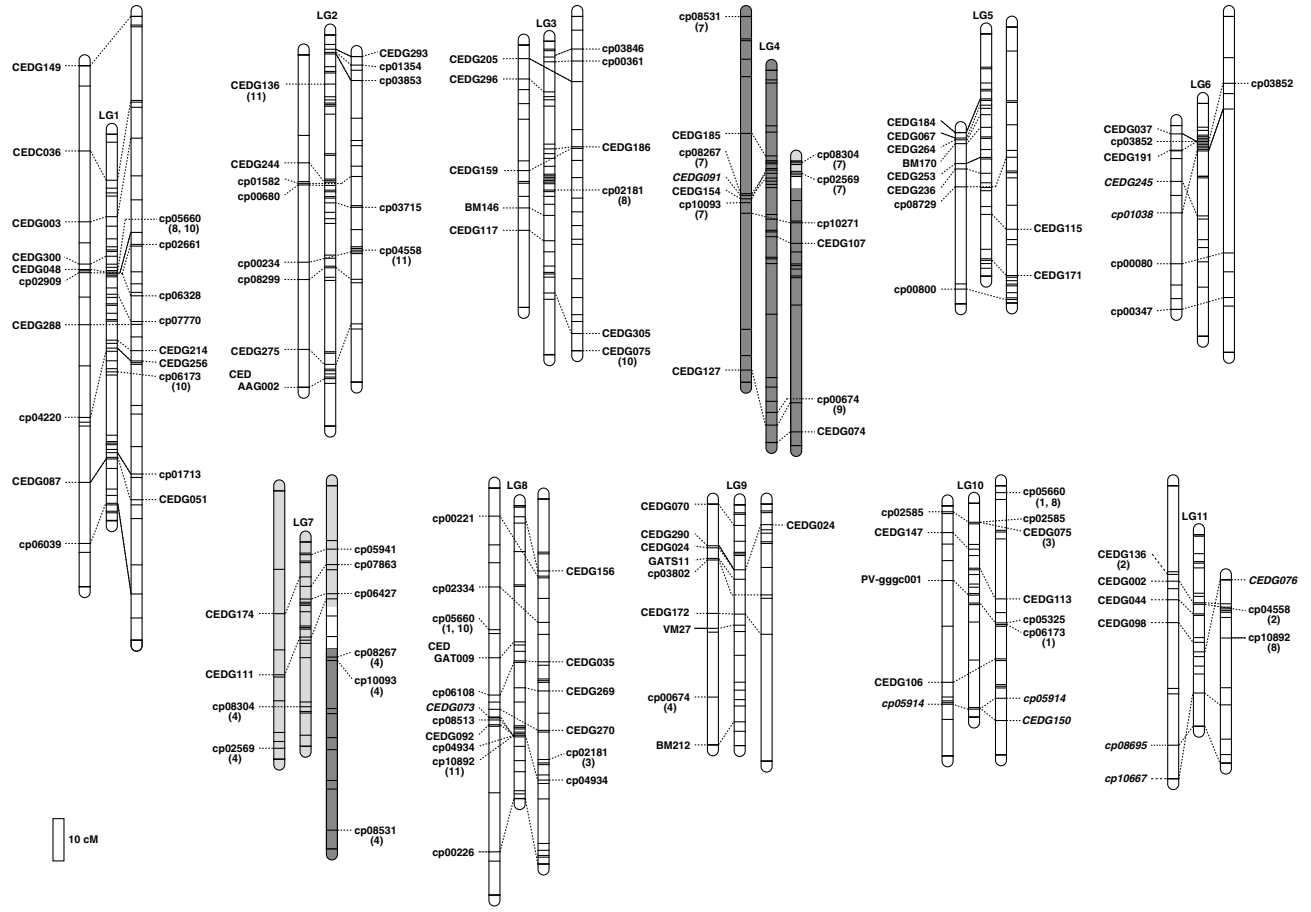

Fig. 4 Comparative map among *V. luteola* × *V. marina* subsp. *oblonga* (*right*), mungbean (*V. radiata*) (*middle*) and yardlong bean [*V. unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*] (*left*) based on common SSR markers. The positions of common markers are connected by *dotted lines*. The markers with *italic* indicate that the position or order within the linkage group is different among three species. Markers followed by a *number in parenthesis* indicate the location of the loci on the linkage groups. For the *colorless regions* on LG4 and LG7 of yardlong bean, we cannot not identify whether these regions correspond to either LG4 (*dark gray*) or LG7 (*pale gray*) in Asian *Vigna* species since there are no common markers between yardlong bean and Asian *Vigna* species. Mungbean and yardlong bean maps were obtained from Isemura et al. (2012) and Kongjaimun et al. (2012a), respectively

