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# Potential of the Common Ice Plant, *Mesembryanthemum crystallinum* as a New High-Functional Food as Evaluated by Polyol Accumulation

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**Abstract** : We measured the concentration of polyols (pinitol, ononitol, and myo-inositol), which are known to have health-promoting and/or disease-preventing functions, in the common ice plant (*Mesembryanthemum crystallinum* L.) cultured under salt- and drought-stressed treatments. In NaCl-treated plant the concentration of pinitol/ononitol increased with increasing NaCl concentration in culture solution. The maximal concentration was 3.6 mg g<sup>-1</sup> FW, which was found in the shoot top, followed by small side shoots (2.1 mg g<sup>-1</sup> FW) of mature plants grown with 400 mM NaCl for 35 ds. The drought stress also accelerated the accumulation of pinitol/ononitol. The maximal concentration was 1.2 mg g<sup>-1</sup> FW, which was found in the shoot top of plants under the stress for 25 ds. The myo-inositol increased in salt-stressed plants at 3 ds after the start of the treatment and then decreased with the lapse of time during stress. The concentration of polyols in the ice plant was comparable to that in the other species reported to accumulate polyols at high levels. Radical scavenging activity evaluated by DPPH assay was increased two-fold by 400 mM NaCl treatment, which was twice as high as that in the leaves of lettuce (*Lactuca sativa* L.). These results indicated the high potential of the ice plant as a polyol-rich high-functional food.

**Key words** : Common ice plant, Myo-inositol, Pinitol, Salt stress.

Drought and soil salinization are the most important factors limiting modern agricultural settings. The utilization of halophytic plants as pasture and fodder (Yeo and Flowers, 1980) or cleaning crop would be an economic solution in such harsh environmental conditions. The common ice plant, *Mesembryanthemum crystallinum* L. is an annual plant native to the Nambian desert in southern Africa and is widely distributed and naturalized in western Australia, southwestern US, the Pacific coast of Mexico and Chile (Bohnert and Cushman, 2000). This plant has high tolerance to drought and salinity, and can complete its life cycle on the soil containing NaCl at a concentration equivalent to that in seawater (ca. 500 mM) (Adams et al., 1998). This species has been utilized as a vegetable in some European countries, but has never been used in Japan. We recently established a stable cultivation system for ice plant to meet the demand as a commercial product, and have sold the ice plant as a vegetable under the registered trademark, “Crystal leaf” or “Barafu”.

In response to osmotic stresses caused by drought and salinity, plants generally accumulate compatible solutes such as sugars, amino acids, polyols, betaines, and ectoines in cytoplasmic compartments (Bohnert

and Shen, 1999). In Pinaceae, Leguminosae and Caryophyllaceae, D-pinitol (3-O-methyl- D-chiro-inositol) is a major solute (Nguyen and Lamant, 1988) and its accumulation is commonly thought to be beneficial for stress adaptation, stabilizing membranes and proteins against osmotic alterations (Jennings and Burke, 1990; Popp and Smirnov, 1995). In *M. crystallinum*, major compatible solutes are proline and polyols (myo-inositol, pinitol and ononitol) in stressed plants. In some cases, pinitol constituted 71% of the soluble carbohydrate fraction and 9.7% dry weight in the long-term stressed leaves (Paul and Cockburn, 1989).

Pinitol biosynthesis occurs through a two-step conversion of myo-inositol (Dittrich and Brandl, 1987). The methyl group of S-adenosyl-L-methionine is first transferred to myo-inositol by inositol methyl transferase (IMT) resulting in the production of ononitol (1-D-4-O-methyl myo-inositol). Ononitol is then converted to pinitol by an epimerase that has yet to be isolated. The genes encoding myo-inositol 1-phosphate synthase (*Inps1*) and myo-inositol O-methyltransferase (*Imt1*) have been isolated from *M. crystallinum* (Rammesmayr et al., 1995) and these genes are transcriptionally induced in response to

water stresses in this halophyte. The genome of the glycophyte, *Arabidopsis thaliana*, lacked *Imt1* and *Inps1* was not upregulated in response to salt stress. Ishitani et al. (1996) suggested that the induction of myo-inositol synthesis is a factor distinguishing halophytes from glycophytes. The transgenic tobacco conferring *Imt1* from *M. crystallinum* increased accumulation of the methylated inositol D-ononitol, and increased salt and drought tolerance, which was evaluated by less inhibitory photosynthetic CO<sub>2</sub> fixation during stress and faster recovery in the stressed plants during de-stress treatment.

Recently, D-pinitol has raised pharmacological interest as a food supplement because of its insulin-like function. Narayanan et al. (1987) showed that administration of pinitol decreased plasma glucose in normal and diabetic albino mice, and Kang et al. (2006) suggested that soybean-derived pinitol was useful in controlling the increase in blood glucose in type 2 diabetic patients who were poorly controlled with hypoglycemic drugs, such as sulfonylurea, metformin and/or insulin. Myo-inositol has been implicated in a variety of cellular processes including growth regulation, membrane biogenesis, signal transduction, ion channel physiology, and membrane dynamics (Loewus and Murthy, 2000). This compound has also been used for medical purposes such as the treatment of liver disease, depression, panic disorder, diabetic neuropathy, and respiratory distress syndrome. In addition to these functions, polyols also function as oxygen radical scavengers. For example, mannitol was found in vitro to act as a scavenger of reactive oxygen species (Shen et al., 1997), thereby protecting the proteins from oxidative damage in drought stressed plants. Smirnoff and Cumbes (1989) found that mannitol, sorbitol, glycerol, ononitol and pinitol were all active scavengers. However, free radical scavenging activity, which is evaluated as typical properties of functional foods, has not been investigated in *M. crystallinum*.

