Evaluation of salt tolerance in Japanese wild radishes (*Raphanus sativus* f. *raphanistroides* Makino)

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In order to evaluate the salt tolerance of Japanese wild radishes called 'Hamadaikon' (*Raphanus sativus* f. *raphanistroides* Makino), seed germination, plant height, root length and fresh weight were examined under the salinity condition, in comparison with cultivated radishes 'Aokubi-Miyashige' and 'Minowase.' The Japanese wild radishes were collected near the beach of Takanabe, Miyazaki, Japan. The artificial seawater and its elements, NaCl, $MgCl_2$, $MgSO_4$, $CaSO_4$, K_2SO_4, were examined in various concentrations for this study. Cultivated radishes showed higher salt tolerance than wild radishes in seed germination. In the wild radishes, higher germination and growth in NaCl were shown at 25℃ than those at 20℃. There were differences among the five wild radish strains. Wild radish A showed tolerance of NaCl at 25℃. Wild radish C showed tolerance of 10 mM $MgCl_2$ and 100 mM $MgSO_4$, but was sensitive to NaCl and $CaSO_4$. Wild radish E showed tolerance of NaCl, $MgSO_4$ and $CaSO_4$ at relatively lower concentrations. Therefore, the wild radishes could be of use for the salt tolerance breeding.

Key words: *Raphanus sativus* f. *raphanistroides* Makino, Japanese wild radish, salt tolerance, seed germination.

INTRODUCTION

Japanese wild radishes called 'Hamadaikon' are widely spread along the coastline of Japan. It was suggested that the wild radishes escaped from the cultivated radishes, and classified as *Raphanus sativus* forma *raphanistroides* (Makino 1909). They were also described as derivatives of a wild species (Aoba 1989), or consisting of descendants of both the wild species and the domesticated plants from escaping ones (Kumazawa 1956). On the other hand, it is reported that escaping cultivated radishes could hardly survive in the wild and would not consist of a population independently from cultivars (Ohnishi 1988). Yamagishi et al. (1998) postulated that the Japanese wild radishes are a part of a large group of cultivated radishes (*R. sativus*) by the random amplified polymorphic DNA (RAPD) analysis and that the wild-radishes were derivatives of common ancestors. The Ogura type male sterile cytoplasm in radish (Ogura 1968) may have originated in Hamadaikon (Japanese wild radishes) and it is distributed widely among them (Yamagishi et al. 1997, 1998).

Radish was reported showing a strong tolerance to salinity (Shimose and Hayashi 1983). In the genus *Brassica*, the salt tolerance was investigated in *B. campestris* (Shimose and Kurosaka 1985a), *B. juncea* (Kumar 1984, Jain et al. 1991, Ibrar et al. 2003), *B. napus* (Rahman et al. 1995), *B. oleracea* (Singh et al. 1982, Shimose and Takenaka 1986), *B. rapa* (Francois 1984, Shimose and Kurosaka 1985b). It is also reported that the salinity tolerance were Chinese kale > Chinese radish > Chinese mustard > Chinese cabbage > leaf lettuce in saline water on soil (Yuvaniyom et al. 1993). However, Japanese wild radishes' salt tolerance has not reported yet. The Japanese wild radishes are expected to be highly tolerant to salinity, because their habitat is severely exposed to seawater and salt breeze.

The objective of this study was to evaluate Japanese wild radishes for materials of plant breeding by investigating how they grow under the salinity conditions.

MATERIALS AND METHODS

Plant materials. Japanese wild radishes called 'Hamadaikon' (*R. sativus* f. *raphanistroides* Makino) growing in the coast of Takanabe (Miyazaki prefecture) were used for this experiment. Five strains, A, B, C, D, and E (named for distinction depending on the place where they grew), were collected from individual colonies in the shore. Two Japanese varieties (*R. sativus* L.), 'Aokubi-Miyashige' (shown as a shortened name of 'Miyashige' in the figures) and 'Minowase' were obtained from a seed company (Takii Co, Ltd) and used for comparison.

Salt treatments. The artificial seawater (Mafuji, 1995) consisting of 2.72% NaCl, 0.38% $MgCl_2$, 0.17% $MgSO_4$, 0.13% $CaSO_4$, 0.086% K_2SO_4 was used. Five concentrations of the artificial seawater, 0 (control), 1/10, 1/5, 1/2, 1/1 were examined. Each element of the artificial seawater was added to Murashige & Skoog (MS) medium according to the set concentrations. The element of $MgSO_4$ is already contained in MS medium at 1.5 mM. MS medium was containing 30g·liter^{-1} sucrose and 8 g·liter^{-1} agar (pH 5.8 before autoclaving).

In order to examine the effects of individual salt, various concentrations of sodium chloride (NaCl), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$), calcium sulfate ($CaSO_4$), or potassium sulfate (K_2SO_4) was added at the concentration of 0 (control), 10, 50, 100 and 200 mM to MS medium. The other conditions of the media were the same as above.

Seed inoculation. Seeds of radishes were surface-sterilized by 70% ethanol for 30 sec, followed by 1% sodium hypochlorite for 30 min. After the treatment, they were rinsed three times with sterile distilled water. A single seed was inoculated in a glass tube of 25 mm in diameter and 120 mm height containing 10 ml of each medium. Each treatment consisted of 10 tubes was replicated four times. The jars were incubated at 20°C under 2000 lx and a 16-hr photo period, 25°C was also examined for the NaCl test. The number of germinating seeds was counted daily for 14 days after the inoculation. The germination rates were converted into relative values (relative germination rate) to the control (salt concentration of 0) as 100.

Measuring the growth and data analysis. Germinated seeds were maintained as described above until 30 days after the germination. Plant height, the longest root length, and fresh weight per plant were measured at the point. All data were converted into relative values to the control (salt concentration of 0) as 100. Data were analysed by analysis of variance (ANOVA) at the level of 5% or 1%. The least significant differences at 5% level ($LSD_{0.05}$) were also calculated.