41.4 % of the variation. This QTL showed an additive effect of −0.86 and a dominant effect of 0.03 in wilt score. For plant recovery score, QTL for this trait, *Saltol1.3*, was detected at 73.0 cM, between the markers CEDG007 and cp04220 with an LOD score of 5.67. *Saltol1.3* accounted for 20.0 % of the variation in plant recovery and showed additive and dominant effects of 0.37 and 0.16 in recovery score, respectively. At all the detected QTLs, alleles from *V. marina* increased the salt tolerance, i.e., increased the percentage of surviving seedlings and plant recovery score, but decreased leaf wilt score.

QTL for domestication-related traits

Single regression analysis for pod length and seed size of the F_2 population data showed that 14 markers from LGs 3, 9 and 11 were associated with pod length and 22 markers from LGs 2, 4, 9, 10 and 11 were associated with 100-seed weight ($P < 0.01$) (supplementary Table S1). The coefficient of determination (R^2) of these markers for pod length data ranged from 5.6 % (CEDG290) to 12.9 % (CEDG002), while for the 100-seed weight data varied between 5.7 % (cp08229) and 21.7 % (CEDG172). MIM on pod length and seed size was also performed; four QTLs were detected, one each on LG03, LG08, LG09 and LG11, for pod length (Fig. 3; Table 3). These QTLs explained the phenotypic variation ranging from 6.76 % (*Pdl9.1*) to 22.45 % (*Pdl11.1*). Four QTLs were identified for 100-seed weight, one each on LG02, LG04, LG09 and LG10. The QTLs accounted for 6.15 % (*Sd100wt2.1*) to 22.7 % (*Sd100wt9.1*) of the variation in seed weight (Fig. 3; Table 3). As in the case of pod length, alleles from *V. marina* at all of the detected QTLs increased seed weight.

Table 2 SSR markers associated with salt tolerance in the $F_{2:3}$ population from the cross between *V. luteola* and *V. marina* subsp. *oblonga* revealed by single regression analysis

Marker name	LG[a]	Percentage of surviving seedling							Leaf wilt score at the vegetative stage							Plant recovery score						
		PVE[b]	*P* value	Marker mean[c]					PVE	*P* value	Marker mean					PVE	*P* value	Marker mean				
				A (A/A)	H (A/B)	B (B/B)	D (A/-)	C (-/B)			A (A/A)	H (A/B)	B (B/B)	D (A/-)	C (-/B)			A (A/A)	H (A/B)	B (B/B)	D (A/-)	C (-/B)
CEDG007	1	NA[d]	NS[e]	48.04	49.43	68.35	NA	NA	11.4	<0.001	6.36	5.96	5.30	NA	NA	17.1	<0.001	1.71	2.20	2.42	NA	NA
cp04220	1	11.97	<0.001	42.05	50.37	72.30	NA	NA	19.3	<0.001	6.41	6.00	5.16	NA	NA	17.1	<0.001	1.75	2.20	2.42	NA	NA
CEDG102	1	12.53	<0.001	41.51	50.22	73.77	NA	NA	20.1	<0.001	6.43	5.99	5.16	NA	NA	14.8	<0.001	1.76	2.24	2.36	NA	NA
VES0077	1	14.03	<0.001	40.88	49.60	74.74	NA	NA	21.2	<0.001	6.44	6.01	5.14	NA	NA	12.4	<0.001	1.78	2.23	2.33	NA	NA
CEDG087	1	35.90	<0.001	24.76	51.45	81.02	NA	NA	32.3	<0.001	6.80	5.98	5.10	NA	NA	NA	NS	1.77	2.28	2.16	NA	NA
cp06039	1	27.96	<0.001	18.82	54.33	73.53	NA	NA	22.6	<0.001	6.83	5.92	5.38	NA	NA	NA	NS	1.88	2.29	2.09	NA	NA
VES0081	1	27.17	<0.001	18.99	NA	NA	NA	60.69	16.3	<0.001	6.77	NA	NA	NA	5.75	NA	NS	1.92	NA	NA	NA	2.14
VES0793	1	9.60	<0.001	NA	NA	68.87	44.98	NA	14.2	<0.001	NA	NA	5.39	6.21	NA	NA	NS	NA	NA	2.30	2.01	NA
CEDG288	1	NA	NS	53.56	50.40	55.21	NA	NA	NA	NS	6.29	5.67	5.88	NA	NA	8.4	<0.001	1.74	2.29	2.17	NA	NA
VES0099	11	NA	NS	NA	NA	51.61	52.60	NA	9.9	<0.001	NA	NA	6.47	5.78	NA	NA	NS	NA	NA	1.98	2.14	NA