The aim of this study is to clarify the possibility of *M. crystallinum* as a functional food with high capacity for accumulation of health-promoting and/or disease-preventing compounds, polyols and determine proper culture conditions to increase accumulation of the compounds. We have cultured *M. crystallinum* under salt- and drought-stress conditions in which polyol synthesis was induced, and measured concentrations of polyols in different organs.

## Materials and Methods

### 1. Water culture and NaCl treatment

The common ice plant was cultured according to previous report (Agarie et al., 2007). NaCl was added to the culture solution at 0, 100, 200 and 400 mM when plants reached the growth stage of 4th leaf (around 40 ds after sowing). The ice plant developed



Fig. 1. Tissues of *M. crystallinum* used for polyol analysis. From left to right, large leaves consisted of 3rd and 4th primary leaves, small leaves consisted of 5th and 6th primary leaves and leaves of side shoots, large stems developing between 3rd and 4th lower leaf pairs, small stems of side shoots developing between upper leaf pairs, and shoot top contained apex of primary axis with the youngest leaf pairs. Scale bar indicates 1 cm.

one primary leaf pair per wk up to seven pairs, and at around 6 wks after germination, the growth of primary axis terminated, and the mature stems and leaves start to appear as side shoots from the axes of the primary leaves. Therefore, at 1, 3, 5 ds after the start of treatment, the 4th and 5th leaves were harvested, and at 10 ds and 15 ds side shoots and shoot top were harvested. At 25, 35, and 45 ds after the start of treatment, the total aerial parts of the plants were divided into five parts: 1) shoot top containing the apex of primary axis with the youngest leaf pairs, 2) small stems of side shoots developing between upper leaf pairs, 3) large stems developing between 3rd and 4th primary leaf pairs, 4) large leaves consisted of 3rd and 4th primary leaves, and 5) small leaves consisted of 5th and 6th primary leaves and leaves developing on side shoots (Fig. 1). They were excised and immediately soaked in the liquid nitrogen, and the frozen samples were preserved at  $-80^{\circ}\text{C}$  until use. For analysis of antioxidant activities (see below), fresh and healthy butterhead lettuce (*Lactuca sativa* L., var. *capitata*) and leaf lettuce (*L. sativa* L., var. *crispa*) were obtained from a local supermarket. Tissues were preserved as described above.

### 2. Soil culture and dehydration treatment

The soil culture was carried out at a greenhouse in Saga University. Seeds were sterilized and sown on MS agar as described by Agarie et al. (2007). The plants were transferred to 4 L plastic pots filled with 1:1 mixture of vermiculite and peat moss, and irrigated with 0.5 × mixture of Otsuka House solution No.1 and No.2 once or twice a week. When plants reached the growth stage of 4th leaf (around 40 ds after sowing), they were divided into two groups, control watered as described above and water-stressed dehydrated by termination of the irrigation. At 10 ds after withdrawing water, the 4th and 5th leaves and side shoots were harvested, and at 15 ds after the start