RESULTS

Germination under the salt treatments. In all salt treatments, significant differences at 1% level among concentrations were detected in seed germination rates (Table 1). Cumulative germination rates of all materials reached maximum in 14 days. The Japanese wild radishes germinated at most 85% in the MS medium, while those of the varieties 'Aokubi-Miyashige' and 'Minowase' showed 100%. There were significant differences between cultivars and wild radishes except for NaCl at 25°C, whereas there was no significant difference between the cultivars.

In the artificial seawater test, all radishes tested showed the highest germination rate without the seawater (control), and no radish germinated in the concentration of 1/1 (Fig. 1). The cultivars, especially 'Miyashige', showed higher germination rates than did the wild radishes throughout the concentrations tested. The two cultivars did not germinate in the seawater medium in the concentration higher than 1/2. Wild radish C did not germinate in all media with the artificial seawater.

In NaCl at 20°C, significant difference at 5% level was detected among the wild radishes due to wild radish D (Fig. 2 and Table 1). The wild radishes showed higher germination rates at 25°C than those at 20°C. No radish germinated in 200 mM NaCl at 20°C. However, at 25°C, wild radish C could germinate in 200 mM, so did 'Minowase.' Significant differences at 1% level were detected between cultivars and wild radishes at 20°C, but not at 25°C (Table 1).

In $MgCl_2$, there was not significant difference among radishes, but the difference between cultivars and wild radishes were detected at 1% level (Table 1). In $MgSO_4$, $CaSO_4$ and K_2SO_4, both cultivars of 'Aokubi- Miyashige' and 'Minowase' showed higher germination rates in all concentrations than the wild radishes (Fig. 3 and 4). In $MgCl_2$ and K_2SO_4, both cultivars could germinate even in 200 mM in which the wild radishes did not germinate. Especially, 'Aokubi-Miyashige' was not affected by $CaSO_4$ on germination through the concentrations tested.

Plant height under the salt treatments. There were significant differences of plant height among concentrations of all salts at 1% level except for $CaSO_4$. Significant differences were detected among wild radishes, and between cultivars and wild radishes in $CaSO_4$ (Table 2). There were also significant differences among wild radishes, and between cultivars and wild radishes in K_2SO_4. The plant heights of all radishes in the artificial seawater became lower along to the concentrations going up (Fig. 5). Significant differences at 5% level were detected between cultivars and wild radishes in seawater. In both 10 mM NaCl at 20°C and 25°C, plant heights of wild radish E were significantly higher than those of cultivars (Fig. 6). In $MgCl_2$, wild radish A showed higher plant heights than the cultivars at 10 and 50 mM, and so did wild radish C at 10 mM (Fig. 7). Wild radish E showed significantly (5% level) higher plant height at 10 mM $MgSO_4$ and at 100 mM $CaSO_4$ (Fig. 8). There were significant differences at 1% level between cultivars and wild radishes in K_2SO_4.

Root length under the salt treatments. There were significant differences among concentrations of all salts at 1% level except for $CaSO_4$. Significant differences in root lengths were detected between cultivars and wild radishes in seawater at 5% level, in NaCl at 20°C at 1% level, and in K_2SO_4 at 5% level (Table 3). Root length showed similar patterns to the plant heights in the seawater (Fig. 9). Wild radish A showed significantly (5% level) higher root length than the cultivars at 10, 50 and 100 mM NaCl at 25°C, and wild radish C and D also showed significant differences at 10 mM NaCl (Fig. 10). Wild radish C showed significantly higher root length than those of the cultivars in $MgCl_2$ at 10 mM. Wild radish E showed significant difference at 10 mM $MgSO_4$ (Fig. 11). Wild radish E also showed significantly larger root length at 10 mM $CaSO_4$ (Fig. 12).

Fresh weight under the salt treatments. There were significant differences among concentrations of all salts at 1% level except for $CaSO_4$. Significant differences in fresh weight were detected between cultivars and wild radishes in seawater at 5% level, in NaCl at 20°C at 1% level, in $CaSO_4$ at 1% level, and in K_2SO_4 at 1% level (Table 4). Fresh weights of wild radish A were significantly (5% level) higher than the other radishes tested at 10 mM NaCl at 20°C (Fig. 13). In $MgCl_2$, wild radish C showed significantly (5% level) higher fresh weights at 10 and 50 mM than those of the cultivars, and so did wild radish A at 50 mM (Fig. 14). Wild radish E and C showed significantly higher fresh weights at 50 mM and 100 mM $MgSO_4$, respectively (Fig. 14). 'Minowase' showed higher fresh weights throughout the concentrations of K_2SO_4 (Fig. 15).

Table 1. F-values from analysis of variance for seed germination among cultivated and wild radishes

Element	Among concentrations	Among radishes	Among wild radishes	Between cultivars	Between cultivars and wild radishes
Seawater	29.920**	3.163*	0.401	0.240	17.135**
$NaCl^{20}$	39.508**	3.673**	2.687*	3.232	8.060**
$NaCl^{25}$	32.710**	2.134	2.482	0.000	2.878
$MgCl_2$	34.419**	2.500	2.155	0.279	6.097**
$MgSO_4$	14.521**	6.057**	0.666	1.499	32.182**
$CaSO_4$	14.286**	11.308**	2.498	0.114	57.742**
K_2SO_4	37.524**	4.550**	0.168	0.115	26.514**

$NaCl^{20}$, $NaCl^{25}$: cultured at 20℃ and 25℃, respectively.
*, **: significant at 5% and 1% level, respectively.

Table 2. F-values from analysis of variance for plant height among cultivated and wild radishes

Element	Among concentrations	Among radishes	Among wild radishes	Between cultivars	Between cultivars and wild radishes
Seawater	31.013**	1.486	0.994	0.037	4.899*
$NaCl^{20}$	33.958**	0.886	1.097	0.304	0.628
$NaCl^{25}$	33.259**	1.715	2.500	0.001	0.288
$MgCl_2$	17.779**	1.635	2.435	0.000	0.072
$MgSO_4$	21.992**	0.263	0.335	0.013	0.228
$CaSO_4$	1.729	4.108**	5.212**	0.286	3.516*
K_2SO_4	73.659**	2.871*	1.539	1.156	9.913**

$NaCl^{20}$, $NaCl^{25}$: cultured at 20℃ and 25℃, respectively.
*, ** : significant at 5% and 1% level, respectively.

