JIFS, 9 : 21 − 25 (2012) Symposium Paper

"Na-no-hana Project" for Recovery from the Tsunami Disaster by Producing Salinity-Tolerant Oilseed Rape Lines: Selection of Salinity-Tolerant Lines of *Brassica* Crops

Shiori NASU, Hiroyasu KITASHIBA and Takeshi NISHIO*

Graduate School of Agricultural Science, Tohoku University
1-1, Tsusumidori-Amamiyamachi, Aoba-ku, Sendai, 981-8555, Japan
***e-Mail: nishio@bios.tohoku.ac.jp**

Keywords: *Brassica napus*, *Brassica juncea*, *Raphanus sativus*, salt tolerance, rapeseed breeding

Received 2 December 2011; accepted 27 December 2011

Abstract

We tested salinity tolerance of 44 lines of *Brassica napus*, *Brassica juncea*, *Raphanus sativus*, and *Raphanus raphanistrum* using a *Brassica oleracea* line, which has been evaluated to be highly tolerant to salinity, as a control. Plants at the 3-4 leaf stage were cultured in pots containing perlite with liquid culture media containing 0 mM, 50 mM, 100 mM, and 200 mM NaCl for three weeks, and then the dry weight of the aerial part was compared. Since most of the lines showed a decrease of growth in the liquid media containing NaCl, salinity tolerance of each line was represented by a ratio of dry weights of plants grown with NaCl to those of plants grown without NaCl. Although the average dry weight ratio for *Raphanus* was slightly higher than that for *B. napus*, variation within species was much higher than the difference between species. In *B. napus*, N-343 and N-119 showed higher dry weight ratios than 'Kirariboshi', which is commonly grown in Japan, and N-343 had dry weights significantly higher than those of the salinity-sensitive lines in 200 mM NaCl, suggesting N-343 to be highly tolerant to salinity. J-105 in *B. juncea* and 'Izumoorochi' in *R. sativus* also showed high salinity tolerance. Use of these salinity-tolerant lines in oilseed production and as materials for plant breeding is herein discussed.

Introduction

More than 20,000 ha of agricultural land in the Tohoku region was damaged by the tsunami disaster in the wake of the Great East Japan Earthquake, making it difficult to grow crops in these fields without removal of salt from the soil. Until such time, salinity-tolerant plants are required for crop production.

Plants in the genus *Brassica* and closely related genera are distributed on the seashore of the Mediteranean Sea and in deserts in Africa and the Mideast, suggesting that they are tolerant to salinity. As expected, Brassicaceae vegetables including cabbage, Chinese cabbage, and radish have been revealed to be more tolerant than other vegetables tested (Osawa 1961). Wild Brassicaceae plants growing at the seashore have been reported to be highly tolerant to salinity (Takahata and Tshunoda 1981). However, Shimose and Sekiya (1991) have reported that salinity tolerances of Chinese cabbage and rape are low and that that of cabbage is intermediate. These differences may suggest the presence of high variation of salinity tolerance within species.

Since Tohoku University has unique genetic resources of *Brassica* crops and its wild relatives, we investigated the salinity tolerance of many lines in *Brassica napus*, *Brassica juncea*, and *Raphanus sativus* to use these genetic resources as materials for developing salinity-tolerant oilseed rape lines, which could be grown in these fields and used for oil production.

Materials and methods

Nine lines of *Brassica napus*, 30 lines of *Brassica juncea*, four lines of *Raphanus sativus*, one line of *Raphanus raphanistrum*, most of which are maintained as genetic resources in the Brassica Seed Bank in the laboratory of plant breeding and genetics in Tohoku University, were used for evaluating salinity tolerance (Table 1). A line of *Brassica olercea*, O-166, which has been reported to have high tolerance to

Table 1. Dry weights of aerial parts of plants cultured with liquid media containing 0 mM, 50 mM, 100 mM, and 200 mM NaCl