[a] Linkage group

[b] Phenotypic variance explained

[c] A = alleles of *V. luteola*, B = alleles of *V. marina* subsp. *oblonga*

[d] Not available

[e] Not significant

Table 3 QTLs detected for salt tolerance as measured from the percentage of surviving seedlings, leaf wilt score and plant recovery score in the $F_{2:3}$ population of *V. luteola* × *V. marina* subsp. *oblonga*

Trait	QTL name	LG[a]	LOD	Position (cM)[b]	Marker interval	PVE (%)[c]	A[d]	D[e]
Percentage of surviving seedlings	*Saltol1.1*	1	13.62	101.6	CEDG087–cp06039	50.70	20.71	0.41
Leaf wilt score at vegetative stage	*Saltol1.2*	1	11.50	98.8	CEDG087–cp06039	41.40	−0.86	0.03
Plant recovery score	*Saltol1.3*	1	5.67	73.0	CEDG007–cp04220	20.00	0.37	0.16
Pod length	*Pdl3.1*	3	5.28	61.0	CEDG117–cp07847	13.84	0.29	0.08
	Pdl8.1	8	3.27	50.0	VES1269–CEDG270	8.81	0.28	0.07
	Pdl9.1	9	2.83	20.0	GATS11–CEDG172	6.76	0.22	0.06
	Pdl11.1	11	6.77	10.0	VES0195–CEDG042	22.45	0.42	0.24
100-seed weight	*Sd100wt2.1*	2	2.53	78.5	CEDG275–CEDAAG002	6.15	0.13	0.07
	Sd100wt4.1	4	7.21	33.6	CEDG185–cp08267	20.60	0.22	0.25
	Sd100wt9.1	9	6.55	29.3	VM27–cp03444	22.70	0.23	0.07
	Sd100wt10.1	10	3.14	25.6	PV-ggc001–CEDG063	7.69	0.13	0.01

[a] Linkage group

[b] Position on the linkage group

[c] Percentage of phenotypic variance explained by the QTL

[d] Additive effect

[e] Dominant effect

Discussion

In this study we developed the first genetic linkage map of wild *Vigna* species, *V. marina* and *V. luteola*. Our linkage map indicated not only that these species were genetically close to each other, but also that the two species were more closely related to Asian *Vigna* than to African *Vigna*. The successive QTL analysis consistently detected one major QTL for salt tolerance and four QTLs involved in pod length and seed size.

Close genetic relation between *V. marina* and *V. luteola*

The genetic map comprises 150 SSR marker loci from cowpea, azuki bean and common bean, and covers 11 linkage groups corresponding to the haploid chromosome number ($n = 11$) of *Vigna* species. Of the mapped 150 markers, 20 (13.3 %) showed significantly distorted segregation. Segregation distortion (SD) is a common phenomenon caused by genetic barriers at both intra- and interspecific crosses. SD in *Vigna* species was reported to be between 12 and 30.8 % (Kaga et al. 2005). Thus, the relatively low SD in our linkage map suggested a close genetic relationship between these two species. RAPD analysis of *V. marina* and *V. luteola* germplasm by Sonnante et al. (1997) demonstrated that *V. marina* subsp. *oblonga* is more closely related to *V. luteola* than the other variants of *V. marina*. Morphological differences between *V. marina* and *V. luteola* are very low (Maréchal et al. 1978), and hybridization between *V. marina* and *V. luteola* yields fertile F_1 hybrids (Palmer et al. 2002, and this study). The main difference between these two species is their ecological habitats: marine for *V. marina* and freshwater for *V. luteola*. Maréchal et al. (1978) noted a possibility that *V. marina* is an adaptive form of *V. luteola* to the halophytic condition. In fact, some botanists proposed that these species should be treated as belonging to the same taxon (Verdcourt 1971). Our results support such a possibility.