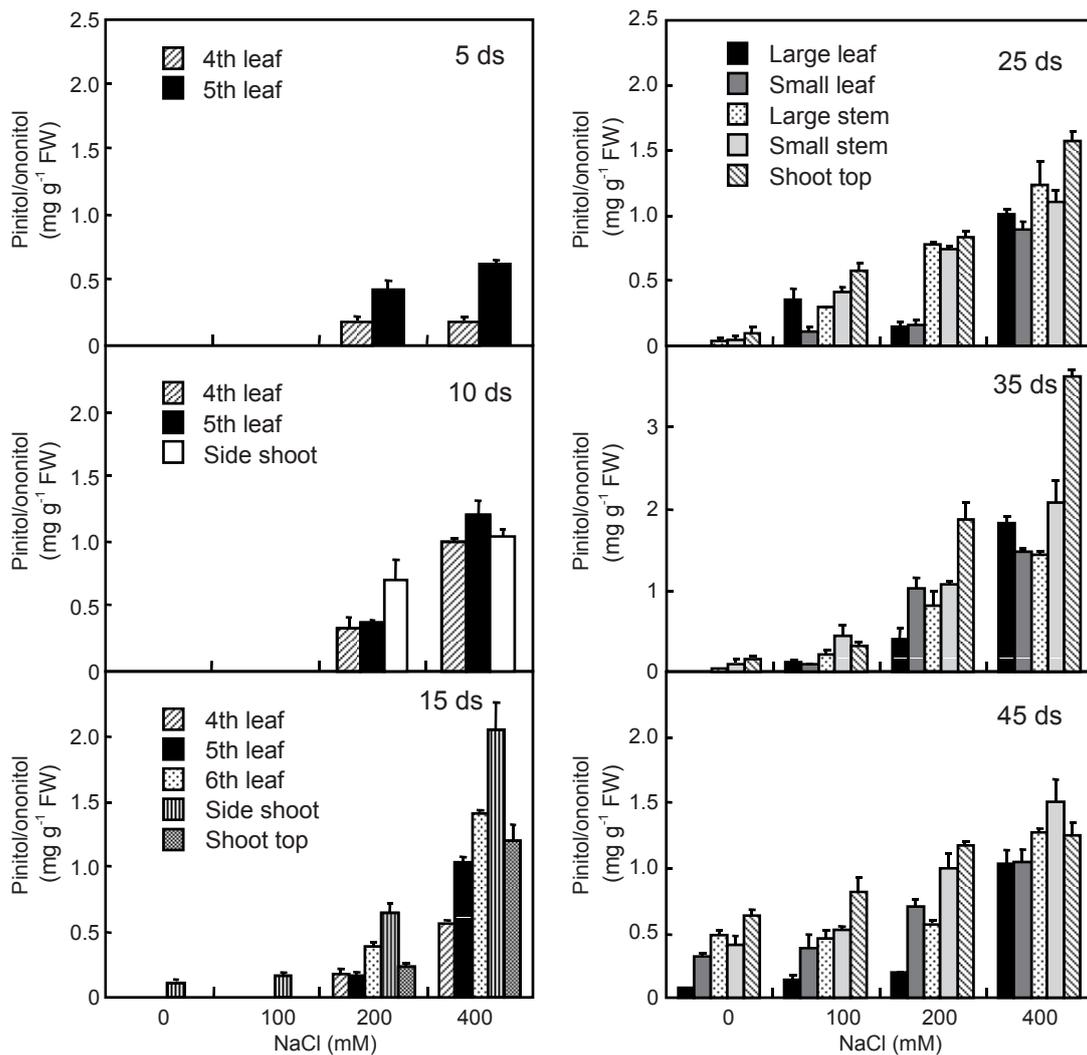


Fig. 2. Effect of salt stress on pinitol/ononitol accumulation in *M. crystallinum*. 5 ds, 10 ds, 15 ds, 25 ds, 35 ds and 45 ds indicate stress duration. Values are means of three duplicates. Vertical bars indicate the standard error of the mean.

of treatments shoot top were harvested additionally. At 25, 35, and 45 ds, the total aerial parts of the plants were divided into five parts as in NaCl treatment. Tissues were immediately soaked in the liquid nitrogen, and the frozen samples were preserved at  $-80^{\circ}\text{C}$  until use.

### 3. Extraction and determination of polyols

Frozen tissues were ground in liquid nitrogen and extracted in 80% (v/v) ethanol at  $80^{\circ}\text{C}$  for 5 min. The homogenate was centrifuged at  $10000\text{ g}$  for 10 minutes, and the supernatant was dried at  $50^{\circ}\text{C}$  under vacuum. Following ethanol extraction tissue pellets were re-suspended in sterile distilled water and were filtered through the  $0.45\text{ }\mu\text{m}$ -pore PTFE membrane (MILLIPORE). The filtrate was analyzed on a Shimadzu HPLC system (LC-10A system, Shimadzu, Japan) equipped with a guard column (Shim-pack SCR-N,  $4.0\text{ mm}\times 50\text{ mm}$ ; Shimadzu, Japan) connected

to an analytical column (Shim-pack SCR-101N,  $7.9\text{ mm}\times 300\text{ mm}$ ; Shimadzu, Japan) and a reflective index detector (RID-6A; Shimadzu, Japan). The columns were heated to  $80^{\circ}\text{C}$ , and MilliQ water as the mobile phase was allowed to flow at the rate of  $0.8\text{ }\mu\text{L min}^{-1}$ . The injection volume was set at  $50\text{ }\mu\text{L}$ . Standard D-pinitol and myo-inositol solutions were prepared in MilliQ water in the range of  $1\text{-}5,000\text{ }\mu\text{g mL}^{-1}$ . From the HPLC profile, D-pinitol and myo-inositol could be quantified. The concentration was calculated by an absolute calibration curve method using commercial D-pinitol and myo-inositol as standard. The concentration was corrected using recovery ratio determined by standard addition.

### 4. Antioxidant activity

Leaves ( $0.1\text{ g}$ ) were homogenized in 50% ethanol, and the homogenate was centrifuged at  $3000\text{ g}$  for 10 minutes. The supernatant was used for the