	Dry weight (g)			
Names of lines	0 mM	50 mM	100 mM	200 mM
Brassica napus				
N343	0.92	0.68	0.52	0.44*
N-119	0.77	0.56	0.48	0.32
Kirariboshi	0.85	0.45	0.27	0.23
Kizakinonatane	1.05	0.66	0.57	0.26
Brassica juncea				
J-105	0.52	0.53	0.46	0.26*
J-124	0.70	0.62	0.38	0.25*
J-130	0.50	0.35	0.33	0.28*
J-106	0.57	0.32	0.31	0.14
J-601	0.55	0.32	0.27	0.12
J-473	0.46	0.32	0.27	0.09
Raphanus sativus				
Izumoorochi	1.20	1.29	0.83	0.61
RAP-SAR-40	1.94	1.81	1.05	0.39

* indicates significant difference from dry weights of the salinity-sensitive cultivars at 5% level.

salinity (Takahata and Tsunoda 1981), was used for comparison.

Seeds were sown on wet filter paper in a Petri dish. After expansion of cotyledons, four plantlets were transplanted into a plastic pot with a diameter of 19 cm and a height of 14.4 cm (1.6 L capacity) containing perlite 1 to 3 mm in diameter. Plants were grown with a liquid culture medium containing 0.75 g Aminohouse No. 1, 0.5 g Otsuka House No. 2, and 0.025 g Otsuka House No. 5 (Otsuka AgriTecno Co. Ltd. Japan) per liter, which is estimated to contain 2.12 mM NO_3, 0.42 mM P_2O_5, 2.17 mM K_2O, 2.05 mM CaO, 0.74 mM MgO, 0.01 mM MnO, 0.05 mM Fe, 0.01 mM B_2O_3, 0.001 mM Zn, 0.0004 mM Cu, and 0.0003 mM Mo. When the surface of perlite was dry, the plants were supplied with 50 ml liquid culture medium. From the 3– to 4-leaf stage, plants were cultured with the liquid culture media containing 0, 50, 100, and 200 mM NaCl. When the surface became dry, 100 ml liquid culture media were supplied. Twenty-one days after treatment with the culture media containing NaCl, aerial parts were air-dried at 85 C for ten days and weighed.

Results

Although dry weights of J-105 in *B. juncea* and 'Izumoorochi' in *R. sativus* treated with 50 mM NaCl were slightly higher than those without NaCl, most of the lines showed a decrease of growth in the liquid media containing NaCl (Fig. 1). Salinity tolerance of each line was represented by the dry-weight ratio, i.e., the ratio of dry weights of plants grown with NaCl to those of plants grown without NaCl. Average dry-weight ratios of *B. napus* in the 50 mM, 100 mM, and 200 mM NaCl treatments were 0.64, 0.49, and 0.36, respectively. Average dry-weight ratios of *B. juncea* in 50 mM, 100 mM, and 200 mM NaCl treatments were 0.62, 0.51, and 0.31, respectively, and those of *Raphanus* in the 50 mM, 100 mM, and 200 mM NaCl treatments were 0.74, 0.53, and 0.40, respectively. In the 50 mM NaCl treatment, average dry-weight ratio of *Raphanus* was somewhat higher than that of *B. napus*, but the difference was not significant because of high varietal differences within these species. Dry-weight ratios of O-166 in *B. oleracea*, which has been evaluated to be highly salinity tolerant (Takahata and Tsunoda 1981), in 50 mM, 100 mM, and 200 mM NaCl treatments were 0.71, 0.52, and 0.32, respectively.

In *B. napus*, differences of dry-weight ratios among nine lines in the 200 mM NaCl treatment were significant ($p<0.05$). Among them, N-343 showed the highest dry-weight ratios of 0.74 and 0.48 in 50 mM and 200 mM NaCl, respectively. In 100 mM NaCl, N-119