Although some QTLs found in this study were located on the same LG with clusters of distorted markers, the locations of those QTLs, especially those for salt tolerance, are far from distorted markers (over 40 cM apart from *Saltol1.1* and *Saltol1.2* or at least about 20 cM for *Saltol1.3*), except for QTL for 100-seed weight on LG4 which is about 6 cM from the nearest distorted marker (Table 3; Fig. 3). In a study on the impact of SD on QTL mapping in F_2 population by Zhang et al. (2010), they found that if distorted marker is not closely linked with QTL, SD will not have significant effect on QTL mapping and its effect decreases rapidly as the marker is further apart from the QTL. Thus, the distorted markers in our study should not significantly affect the mapping result.

Genetic relation of *V. luteola* and *V. marina* with other *Vigna* species

In the present study, 561 (42.0 %) of the 1,336 SSR primer pairs from *Vigna* and *Phaseolus* species amplified DNA fragments of *V. marina* and *V. luteola*. This result confirmed the high transferability of SSR markers within the genus *Vigna* (Tangphatsornruang et al. 2009; Somta et al. 2009; Kongjaimun et al. 2012a). However, it was beyond

our expectation that the SSR markers from azuki bean were more transferable to *V. luteola* and *V. marina* than SSR markers from cowpea (Table 1), because these two species have been considered as African *Vigna* and thus belonging to the same section as cowpea. The closer relation of *V. luteola* and *V. marina* to Asian *Vigna* was also suggested by the comparative linkage map (Fig. 4). Compared to yardlong bean linkage map (Kongjaimun et al. 2012a), our map contained one reciprocal translocation between LG4 and LG7 that was not found between the linkage map of this study and mungbean (Isemura et al. 2012). Thus, the taxonomy of *V. luteola* and *V. marina* might need to be reconsidered. More comprehensive analyses such as phylogenomic approaches will be required to conclude this issue.

QTLs related to the domestication syndrome

The QTLs for domestication-related traits such as seed and pod size have been identified in several *Vigna* crops (Isemura et al. 2007, 2010, 2012; Kaga et al. 2008; Kongjaimun et al. 2012b). Their results revealed that many detected QTLs are conserved among *Vigna* species. In this study, seed size and pod length of *V. luteola* × *V. marina* were each controlled by four QTLs. The QTL for pod length on LG3 of *V. marina* overlapped that of rice bean (Isemura et al. 2010), while the three QTLs for pod length in *V. marina* were on the same LGs as in yardlong bean (LG3, LG8 and LG11) (Kongjaimun et al. 2012a, b). For 100-seed weight, two QTLs each on LG2 and LG4 of *V. marina* and rice bean were in the same location, while all the four QTLs of *V. marina* were located on the same LGs (LG2, LG4, LG9 and LG10) as in yardlong bean (Kongjaimun et al. 2012b). The overlap of these QTLs with crop species may support the hypothesis that large seed size of *V. marina* is the result of human selection (Smartt 1978). However, since almost all accessions of *V. marina* have larger seeds than *V. luteola*, it is also possible that this trait might be more adaptive to survive in marine beach and/or to distribute seeds across the ocean.

Salt tolerance QTLs in *V. marina*

The continuous distribution of percentage of survival plants at the seedling stage, score of leaf wilt and plant recovering ability in the $F_{2:3}$ population suggested the possibility of oligogenes or polygenes controlling the traits. The transgressive segregation observed for the above traits except plant recovery rate in the $F_{2:3}$ population suggested that alleles from both *V. marina* and *V. luteola* increased salt tolerance. However, only one QTL for each trait was consistently detected by both single regression analysis and MIM on LG1. Another QTL with relatively small effect was detected by single regression analysis on LG11 for leaf wilt, but still only half of the phenotypic variance can be explained (Table 2). Further analysis with larger population may resolve undetected minor QTLs.