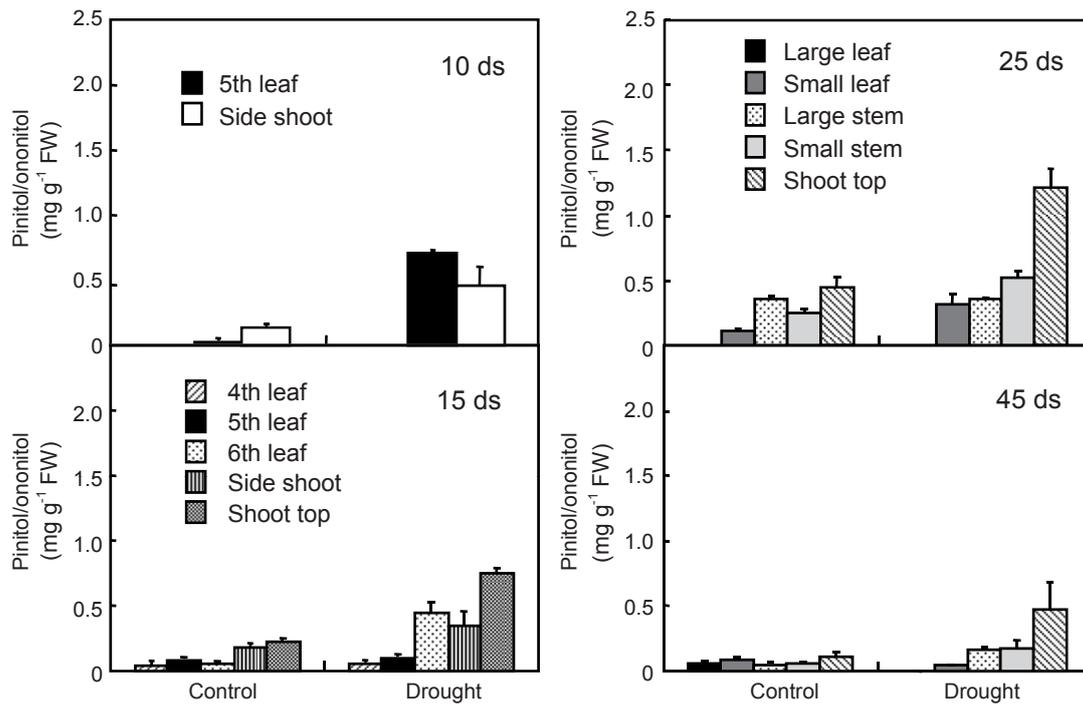


Fig. 3. Effect of drought stress on pinitol/ononitol accumulation in *M. crystallinum*. 10 ds, 15 ds, 25 ds and 45 ds indicate stress duration. Values are means of three duplicates. Vertical bars indicate the standard error of the mean.

determination of antioxidant activities, which were assessed on the basis of their abilities to scavenge the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical (Blois, 1958). The leaf extracts (0–100  $\mu$ L) were added to the reaction mixture DPPH dissolved in ethanol and MES (pH 6.0), and the reaction mixture based on diluted to a final volume of 1.2 mL contained 50  $\mu$ M DPPH, 25mM MES (pH 6.0), and then held at room temperature for 2 min. Absorbance was measured at 520 nm (A520) using UV-visible recording spectrometer (UV-160A, Shimadzu, Japan). The scavenging activities of DPPH were expressed as volumes of extracts required giving a 50% reduction in A520 relative to that of the control contained DPPH without extract.

## Results

### 1. Pinitol/ononitol accumulation in salt- and drought-stressed ice plant

The peak of HPLC chromatogram for extract was the same as that of the commercial D-pinitol. The chemical characteristics of D-pinitol and D-ononitol are similar, and thus in this study the metabolite peak was indicated as pinitol/ononitol, as in the other report which analyzed polyols in different cell types of plant and suspension culture cells of the ice plant (Adams et al., 1992). The correlation between the peak area of chromatogram (A) and concentration of standard (S) was given by a regression:  $A=49322S$  ( $r^2=1.0$ ). The recovery rate determined by standard addition method

was 91.0%, and the concentration was corrected using this value.

Pinitol/ononitol was accumulated in the salt-stressed plants (Fig. 2). The concentration of pinitol/ononitol increased with increasing NaCl level. During the first 10 d of salt stress, pinitol/ononitol increased only in the plants treated with 200 mM and 400 mM NaCl, and not in control and 100 mM NaCl. At 15 d after the start of treatment pinitol/ononitol started to accumulate in the control and 100 mM NaCl, but the pinitol/ononitol concentration in stems were 5% and 7.6% of those in the plant grown with 400 mM NaCl, respectively. At this growth stage the concentration varied among organs, exhibiting the following descending order: side shoot > 6th leaf > shoot top > 5th leaf > 4th leaf. In the tissues of shoot top, small stems, large stems, large leaves and small leaves harvested at 25, 35, and 45 ds after treatment, overall concentration of pinitol/ononitol also increased with strength of salinity. In the plants grown with 400 mM NaCl for 35 ds, the concentration was highest in the shoot top (3.6 mg g<sup>-1</sup> FW), followed by those in small stems.

In the drought-stressed plants the pinitol/ononitol was not detected at 1, 3 and 5 ds after the start of treatment (data not shown). At 10 ds, however, pinitol/ononitol accumulated in both control and drought-stressed plants was higher in drought-stressed plants (Fig. 3). The concentration of pinitol/ononitol was higher in younger tissues, especially in shoot top. The concentration varied among tissues in the following