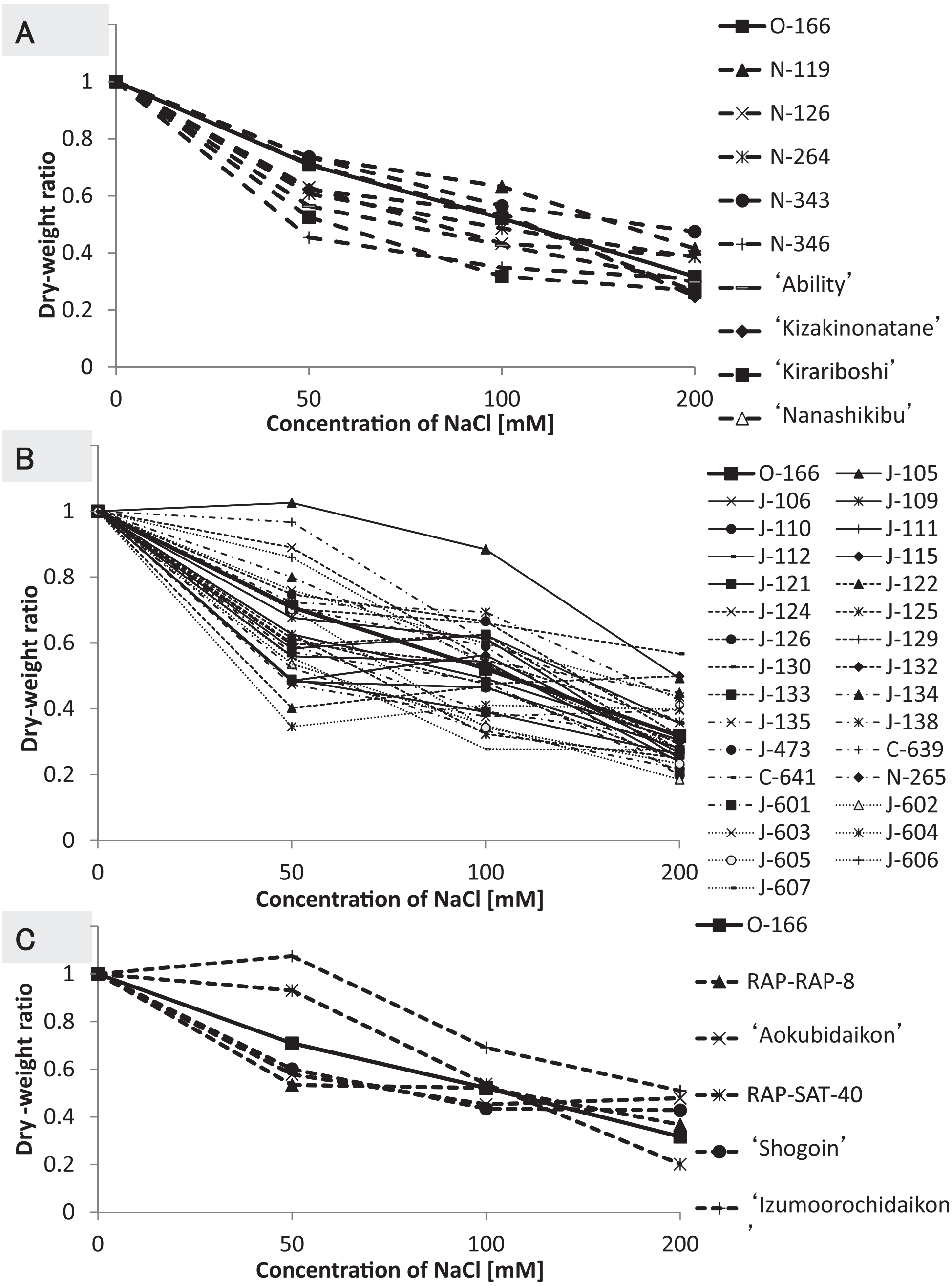

Fig. 1. Salinity tolerance of plants in *B. napus* (A), *B. juncea* (B), and *Raphanus* (C) represented by dry weight ratios of the aerial parts.
O-166 in *Brassica olercea*, which has been reported to have high tolerance to salinity, was used for comparison.

showed the highest value. The dry-weight ratios of these lines were higher than those of O-166 for all the concentrations of NaCl.