Table 3. F-values from analysis of variance for root length among cultivated and wild radishes

Element	Among concentrations	Among radishes	Among wild radishes	Between cultivars	Between cultivars and wild radishes
Seawater	14.665**	1.550	0.942	0.118	5.413*
$NaCl^{20}$	98.807**	3.693**	0.973	0.167	18.097**
$NaCl^{25}$	28.565**	1.971	2.235	2.417	0.466
$MgCl_2$	33.806**	1.339	1.962	0.155	0.029
$MgSO_4$	27.602**	0.508	0.482	0.567	0.558
$CaSO_4$	1.667	3.545*	4.446**	0.735	2.751
K_2SO_4	37.484**	1.804	1.371	0.109	5.231*

$NaCl^{20}$, $NaCl^{25}$: cultured at 20℃ and 25℃, respectively.
*, **: significant at 5% and 1% level, respectively.

Table 4. F-values from analysis of variance for fresh weight among cultivated and wild radishes

Element	Among concentrations	Among radishes	Among wild radishes	Between cultivars	Between cultivars and wild radishes
Seawater	18.331**	1.508	0.978	0.922	4.214*
$NaCl^{20}$	79.786**	2.964*	2.099	1.197	8.190**
$MgCl_2$	31.312**	2.041	3.047*	0.003	0.056
$MgSO_4$	17.198**	1.837	0.759	4.616*	3.371
$CaSO_4$	1.056	7.378**	8.708**	1.408	8.026**
K_2SO_4	44.924**	4.410**	0.334	0.516	24.612**

$NaCl^{20}$: cultured at 20℃.
*, **: significant at 5% and 1% level, respectively.

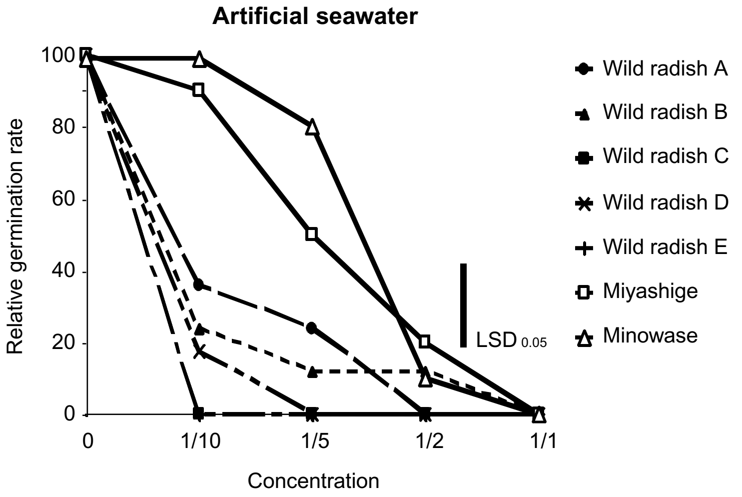

Fig. 1. Comparison of relative seed germination rates under the artificial seawater treatment among cultivated and wild radishes. The vertical bar indicates the least significant difference (LSD) at 5% level.

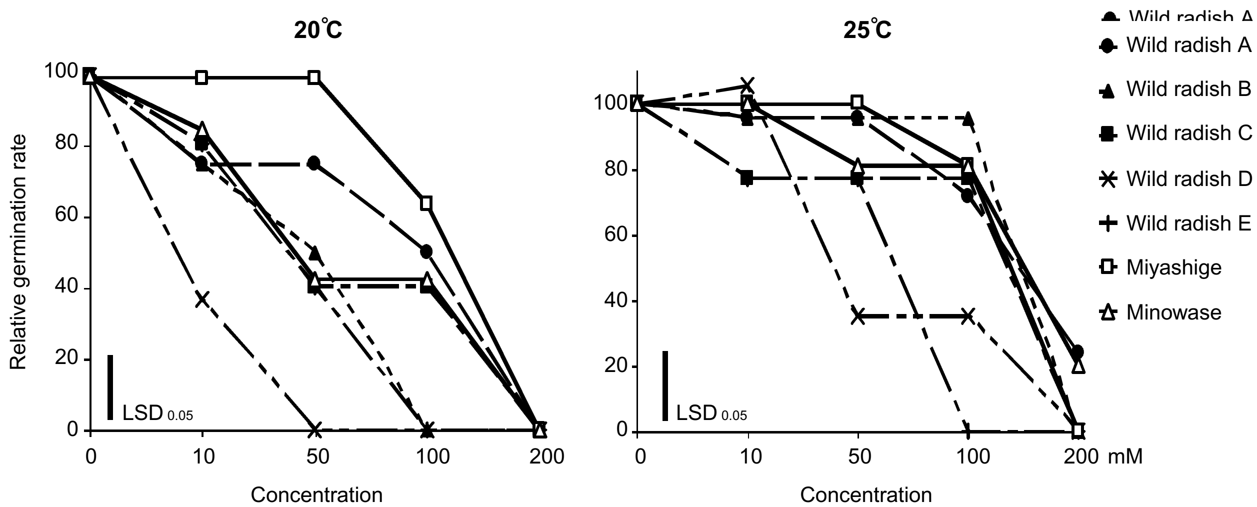

Fig. 2. Comparison of relative seed germination rates under NaCl treatment among cultivated and wild radishes cultured at 20°C and 25°C. The vertical bar indicates LSD at 5% level.