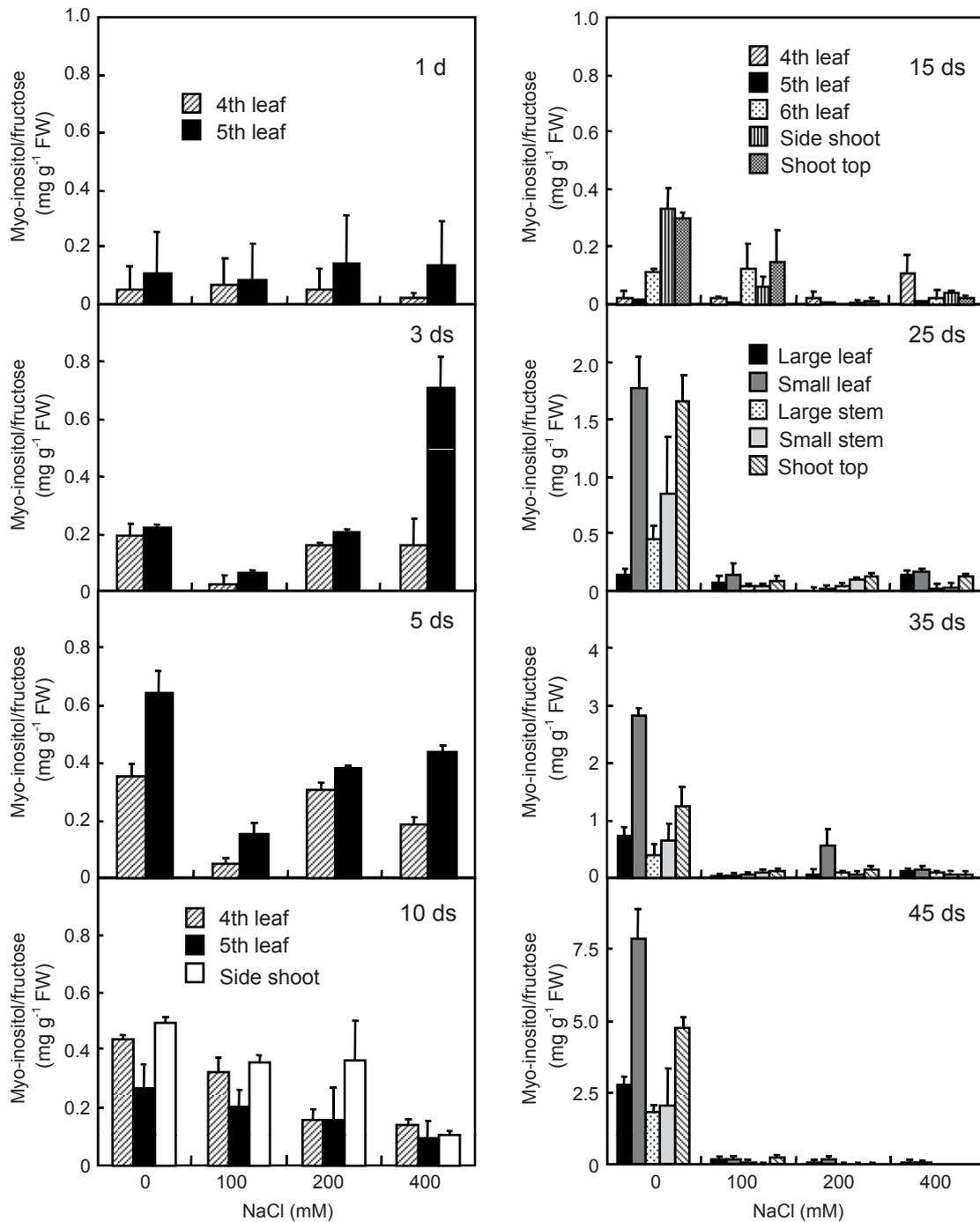


Fig. 4. Effect of salt stress on myo-inositol/fructose accumulation in *M. crystallinum*. 1 d, 3 ds, 5 ds, 10 ds, 15 ds, 25 ds, 35 ds and 45 ds indicate stress duration. Values are means of three duplicates. Vertical bars indicate the standard error of the mean.

order: shoot top>small stems>large stems>small leaves>large leaves. The maximal value was 1.2 mg g<sup>-1</sup> FW, which was found in the shoot top of drought-stressed plant at 25 ds after the start of treatment. The overall concentration of pinitol/ononitol was higher in the drought-stressed plants than in control, but it was lower than that of salt-stressed plants.

## 2. Inositol/fructose accumulation in salt- and drought-stressed ice plant

The peak area in the HPLC chromatogram of the extract was the same as that of the commercial myo-inositol. Under salt stress, accumulation of polyols in *M. crystallinum* was associated with low levels of fructose and glucose (Adams et al., 1992; Paul and Cockburn, 1989), because polyols biosynthesis originated from the glucose-6-phosphate pool (Bohnert, 1995). However,