In *B. juncea*, significant differences ($p<0.05$) of dry-weight ratios among the tested lines were observed in the 100 mM and 200 mM NaCl treatments. In 200 mM NaCl, the difference between the highest and the lowest dry-weight ratios was quite large, i.e., 0.57 in J-130 and 0.21 in J-601. The line J-105, which showed slightly higher growth in 50 mM NaCl than that in 0 mM NaCl, showed the highest dry-weight ratio, 0.88, also in 100 mM NaCl and the third highest value, 0.50, in 200 mM NaCl. J-105 was considered to be the most salinity-tolerant line among the tested *B. juncea* lines.

'Izumoorochi' in *R. sativus* exhibited high dry-weight ratios in all the tested NaCl concentrations, i.e., 1.08, 0.69, and 0.51 in 50 mM, 100 mM, and 200 mM

NaCl, respectively. The average dry-weight ratios of 50 mM, 100 mM, and 200 mM NaCl were the highest in 'Izumoorochi' among all the examined lines of *B. napus*, *B. juncea*, and *Raphanus*. Significant differences of dry-weight ratios between lines in *Raphanus* were not observed in any tested NaCl concentrations.

Some lines showing high dry-weight ratios had low dry weights in the control with 0 mM NaCl. Therefore, dry weights of aerial parts of the selected lines were compared with those of lines with low salinity tolerance (Table 1). The dry weight of N-343 was comparable to those of the salinity-sensitive lines, i.e., 'Kirariboshi' and 'Kizakinonatane', in the control, and N-343 had a significantly higher dry weight ($p<0.05$) in 200 mM NaCl than those of the salinity-sensitive lines. On the other hand, the dry weights of N-119 in the control were lower than those of the salinity-sensitive lines. In *B. juncea*, J-105, J-124, and J-130 had significantly higher dry weights than those of salinity sensitive lines, i.e., J-106, J601, and J-473, in 200 mM NaCl, and dry weights of J-105, J-124, and J-130 in 0 mM NaCl were comparable to those of the salinity sensitive lines.

Discussion

Salinity tolerance evaluated with dry-weight ratios in *Raphanus* seemed to be higher than *B. napus*, as reported by Shimose and Sekiya (1990), but varietal differences within a species were too high to reveal the difference between these two species. Most of the Brassicaceae crops are allogomous plants, some of which have self-incompatibility (Kitashiba and Nishio 2010), and are therefore considered to have high variation in many genetic traits in a species. High variation within a species of *B. napus*, *B. juncea*, and *R. sativus* was found in the salinity tolerance trait. In *B. juncea*, high variation of salinity tolerance in a species has been revealed, and salinity tolerant lines have been developed in India (Purty et al. 2008). Data on salinity tolerances of different plant species without names of cultivars or lines in the Brassicaceae crops should be handled with care.

N-343 and N-119 in *B. napus* showed high dry-weight ratios. N-343 is a rutabaga cultivar, and not cultivated for rapeseed production. Evaluation of seed yield of N-343 is required. N-119 is an old Japanese cultivar of rape grown in the Tohoku region. Therefore, N-119 may be useful for rapeseed production in fields damaged by the tsunami. As a breeding material for salinity tolerance, N-343 is considered to be promising.

J-105 was found to be the most salinity-tolerant line among *B. juncea* lines examined in the present study. This line was provided to the Tohoku University Brassica Seed Bank by Takii Seed Co. under the name "long standing mustard" in 1943. There is no other description of this line. The plant looked like a leafy vegetable. Evaluation of seed yield is required for using it in oilseed production.

'Izumoorochi' was found to have the highest salinity tolerance among the lines examined in the present study. 'Izumoorochi' is a selected line having thick roots from a wild radish, "Hamadaikon", which grows on seashores in Japan (Ban et al. 2009). Some plants of Hamadaikon can grow on the beach near seawater. There is a possibility that a plant having much higher salinity tolerance than 'Izumoorochi' is found in Hamadaikon. However, *R. sativus* is mainly used as a root crop and not so much as an oil crop. It has strong self-incompatibility, and yield of seeds is much lower than *B. napus* and *B. juncea*. Salinity-tolerant *R. sativus* lines cannot be used directly for producing oil. Incorporation of salinity-tolerance genes of the salinity-tolerant *R. sativus* lines into *B. napus* or *B. juncea* is required. Since *R. sativus* can be crossed with *Brassica* species, genetic traits in *R. sativus* can be used in *Brassica* breeding by conventional cross breeding. Cytoplasm of *R. sativus* has been used for developing cytoplasmic male-sterile lines in *Brassica* (Koizuka et al. 2003), and single-chromosome addition lines in *B. napus* with *R. sativus* chromosomes have been developed (Akaba et al. 2009). Since genome research on *R. sativus* is actively performed (Li et al. 2011), DNA markers linked with a gene responsible for salinity tolerance will be eventually identified.