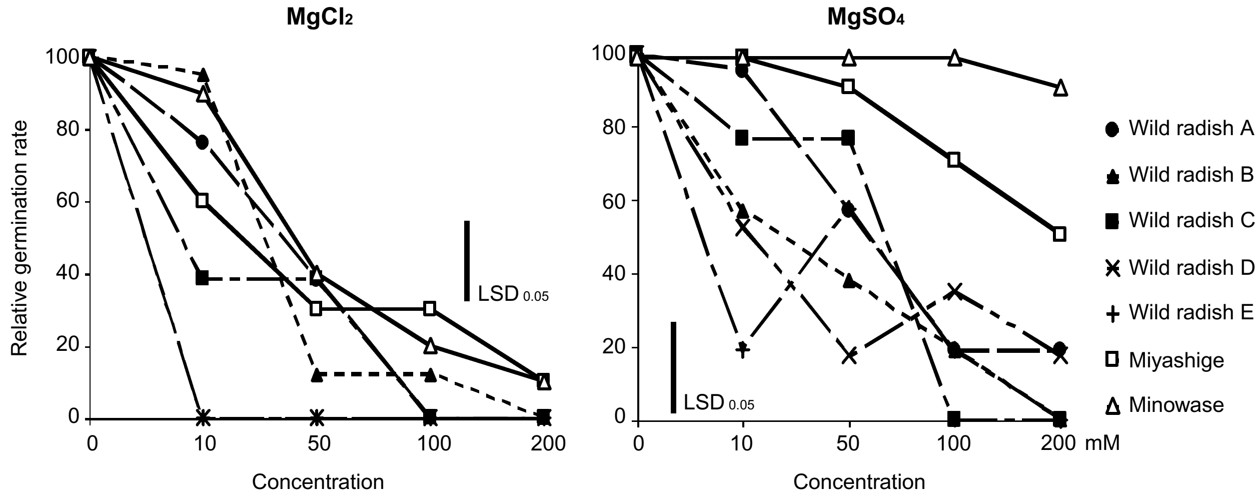

Fig. 3. Comparison of relative seed germination rates under $MgCl_2$ or $MgSO_4$ treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

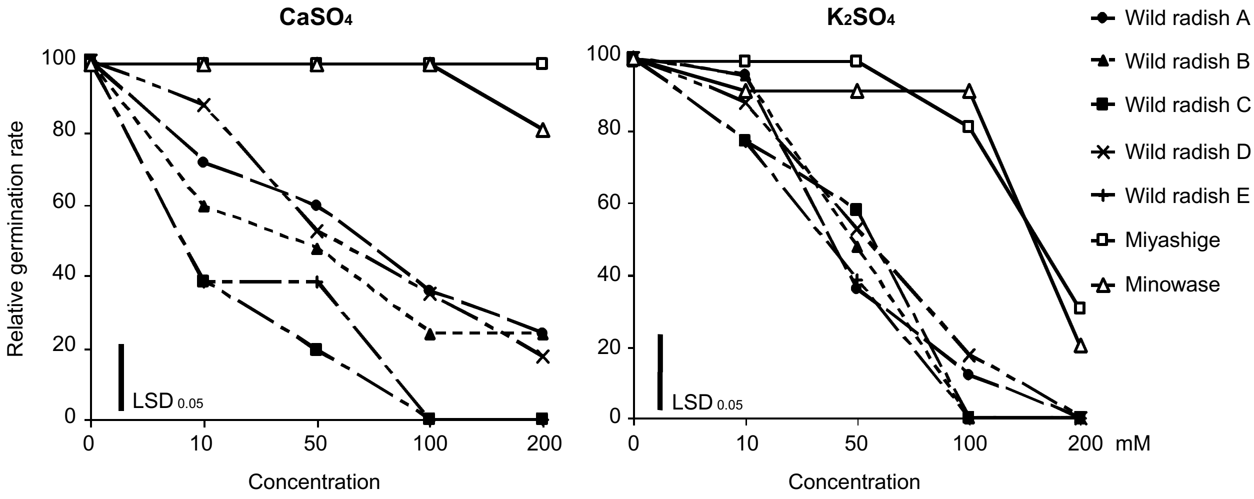

Fig. 4. Comparison of relative seed germination rates under $CaSO_4$ or K_2SO_4 treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

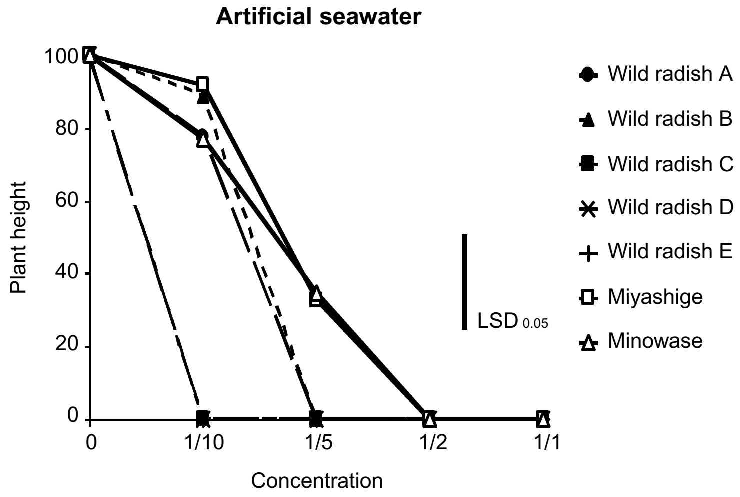

Fig. 5. Comparison of plant heights under the artificial seawater treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