Salinity tolerance in plants appears to be a developmentally regulated and stage-specific trait (Ashraf and Foolad 2013). Salinity tolerance varies highly during the plant's life cycle. There seems to be no correlation between tolerances at different growth stages (see Foolad 2004, for review). QTL mapping for salt tolerance in barley (Mano and Takeda 1997), tomato (Foolad 1999; Zhang et al. 2003) and *Arabidopsis* (Quesada et al. 2002) showed that QTLs controlling salinity tolerance at the germinating stage were different from those conferring the tolerance at other growth stages. In contrast, our study showed that salt tolerance at the seedling stage and vegetative stage were each controlled by a single major QTL (*Saltol1.1* and *Saltol1.2*, respectively). These results were the same as in soybean, a closely related species to *Vigna*. Lee et al. (2004) and Tuyen et al. (2010) reported that only one major QTL controlled salt tolerance in both cultivated soybean and wild soybean (*Glycine soja* Sieb. & Zucc.). The proximate positions of the *Saltol1.1* and *Saltol1.2* suggest that they are on the same locus and that *V. marina* uses the same mechanism to cope with salt stress at both seedling and vegetative stages. The result agreed with the fact that *V. marina* lives on marine habitat that is affected by salinity throughout the whole life cycle. Moreover, the single QTL with large effect identified in this study may play important roles in the divergence of *V. marina* from *V. luteola*, where adaptation from riverbank to seashore had taken place.

An interesting feature of salt tolerance in *V. marina* found in the present study is plant recovering ability. Recovery was expressed as development and production of new leaves and branches under salt stress (300 mM NaCl) after some or all leaves had dropped off. The QTL for recovering ability was located relatively far (>25 cM) from the QTLs controlling plant survival at the seedling stage and leaf wilt at the vegetative stage, so they are possibly different loci. This QTL might also enable *V. marina* to adapt and survive under salt-affected conditions.

The salt tolerance QTLs identified in this study are novel and potentially useful for developing salt-tolerant *Vigna* crops in the future using marker-assisted selection (MAS) or genetic engineering. Since F_1 seeds from the cross between *V. luteola* and cowpea were obtained (Sen and Bhowal 1960), it may be possible to transfer the gene(s) for salt tolerance of *V. marina* to cowpea by MAS. To do so, fine mapping of the QTLs controlling salt tolerance on LG1 is required to more accurately resolve the position of QTLs and validate their effects. Fine mapping is also important for gene cloning of such QTLs for gene transformation to other crops. Available and expected whole genome sequence of common bean

(*Phaseolus vulgaris*-JGI v1.0; http://www.phytozome.net/cgi-bin/gbrowse/commonbean) and mungbean (Lee 2012), respectively, can be exploited to fine map salt-tolerant QTLs in *V. marina*. Based on a BLAST search against the whole genome sequence of common bean (*Phaseolus vulgaris* L.) in Phytozome v9.1 database (http://www.phytozome.net/commonbean) using sequences of cp06039 and CEDG007 flanking QTL *Saltol1.1* and *Saltol1.2*, we found that both markers were on the same chromosome located at Chr03:25,977,425..25,977,477 and Chr03:49,028,227..49,028,291, respectively. This chromosomal region (Chr03:25,977,425.. 49,028,291) is the location of one annotated gene (*Phvul.003G171500*) that encodes for vacuolar H+ pyrophosphatase and three annotated genes (*Phvul.003G143800*, *Phvul.003G159200* and *Phvul.003G193800*), each encoding for plasma membrane H+-transporting ATPase. These enzymes/genes have been reported to be involved in salt tolerance in several plant species (Flowers and Colmer 2008). Therefore, these genes can be used as candidate gene mapping for salt tolerance in *V. marina* spp. *oblonga* in the future.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All the experiments carried out in this study comply with the current laws of both Thailand and Japan.

References

Arraouadi S, Badri M, Abdelly C, Huguet T, Aoudi ME (2012) QTL mapping of physiological traits associated with salt tolerance in *Medicago truncatula* recombinant inbred lines. Genomics 99:118–125

Ashraf MA, Foolad MR (2013) Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. Plant Breed 132:10–20

Blair MW, Pedraza F, Buendia HF, Gaitán-Solís E, Beebe SE, Gepts P, Thome J (2003) Development of a genome-wide anchored microsatellite map of common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 107:1362–1374

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

FAO (2005) Global network on integrated soil management for sustainable use of salt-affected soils. FAO land and plant nutrition management service, Rome

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963

Foolad MR (1999) Comparison of salt tolerance during seed germination and vegetative growth in tomato by QTL mapping. Genome 42:727–734

Foolad MR (2004) Recent advances in genetics of salt tolerance in tomato. Plant Cell Tissue Organ Cult 76:101–119