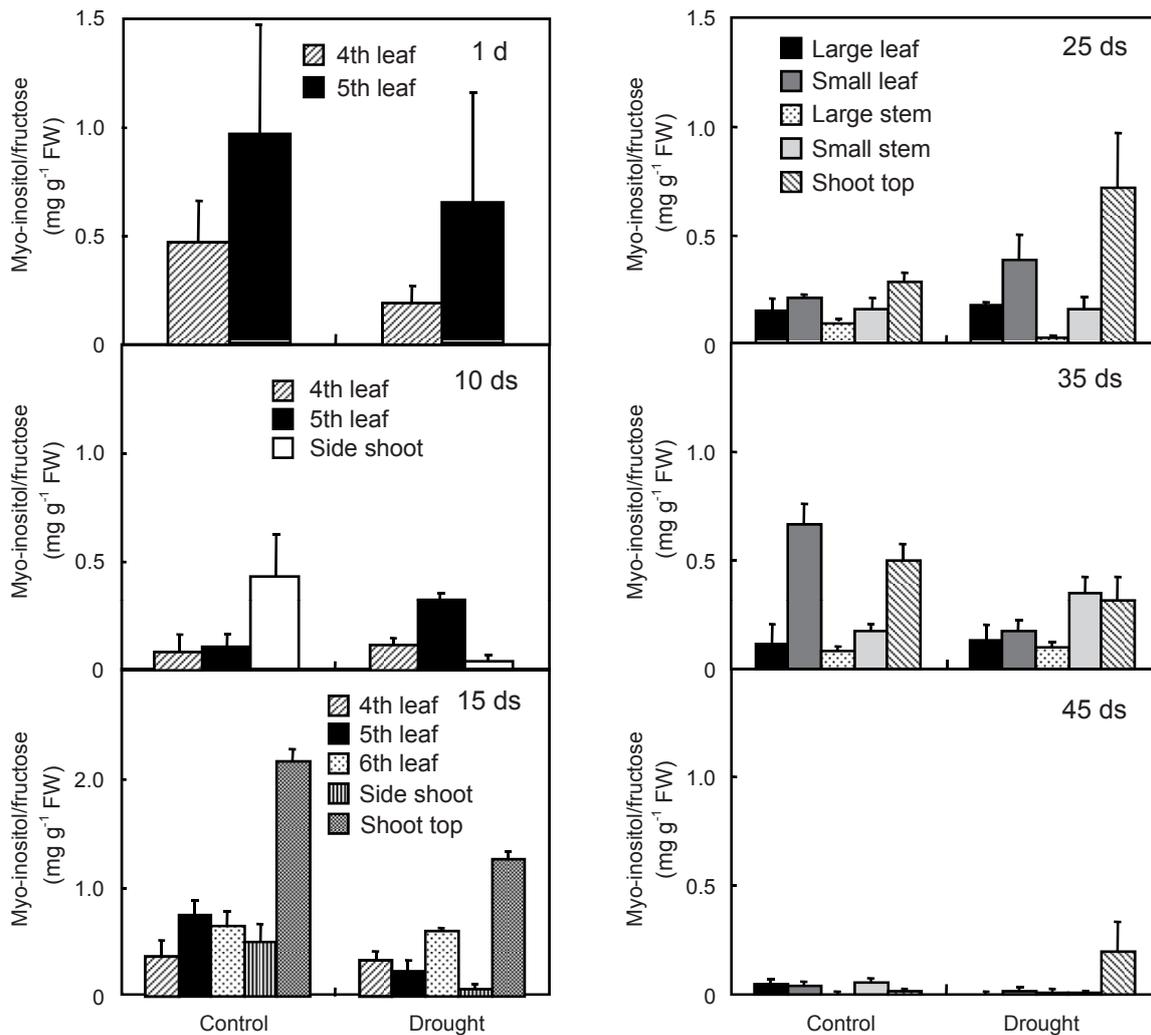


Fig. 5. Effect of drought stress on myo-inositol/fructose accumulation in *M. crystallinum*. 1 d, 10 ds, 15 ds, 25 ds, 35 ds and 45 ds indicate stress duration. Values are means of two duplicates. Vertical bars indicate the standard error of the mean.

we indicated the metabolic peak as inositol/fructose in this study, because the chemical characteristics of myo-inositol are similar to those of fructose. The correlation between the area of chromatogram (A) and concentration of standard (S) was shown by a regression:  $A=55343S$  ( $r^2=0.999$ ). The recovery rate was 88.8%, and the concentration was corrected using this value.

There was no difference in the concentration of inositol/fructose among treatments at 1d after the start of salt stress (Fig. 4). The highest value ( $0.7 \text{ mg g}^{-1} \text{ FW}$ ) in young seedlings was observed in the 5th leaves of plants treated with 400 mM NaCl for 3 ds, but it decreased as elapsed time. At 5, 10, and 15 ds after treatments the concentration of inositol/fructose tended to be higher in the control than in the salt-stressed plants. At 25, 35, and 45 ds after the start of treatment, the highest concentration in mature plants was found in the small leaves of control, which was 1.7, 2.8, and  $7.9 \text{ mg g}^{-1} \text{ FW}$ , respectively.

In the drought-stressed plants inositol/fructose also tended to be higher in control (Fig. 5). It decreased slightly with the lapse of time with 10 d after the start of treatment in both control and stressed plants. The higher concentration of inositol/fructose was found in shoot top in both control and stressed plants at 15 d. The concentrations were  $2.2 \text{ mg g}^{-1} \text{ FW}$  in control and  $1.3 \text{ mg g}^{-1} \text{ FW}$  in stressed plants. At 25 d, inositol/fructose concentration was higher in shoot top of drought stress plants, but it decreased with the lapse of time, and the concentration of inositol/fructose was higher in control at 35 ds. At 45 ds after the start of treatment the concentration decreased in all tissues except in shoot top of stressed plants.

### 3. Antioxidant activity

Fig. 6A shows antioxidant activities in the leaves of salt-stressed plants evaluated by DPPH assay. The lower value indicates the higher antioxidant activity. Antioxidant activity in the leaves treated with 400 mM