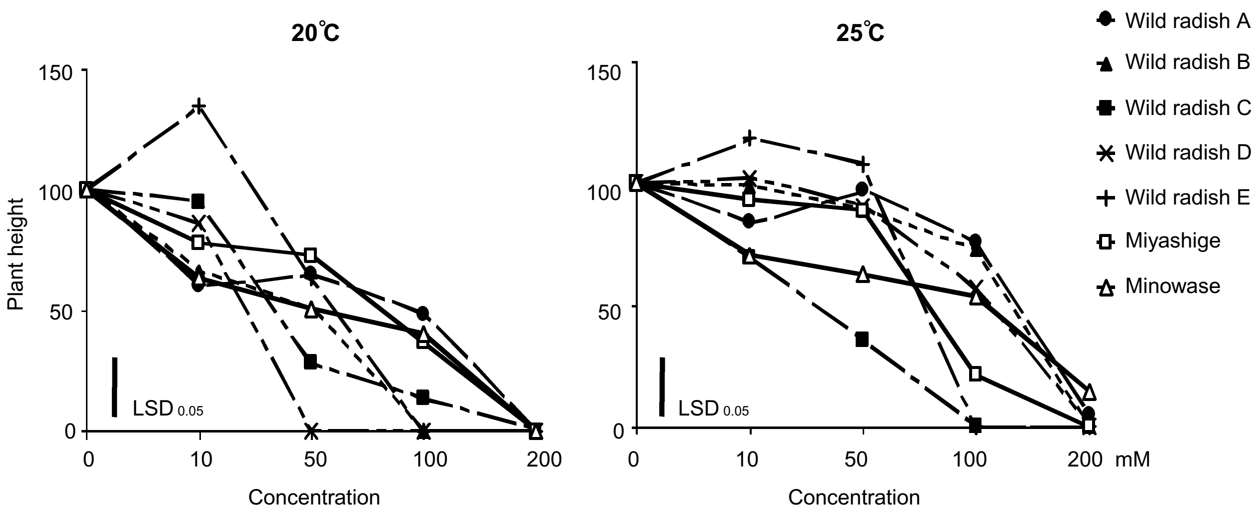

Fig. 6. Comparison of plant heights under NaCl treatment among cultivated and wild radishes cultured at 20℃ and 25℃. The vertical bar indicates LSD at 5% level.

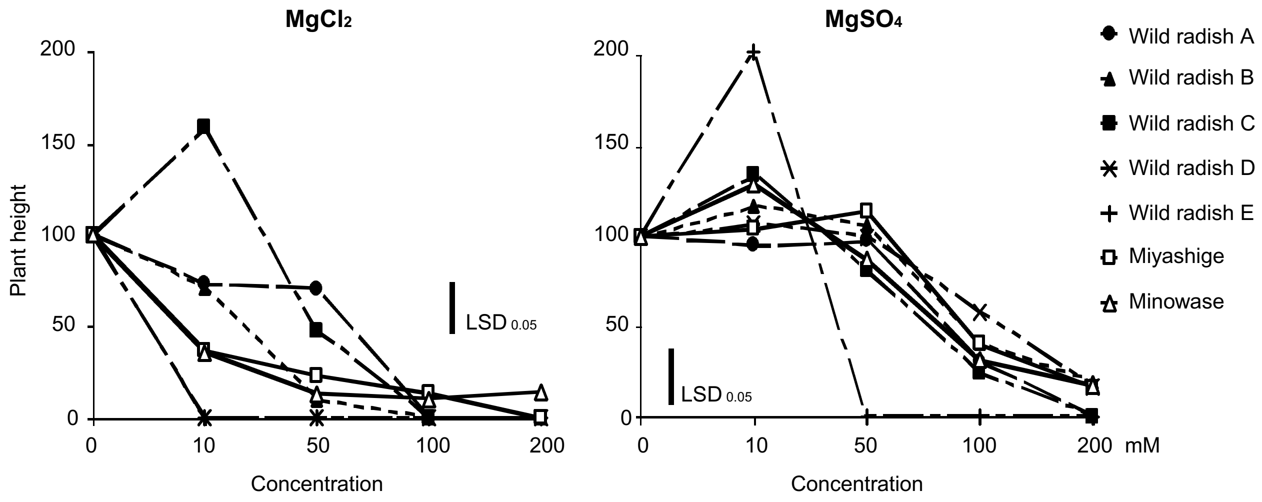

Fig. 7. Comparison of plant heights under $MgCl_2$ or $MgSO_4$ treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

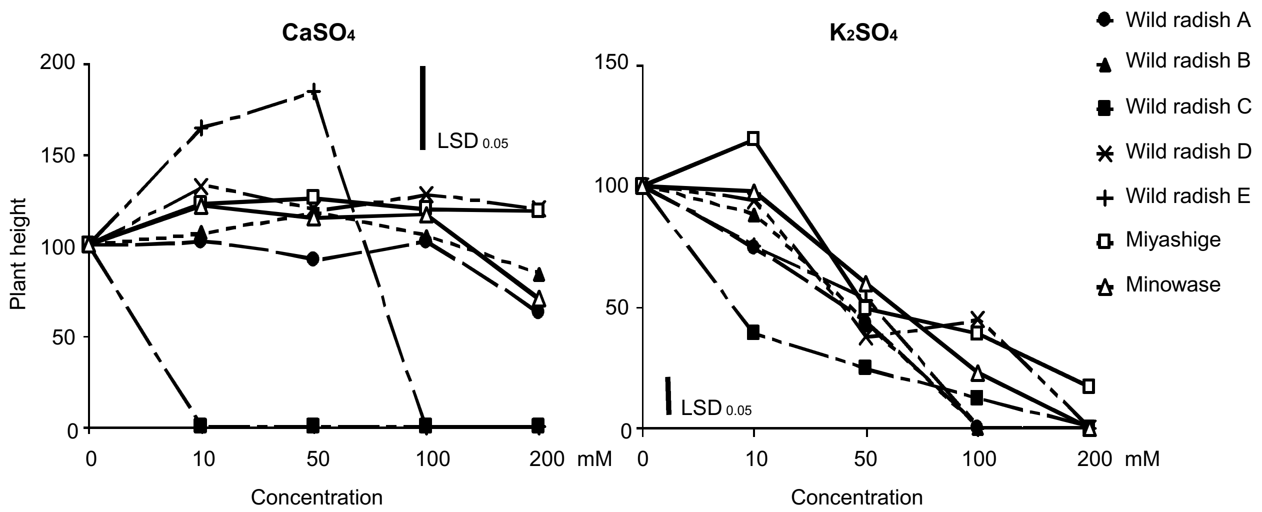

Fig. 8. Comparison of plant heights under $CaSO_4$ or K_2SO_4 treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