Gaitán-Solís E, Duque MC, Edwards KJ, Thome J (2002) Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization, and cross-species amplification in *Phaseolus* ssp. Crop Sci 42:2128–2136

Greenway H, Munns H (1980) Mechanisms of salt tolerance in non halophytes. Annu Rev Plant Physiol 31:149–190

Han O, Kaga A, Isemura T, Wang XW, Tomooka N, Vaughan DA (2005) A genetic linkage map for azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. Theor Appl Genet 111:1288–1299

Isemura T, Kaga A, Konishi S, Ando T, Tomooka N, Han OK, Vaughan DA (2007) Genome dissection of traits related to domestication in azuki bean (*Vigna angularis*) and comparison with other warm-season legumes. Ann Bot 100:1053–1071

Isemura T, Kaga A, Tomooka N, Shimizu T, Vaughan DA (2010) The genetics of domestication of rice bean, *Vigna umbellata*. Ann Bot 106:927–944

Isemura I, Kaga A, Tabata S, Somta P, Srinives P, Shimizu T, Jo U, Vaughan DA, Tomooka N (2012) Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS ONE 7(8):e41304

Kaga A, Vaughan DA, Tomooka N (2005) Molecular markers in *Vigna* improvement: understanding and using gene pools. In: Lorz H, Wanzel G (eds) Biotechnology in agriculture and forestry, vol 55., Molecular marker systemsBerlin, Springer, pp 171–187

Kaga A, Isemura T, Tomooka N, Vaughan DA (2008) The genetics of domestication of the azuki bean (*Vigna angularis*). Genetics 178:1013–1036

Kao CH, Zeng ZB, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. Genetics 152:1203–1216

Kearsey MJ, Pooni HS (1996) The genetical analysis of quantitative traits. Chapman and Hall, London

Kongjaimun A, Kaga A, Tomooka N, Somta P, Shimizu T, Shu Y, Vaughan DA, Srinives P (2012a) An SSR-based linkage map of yardlong bean [*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verc.] and QTL analysis of pod length. Genome 55:81–92

Kongjaimun A, Kaga A, Tomooka N, Somta P, Vaughan DA, Srinives P (2012b) The genetics of domestication of yardlong bean [*Vigna unguiculata* (L.) Walp. ssp. unguiculata cv.-gr. sesquipedalis]. Ann Bot 109:1185–1200

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lawn RJ, Cottrell A (1988) Wild mungbean and its relatives in Australia. Biologist 35:267–273

Lee SH (2012) Toward completion of genome sequence of mungbean. In: Abstract book, VI International Conference on Legume Genetics and Genomics, 2–7 October 2012, Hyderabad, India

Lee GJ, Boerma HR, Villagarcia MR, Zhou X, Carter TE Jr, Li Z, Gibbs MO (2004) A major QTL conditioning salt tolerance in S-100 soybean and descendant cultivars. Theor Appl Genet 109:1610–1619

Leonforte A, Sudheesh S, Cogan NOI, Salisbury PA, Nicolas ME, Materne M, Forster JW, Kaur S (2013) SNP marker discovery, linkage map construction and identification of QTLs for enhanced salinity tolerance in field pea (*Pisum sativum* L.). BMC Plant Biol 13:161. doi:10.1186/1471-2229-13-161

Lewis G, Schrire B, Mackinder B, Lock M (2005) Legumes of the world. Kew, Royal Botanic Gardens

Li CD, Fatokun CA, Ubi B, Singh BB, Scoles GJ (2001) Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. Crop Sci 41:189–197

Lobato AKS, Santos Filho BG, Costa RCL, Goncalva-Vidigal MC, Moraes EC, Oliveira Neto CF, Rodriges VLF, Cruz FJR, Ferreira AS, Pita JD, Barreto AGT (2009) Morphological, physiological and biochemical responses during germination of the cowpea (*Vigna unguiculata* cv. Pitiuba) seeds under salt stress. World J Agric Sci 5:590–596

Lodhi MA, Ye GN, Weeden NF, Reisch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13

Mano Y, Takeda K (1997) Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). Euphytica 94:263–272

Maréchal R, Mascherpa JM, Stainer F (1978) Extude taxonomique d'un groupe d'espèces des genres *Phaseolu*s et *Vigna* (*V. unguiculata*) sur la base de données morphologiques et polliniques, traitées pour l'analyse informatique. Boissiera 28:160–272