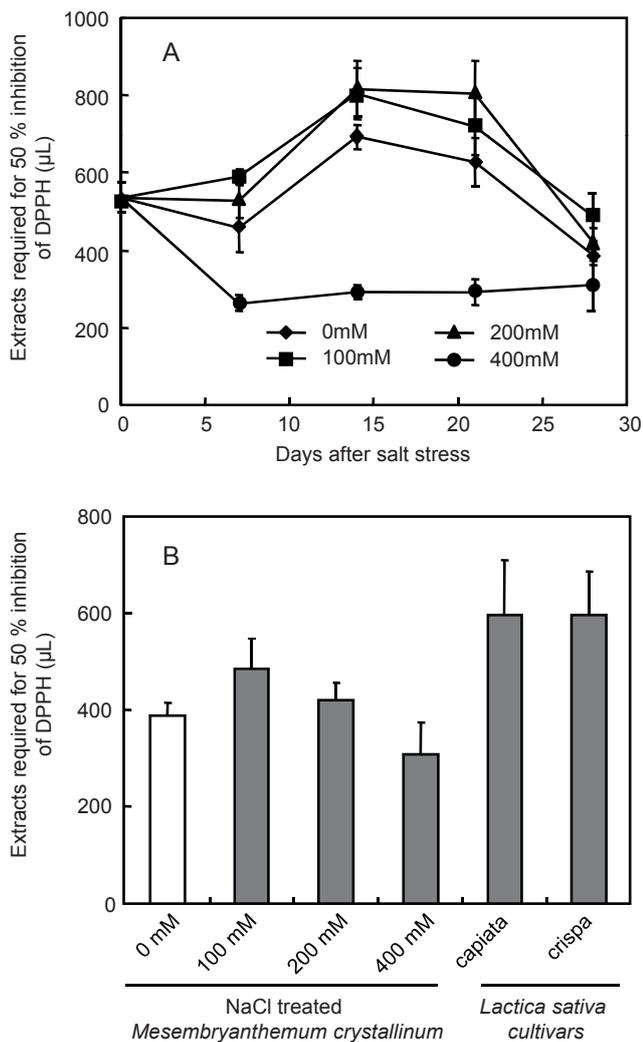


Fig. 6. Antioxidant activities of *M. crystallinum* and *Lactuca sativa* L. evaluated by abilities to scavenge stable free radical DPPH. The scavenging activities of DPPH were expressed as volumes of extracts required to give a 50% reduction in absorbance of DPPH relative to that of DPPH without extract. The lower value indicates the higher antioxidant activity. A, changes in antioxidant activities of leaves of *M. crystallinum* after salt stress with different levels of NaCl. B, comparison of antioxidant activities in the leaves of lettuce and *M. crystallinum* stressed with NaCl for 4 weeks. Values are means of three duplicates. Vertical bars indicate the standard error of the mean.

NaCl was two-fold higher than that in the control (i.e. the volume of extract required to give a 50% reduction in DPPH was almost half of control), and it was kept at least for 4 wks. The antioxidant activity of ice plant was two times higher than that in lettuce. The 50% DPPH inhibiting volume of the extract from the leaves treated with 0, 100, 200 and 400 mM NaCl for 4 wks were 389.0, 486.3, 420.4, and 308.5  $\mu\text{L}$ , respectively, and they were almost half of those in the leaves of butterhead lettuce (*Lactuca sativa* L., var. *capitata*) and leaf lettuce (*L. sativa*, var. *crispa*), which were 595.6

and 597.0  $\mu\text{L}$ , respectively (Fig. 6B).

## Discussion

Both salt- and drought-stresses clearly induced pinitol/ononitol accumulation. The relative water content in the stressed plants was 2–3% lower than that in the control (data not shown), but the degree of increase in polyol content was higher than that of decrease in the water contents. Thus the polyol may be increased by net polyol accumulation, not by the result of simple passive increase in concentration due to dehydration. This idea was supported by the fact that the levels of enzyme expression and mRNA transcript responsible for polyol biosynthesis were elevated in response to salt stress (Vernon and Bohnert, 1992) or drought stress (Keller and Ludlow, 1993). The positive correlation between pinitol accumulation and salt tolerance was observed in various plants species such as maritime pine (*Pinus pinaster*) (Nguyen and Lamant, 1988), mangrove (Popp and Smirnov, 1995), dhaincha (*Sesbania bispinosa*) (Gorham et al., 1988), and *Honkenya peploides* (Gorham et al., 1981).

In the present study, the maximal concentration of pinitol/ononitol was 3.6  $\text{mg g}^{-1}$  FW (equivalent to 18.6  $\mu\text{mol g}^{-1}$  FW and 58.5 mg per unit dry weight), was observed in the shoot top of the plants treated with 400 mM NaCl at 35 ds (Fig. 2). In soybean leaves pinitol was a major low molecular weight carbohydrate (Phillips et al., 1982; Streeter and Strimbu, 1998), and was accumulated in the range from 20 to 25 mg per unit dry weight. Thus the maximal concentration of pinitol/ononitol in the ice plant was higher than that in soybean. This was similar to a previous report, which showed that the pinitol concentration was about 10  $\mu\text{mol g}^{-1}$  FW in the leaves of the plants treated with 400 mM NaCl for 20 days (Paul and Cockburn, 1989). They estimated that the pinitol concentration in the chloroplast was 230 mM, which was higher than in cytosol and vacuoles, and suggested that pinitol acts as a compatible solute providing osmotic adjustment in chloroplasts to counteract the high concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in the vacuole. Proline is accumulated in salt-stressed leaves of *M. crystallinum* (Sanada et al., 1995), but mainly in cytosol (Demmig and Winter, 1986).