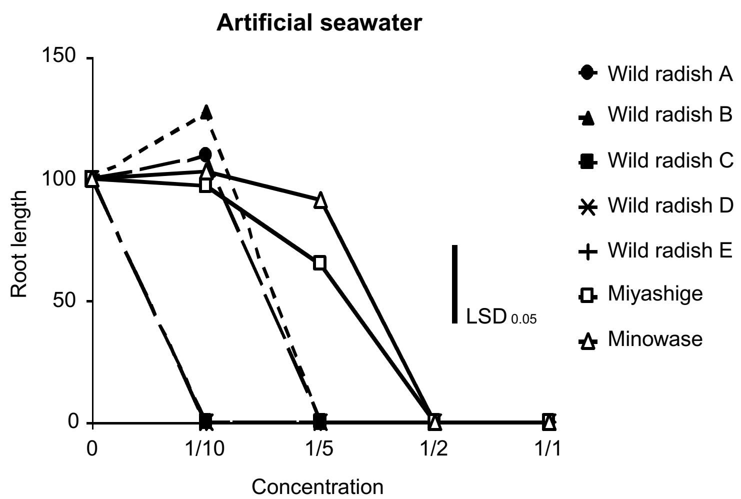

Fig. 9. Comparison of root lengths under the artificial seawater treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

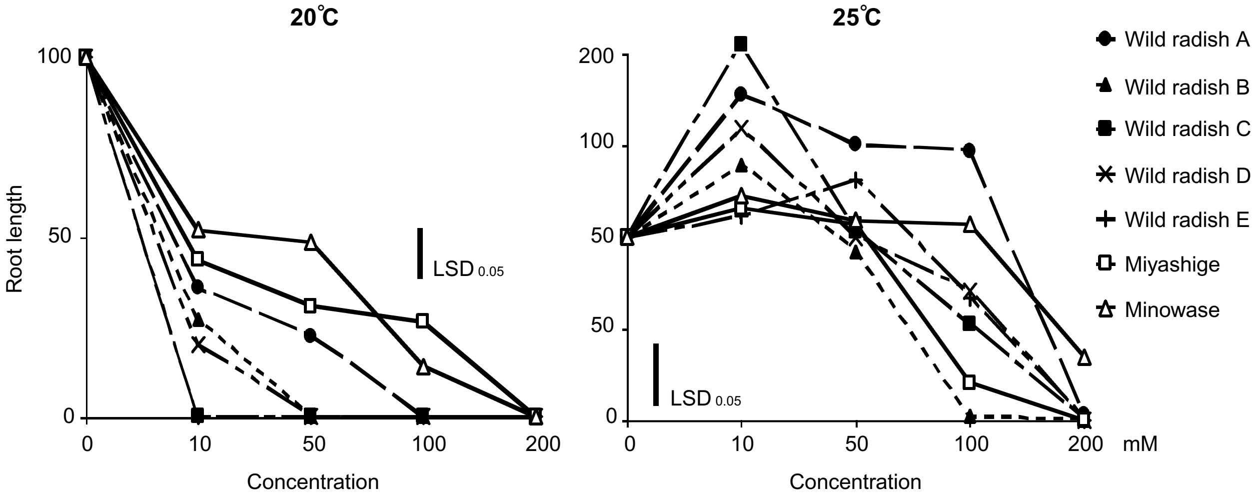

Fig. 10. Comparison of root lengths under NaCl treatment among cultivated and wild radishes cultured at 20℃ and 25℃. The vertical bar indicates LSD at 5% level.

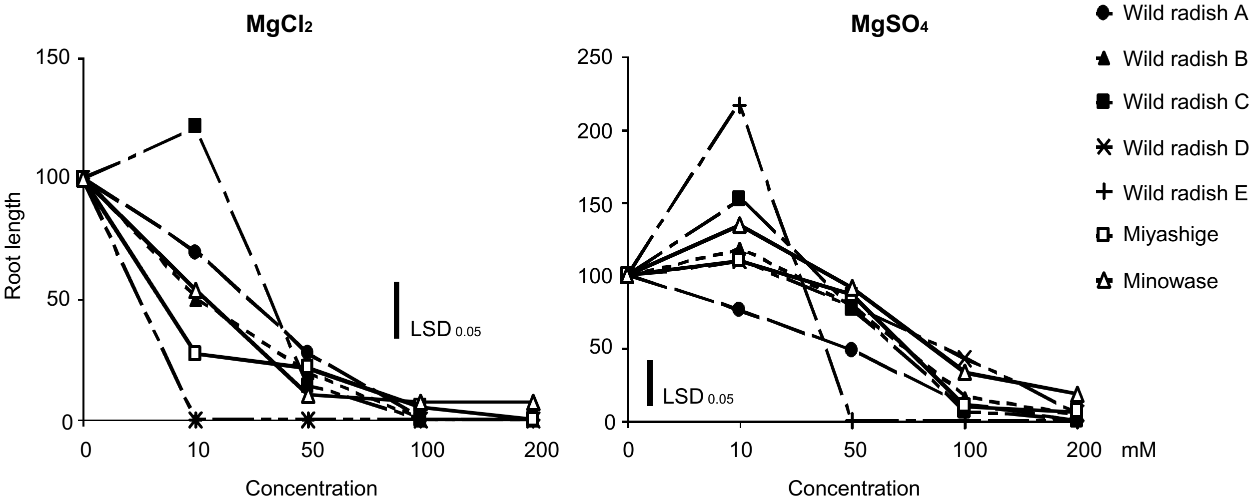

Fig. 11. Comparison of root lengths under $MgCl_2$ or $MgSO_4$ treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