Mehra KL, Ibrahim AH (1989) Genetic resources in the Maldives. FAO/IBPGR Plant Genet Resour Newsl 75(76):42–43

Padulosi S, Ng NQ (1993) A useful and unexploited herb, *Vigna marina* (Leguminosae-Papilionideae) and the taxonomic revision of its genetic diversity. Bull Jard Bot Nat Belg 62:119–126

Palmer JL, Lawn RJ, Adkins SW (2002) An embryo-rescue protocol for *Vigna* interspecific hybrids. Aust J Bot 50:331–338

Quesada V, Martinez SG, Piqueras P, Ponce MR, Micol JL (2002) Genetic architecture of NaCl tolerance in *Arabidopsis*. Plant Physiol 130:951–963

Rogers ME, Craig AD, Munns R et al (2005) The development of fodder plants for the salt-affected areas of southern and eastern Australia: an overview. Aust J Exp Agric 45:301–329

Samineni S (2010) Physiology, genetics and QTL mapping of salt tolerance in chickpea (*Cicer arietinum* L.). Ph.D. Thesis, The University of Western Australia

Sen NK, Bhowal JG (1960) Cytotaxonomic studies on *Vigna*. Cytologia 25:195–207

Smartt J (1978) The evaluation of pulse crops. Econ Bot 32:185–198

Somta P, Seehalak W, Srinives P (2009) Development, characterization and cross-species amplification of mungbean (*Vigna radiata*) genic microsatellite markers. Conserv Genet 10:1939–1943

Sonnante G, Spinosa A, Marangi A, Pignone D (1997) Isozyme and RAPD analysis of the genetic diversity within and between *Vigna luteola* and *V. marina*. Ann Bot 80:741–746

Tangphatsornruang S, Somta P, Uthaipaisanwong P, Chanprasert J, Sangsrakru D, Seehalak W, Sommanas W, Tragoonrung S, Srinives P (2009) Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). BMC Plant Biol 9:137

Tomooka N, Vaughan DA, Moss H, Maxted N (2002) The Asian *Vigna*: genus *Vigna* subgenus *Ceratotropis* genetic resources. Kluwer Academic Publishers, Netherlands

Tomooka N, Kaga A, Isemura T, Vaughan D (2011) *Vigna*. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, Legume crops and forages. Springer, Berlin, pp 291–311

Tuyen DD, Lal SK, Xu DH (2010) Identification of a major QTL allele from wild soybean (*Glycine soja* Sieb. & Zucc.) for increasing alkaline salt tolerance in soybean. Theor Appl Genet 121:229–236

Van Ooijen JW (2006) JoinMap version 4.0, software for the calculation of genetic linkage maps. Kyazma B.V, Wageningen

Verdcourt B (1970) Studies in the Leguminosae-Papilionoideae for the 'Flora of Tropical East Africa', IV. Kew Bull 24:507–569

Verdcourt B (1971) *Phaseoleae*. In: Milne-Redhead E, Polhill RM (eds) Flora of tropical East Africa, Leguminosae (part 4), Papilionideae (2). Crown Agents for Overseas Governments, London, pp 625–627

Wang XW, Kaga A, Tomooka N, Vaughan DA (2004) The development of SSR markers by a new method in plants and their application to gene flow studies in azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. Theor Appl Genet 109:352–360

Wang S, Basten CJ, Zeng ZB (2007) Windows QTL Cartographer 2.5. North Carolina State University, Raleigh

Wilson C, Liu X, Lesch SM, Suarez DL (2009) Growth response of major U.S. cowpea cultivars. I. Biomass accumulation and salt tolerance. Hortic Sci 41:225–230

Win KT, Oo AZ, Hirasawa T, Ookawa T, Yutaka H (2011) Genetic analysis of Myanmar *Vigna* species in response to salt stress at the seedling stage. Afr J Biotechnol 10:1615–1624

Yu K, Park J, Poysa V, Gepts P (2000) Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J Hered 91:429–434

Zhang LP, Lin GY, Foolad MR (2003) QTL comparison of salt tolerance during seed germination and vegetative growth in a *Lycopersicon esculentum* × *L. pimpinellifolium* RIL population. Acta Hort 618:59–67

Zhang L, Wang S, Li H, Deng Q, Zheng A, Li S, Li P, Li Z, Wang J (2010) Effects of missing marker and segregation distortion on QTL mapping in F_2 populations. Theor Appl Genet 121:1071–1082