Salt-stress was more effective in inducing pinitol/ononitol accumulation than drought stress (Figs. 2, 3). The salt-stimulated accumulation of pinitol/ononitol may be one of euhalophytic traits of *M. crystallinum*. This species is a sodium accumulator, accumulating large amount of NaCl with a gradient from roots to the growing shoot apices (Bohnert and Cushman, 2000), and also “salt-loving plant”, showing optimal growth in moderate salinity conditions ranging from 100 to 300 mM NaCl (Agarie, 2007). The incorporated salt provided a turgor gradient along the axes of the mature ice plant, leading to the accelerated growth

of new cells (Lüttge, 1993). The sodium transport from root cells into the leaf mesophyll was based on sodium/inositol symporters that function in conjunction with sodium/proton antiporters (Nelson et al., 1998; 1999). Therefore, the sodium-stimulated growth enhancement and sodium-dependent inositol incorporation into younger tissues may be involved in the salt-stimulated pinitol synthesis in shoot top of the salt-stressed ice plants. The gradient of concentration from roots (concentration low) to the youngest actively growing portions may be correlated with activity of photosynthesis and photorespiration. Pinitol is derived from methylation of myo-inositol to the intermediary product ononitol (Vernon and Bohnert, 1992) and ononitol appears to be epimerized (Dittrich and Brandl, 1987) to pinitol (Adams et al., 1992). Therefore, pinitol formation is closely related to the primary photosynthetic carbon metabolism, directly through hexose-P and indirectly through transitory starch and sucrose. Bohnert and Jensen (1996) argued that pinitol synthesis through epimerization of ononitol would have to be supported by increased availability of methyl group, which could be derived from photorespiration.

The hexose level was reduced under stress (Paul and Cockburn, 1989; Adams et al., 1992) because myo-inositol was synthesized from glucose-6-phosphate (Bohnert, 1995). Therefore the inositol/fructose in control plants, e.g. at 45 ds in salt stress experiment (Fig. 4) and at 15 ds and 35 ds in drought stress experiment (Fig. 5), was mainly composed of fructose, whereas in the stressed plant, e.g. in the 5th leaves of the plants treated with 400 mM for 3 ds (Fig. 4) and shoot top of the plants drought-stressed for 25 ds (Fig. 5), the inositol/fructose was mainly composed of myo-inositol. The concentration of inositol/fructose in salt- and drought-stressed plants was about  $0.7 \text{ mg g}^{-1} \text{ FW}$  ( $4 \mu\text{mol g}^{-1} \text{ FW}$ ), which was similar to that in cowpea (*Vigna sinensis*), which has been reported to accumulate myo-inositol at higher level (Keller and Ludlow, 1993). However, in *M. crystallinum*, the concentration of myo-inositol decreased with the lapse of time (Figs. 2, 3, 4, 5), because myo-inositol is the precursor of D-ononitol and D-pinitol, and was consumed to synthesize these compounds.

Our results clearly showed that the ice plant accumulated polyols comparably with species that have been reported to accumulate them at higher levels. NaCl treatment was more effective than drought-stress in inducing polyol synthesis. Younger parts of the plant, easily accumulate polyols, preferably in shoot top (pinitol/ononitol) and small leaves (inositol/fructose), under stress conditions. An advantage of ice plant as a functional food is that polyols can be taken from raw tissues directly without processing. Other high polyol containing foods, such as beans need to be cooked, but the ice plant can be eaten in a salad. The

health-related efficacy of polyol in the human body can be expected by consumption of the ice plant.

In addition to accumulation of polyols, the ice plant has interesting traits as a functional food. First, under the conditions inducing accumulation of pinitol/ononitol, this species shift photosynthetic mode from C3 to CAM. In the leaves of ice plant performing CAM, malate and citrate accumulate at higher level (Winter et al., 1978; Herppich et al., 1995). Malate and citrate are key metabolites in Krebs cycle and involved in energy production in mitochondria. Citrate has a function to reduce kidney stone formation (Pak, 1987; Levine et al., 1994), and malate is an effective chelator of aluminum, reducing the accumulation of aluminum in various tissues. Second, the ice plant has high antioxidant activity (Fig. 6). In the leaves of ice plant treated with 400 mM NaCl, the volume of extracts required to give 50% reduction in DPPH was almost half of that in butterhead lettuce (*Lactuca sativa* L., var. *capitata*) and leaf lettuce (*L. sativa* L., var. *crispa*). Smirnoff and Cumbes (1989) showed polyols function as scavenger of reactive oxygen species, thus the increased free radical scavenging activity may be at least partly due to accumulation of polyols. In the stressed leaves, in addition to the polyols, flavonols and betacyanin also accumulated (Ibdah et al., 2002), and activity of scavenging enzymes such as superoxide dismutase (SOD), which catalyses the disproportionation of superoxide radicals to  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ , increased (Miszalski et al., 1998). These compounds may also be responsible for the increased radical scavenging activity in the ice plant. Third, the ice plant accumulates proline as compatible solutes in response to osmotic stress (Heun et al., 1981; Demmig and Winter, 1986; Sanada et al., 1995). Proline is a non-essential amino acid involved in the production of collagen (Watanabe et al., 1999) and wound healing, and also is a precursor of hydroxyproline which functions to maintain and heal cartilage, and strengthen joints, tendons, and muscles (Balch and Balch, 2000). Proline and the other compounds described above such as citrate, malate, SOD, and polyols have been produced through industrial processes as commercial supplement tablets from different biological resources. Therefore, the ice plant is considered to have a high potential as a functional food having ability to synthesize and accumulate the beneficial compounds simultaneously in a single plant.

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