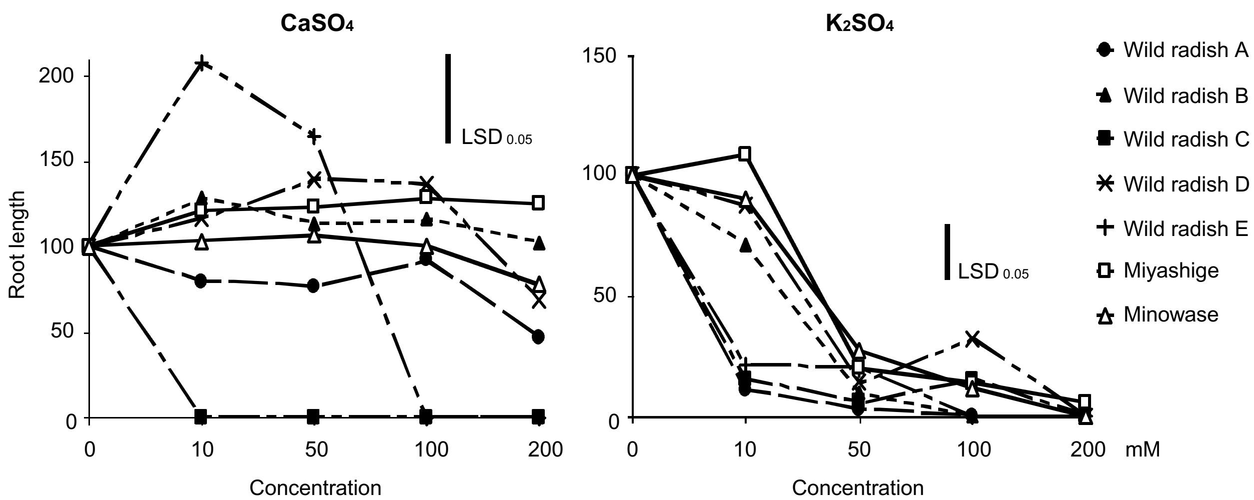

Fig. 12. Comparison of root lengths under $CaSO_4$ or K_2SO_4 treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

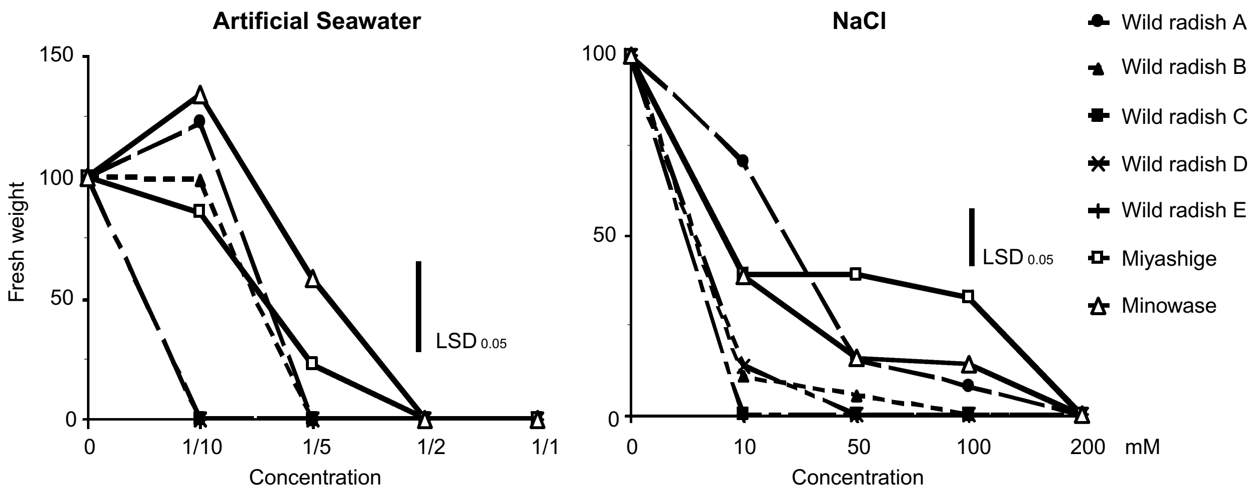

Fig. 13. Comparison of fresh weights under the artificial seawater or NaCl treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

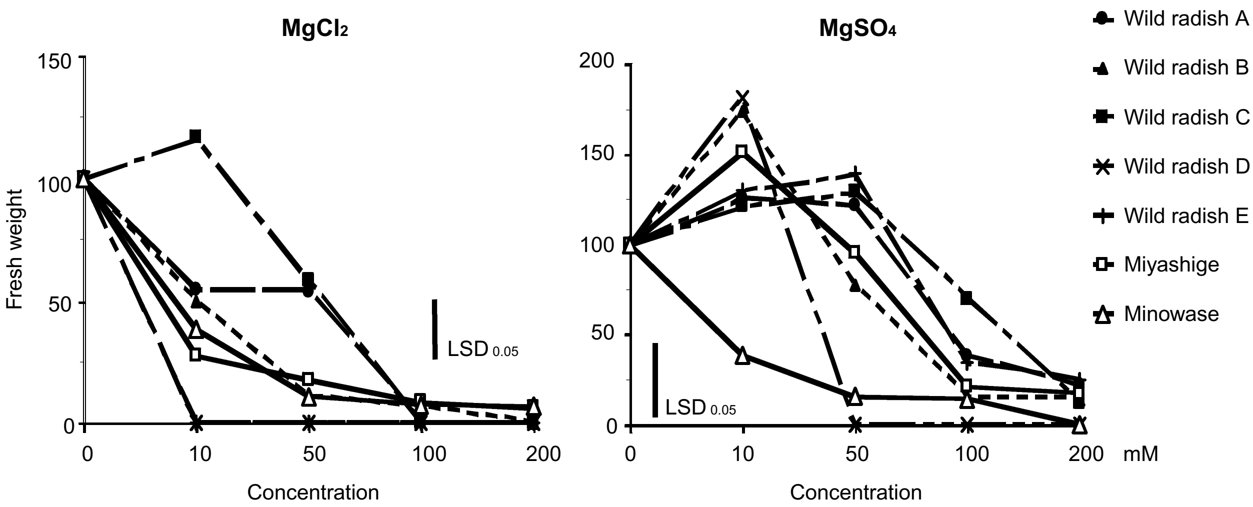

Fig. 14. Comparison of fresh weights under $MgCl_2$ or $MgSO_4$ treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