Salinity tolerance evaluated in the present study is of the young plant stage. Salinity tolerance of each line may change depending on plant stage. In fact, some accessions in *Arabidopsis thaliana* showing high salinity tolerance at the germination stage have been reported to be highly sensitive to salinity stress during vegetative growth (Quesada et al. 2002). Furthermore, lines with a low yield of seeds are not useful, even if they have high salinity tolerance. Therefore, field trials are indispensable for selecting salinity tolerant lines for oilseed production. We selected ten lines and are presently growing them in fields with high salinity. Assessing salinity tolerance, seed yield, adaptability, plant biomass, and uptake of salts, we will select a line usable as a rapeseed line for oil production in these fields and as a material for breeding of a more promising salinity-tolerant high-yield rapeseed line.

Acknowledgement

This study was performed under Rapeseed Project for Restoring Tsunami-Salt-Damaged Farmland supported by Implementation-Support Program of JST.

References

Akaba, M., Y. Kaneko, Y. Ito, Y. Nakata, S.W. Bang and Y. Matsuzawa (2009) Production and characterization of *Brassica napus-Raphanus sativus* monosomic addition lines mediated by the synthetic amphidiploid "*Raphanobrassica.*" Breed. Sci., 59: 109-118.

Ban, T., N. Kobayashi, H. Hontani, M. Kadowaki and S. Matsumoto (2009) Domestication and utilization of Japanese wild radish. Hort. Res., 8: 413-417 (in Japanese with English Abstract).

Koizuka, N., R. Imai, H. Fujimoto, T. Hayakawa, Y. Kimura, J. Kohno-Murase, T. Sakai, S. Kawasaki and J. Imamura (2003) Genetic characterization of a pentatricopeptide repeat protein gene, orf687, that restores fertility in the cytoplasmic male-sterile Kosena radish. Plant J., 34: 407-415.

Kitashiba, H. and T. Nishio (2009) Self-incompatibility. In: Biology and Breeding of Crucifers (ed. by Gupta), CRC Press, New York, pp. 99-112.

Li, F., Y. Hasegawa, M. Saito, S. Shirasawa, A. Fukushima, T. Ito, H. Fujii, S. Kishitani, H. Kitashiba and T. Nishio (2011) Extensive chromosome homoeology among Brassiceae species were revealed by comparative genetic mapping with high-density EST-based SNP markers in radish (*Raphanus sativus* L.). DNA Res., 18: 401-411.

Osawa, T. (1961) Studies on the salt tolerance of vegetable crops in sand culture. Journal of Japanese Society of Horticultural Science, 30: 53-64.

Purty, R. S., G. Kumar, S. L. Singla-Pareek and A. Pareek (2008) Towards salinity tolerance in Brassica: an overwiew. Physiol. Mol. Plants, 14: 39-49.

Quesada, V., S. Garcia-Martinez, P. Piqueras, M. R. Ponce and J. L. Micol (2002) Genetic architecture of NaCl tolerance in Arabidopsis. Plant Physiol., 130: 951-963.

Shimose, N. and J. Sekiya (1990) Salt tolerance of higher plants and uptake of inorganic nutrients. Science Reports of Faculty of Agriculture, Okayama University, 77: 21-29.

Takahata, Y. and S. Tsunoda (1981) Difference in salt tolerance among species of *Brassica* and allied genera with special reference to their photosynthetic response. Japanese Journal of Breeding, 31: 383-394.