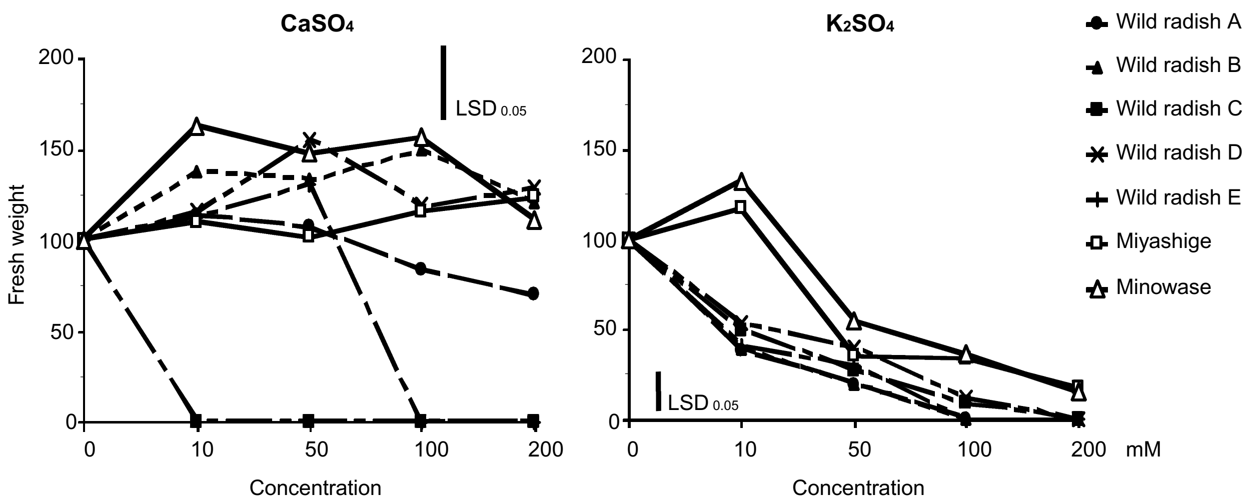

Fig. 15. Comparison of fresh weights under $CaSO_4$ or K_2SO_4 treatment among cultivated and wild radishes. The vertical bar indicates LSD at 5% level.

Table 5. Conditions that the wild radishes showed significantly higher values than the cultivated radishes at 5% levels

Strains	Plant height	Root length	Fresh weight
Wild radish A	$MgCl_2$: 10, 50 mM	$NaCl^{25}$: 10, 50, 100 mM	$NaCl^{20}$: 10 mM $MgCl_2$: 50 mM
Wild radish B	$MgCl_2$: 10 mM		
Wild radish C	$MgCl_2$: 10 mM	$NaCl^{25}$: 10 mM $MgCl_2$: 10 mM	$MgCl_2$: 10, 50 mM $MgSO_4$: 100 mM
Wild radish D		$NaCl^{25}$: 10 mM	
Wild radish E	$NaCl^{20}$: 10 mM $NaCl^{25}$: 10 mM $MgSO_4$: 10 mM $CaSO_4$: 50 mM	$MgSO_4$: 10 mM $CaSO_4$: 10 mM	$MgSO_4$: 50 mM

$NaCl^{20}$, $NaCl^{25}$: cultured at 20℃ and 25℃, respectively.

DISCUSSION

The cultivated radishes, 'Aokubi-Miyashige' and 'Minowase' were proved to possess higher salt tolerance than the wild radishes in seed germination. This result is consistent with the report showing radish's strong tolerance to salinity (Shimose and Hayashi 1983). However, salt tolerances in other characters were detected in the wild radish strains. Wild radish A showed significantly larger root lengths than those of cultivars at 10, 50 and 100 mM NaCl at 25℃ (Table 5). Wild radish C showed significantly larger fresh weights than those of cultivars at 10 and 50 mM $MgCl_2$, and 100 mM $MgSO_4$, but it was sensitive to NaCl and $CaSO_4$. Wild radish E showed significant tolerances of NaCl, $MgSO_4$ and $CaSO_4$ at relatively lower concentrations, such as 10 and 50 mM, in plant height, root length or fresh weight. Therefore, wild radishes could be used for breeding materials of salt tolerance.

Wild radishes showed higher germination rate and growth at 25℃ than those of at 20℃ in NaCl (Fig. 2, 6 and 10). It is suggested that wild radishes would adapt to higher temperature in the presence of NaCl. In other words, it could be expected that the wild radishes' ability of tolerance to salinity would be expressed at higher temperature. Several salt tolerant genes may be expressed depending on temperature. Heat treatment (Musil and Witt 1991) and osmotic condition (Drew and Dearman 1993) should be considered to be effective on germination. Thus, further experiment in 30℃ or over is needed. As the response to salt condition in the wild radishes varied in this study, more variants can be expected for the breeding materials in the wild.

It is reported that Na^+/H^+ vacuolar antiporter gene from *B. napus* (BnNHX1) enhanced salt tolerance in transgenic tobacco plants, which produced as much seeds in the presence of 200 mM NaCl as in the absence of the salt (Wang et al. 2004). Pollen germination percentage in NaCl was reported as a reliable index for whole plant's tolerance to NaCl in oilseed *Brassicas* (Tyagi and Rangaswamy 1993). There could be more accurate indices to evaluate salt tolerance for radish.

The mechanisms for salt tolerance in plants have been described on many aspects. For example, the effect of salt stress occurred in total leaf soluble sugars and a slight increase in total free amino acids in *B. napus* (Qasim et al. 2003). The maintenance of a high K/Na ratio was a mechanism for salt tolerance in *Brassica* species (He and Cramer 1993). It is suggested that the desaturation of fatty acids is responsible for the salt tolerance in tobacco cells during germination period (Kobayashi et al. 2001).

It was described that 'Minowase' is distantly related to the Japanese wild radishes (Yamagishi et al. 1998). It is suggested that the cultivated radishes have been improved their salt tolerance than those of the wild radishes especially in seed germination. However, there were differences among wild radish strains. Tolerances of NaCl, $MgCl_2$, $MgSO_4$ and $CaSO_4$ were detected in some wild radishes. In addition, there must be other factors for the reasons why the Japanese wild radishes can grow in the seashore. This idea needs to be examined further by checking environmental factors, for instance, temperature, wind, light including ultraviolet and so on. The reactions of wild radishes to those factors might offer valuable information to cope with the recent abnormal climate.

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