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Cultivation Research for High–glycyrrhizin Licorice by Applying Low Temperature and Ca^{2+} Ion as Environmental Stress Based on Field Investigation

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Wild Licorice (*Glycyrrhiza uralensis*) is important medical plant. It is distributed in the Asia, especially in the semi–arid zone of China and Mongolia. However wild Licorice contains the high quality of glycyrrhizin (GL), which is the active ingredient holding in the root, the cultivated Licorice in China and Japan contains it low.

The objective of this study is to establish the cultivating method of the high quality Licorice. First, field investigation of the high quality Licorice was conducted at semi–arid area of the South Mongolia in May 2010. There were many large and high quality Licorices in South Mongolia. The average low and high temperature in winter was very low.

Second, to clear the soil properties at the area of growing wild Licorice, soil physical and chemical experiment were conducted. All of soil textures were 'sand', and the saturated and unsaturated hydraulic conductivities showed well drainage and the available moistures were little. The concentrations of Ca in south Mongolia was high, that value was 111.0 mg/100 g.

Third, the plastic tube cultivation experiments to evaluate the effects of increasing GL in Licorice by applying Ca^{2+} ion and low temperature as an environmental stress were conducted. In the cultivation experiment, applying Ca^{2+} ion made GL of Licorice increased a little. On the other hand, The concentration of GL in Licorice applied the low temperature for 7 days increased twice than growing at 20 °C constant conditions, although the root diameter was almost equal.

These results will contribute in the semi–arid area growing wild Licorice because the soil properties growing high quality Licorice was clarified. It is considered that environmental stresses, especially low temperature, were effective to increase GL in Licorice.

INTRODUCTION

There are many kinds of medical plants that have been used for a long time in Japan. Recently, almost of that medical plants in Japan are imported. Especially, *Glycyrrhiza uralensis* (L) (hereinafter called Licorice) is important because 70% of the traditional medicine 'Kampo' contain its ingredients called "Glycyrrhizin" (hereinafter called GL). Licorice is distributed in the Asia, especially in the semi–arid zone of China and Mongolia. Recently, the overharvesting of wild Licorice has caused a problem of land deterioration and/or desertification, and some exporting countries have begun restricting the export of wild Licorice. Yamamoto and Tani (2005) reported that the Chinese government restricted the collection of Licorice by people living in regions other than 3 northern regions in 1984. And in 2000, the Chinese government restricted the collection of wild Licorice (Yamamoto and Tani, 2002). Therefore, the exhaustion of medical plants resource like Licorice was concerned. On the other hand, there are high demands of Licorice continuously. Parker (2007) reported the value of the Licorice trade in 2007 was established at 42 million US $. Hayashi and Sudo (2009) summarized the economic importance that Licorice extracts were used as cosmetics, food additives, tobacco flavors, and confectionery foods.

There are two activities against these circumstances, one is to grow Licorice at semi–arid region combating desertification and the other is to product Licorice in Japan. Abe *et al*. (2005) indicated the cultivation of arid medical plants was expected to be an alternative income source. Yamamoto and Tani (2005) reported the 4th year after seeding conformed to the JP XV standard (The Japanese Pharmacopoeia XV, 2006) for GL content in China. (In order to use the Licorice as a medical plants in Japan, GL content rate have to over 2.5%.) Zhang and Xiong (2008) reported that N^+ ion beam irradiation made Licorice salt tolerant.

In domestic, Kusano *et al*. (2003) suggested that selecting excellent type of Licorice lead to product high quality of Licorice. Shibano and Ozaki (2011) indicated the possibility to product the high quality Licorice for 2 years selecting excellent type. As another approach, the nutriculture had been studied. Kakutani *et al*. (1997) reported the nutriculture using Rockwool could increase GL in Licorice well comparably. Sato *et al*. (2004)

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reported the difference of nutrient solution concentration made GL in Licorice increased. In this way, the cultivation method had been developing. But, the effect of environmental stress such as temperature for GL in Licorice is not obvious.

The objective of this study is the preventing desertification to establish the cultivating method of the high quality Licorice. First, field investigation of the high quality Licorice was conducted at semi–arid area of the South Western Mongolia in May 2010. The vegetation of wild Licorice and the other weeds was also surveyed. Second, to clear the soil properties at the area of growing wild Licorice, soil physical experiment such as particle size distribution, soil water retention, saturated hydraulic conductivity, unsaturated hydraulic conductivity were conducted. Third, the plastic tube cultivation experiments to evaluate the effects of increasing GL in Licorice by applying Ca^{2+} ion and low temperature as an environmental stress were conducted.

FIELD INVESTIGATION AND CULTIVATION EXPERIMENT

Investigation for soil properties growing wild Licorice in Mongolia

Field investigation about soil properties growing wild Licorice was conducted in Bayankhongor prefecture, South Mongolia at May 2010. Fig. 1 shows the investigation field which called Site S_1, S_2 and S_3 at Bayankhongor. There were some Licorice and native plants near Site S_1, on the other hand only Licorice was growing Site S_3. There was nothing to grow plants at Site S_2. Ground water level was −3.0 m near Site S1 and −1.9 m near Site S_3. Fig. 2 showed the average high temperature, average low temperature and precipitation near investigation Site. The total precipitation showed 129.6 mm so that there was the arid region. The both low and high temperature was very low in winter, although Licorice was alive. Relative humidity was about 30% at the investigating time.

Three soil Samples near wild Licorice root, Site S_1 (60 cm in depth), Site S_1 (80 cm in depth) and Site S_3 (50 cm in depth) were collected at study site. In order to compare with Japanese soil, silica sand (size 7) and decomposed granite soil were also analyzed. That five sample were analyzed about saturated hydraulic conductivity, unsaturated hydraulic conductivity, soil texture and soil moisture characteristic curve. The saturated hydraulic conductivity was measured using Falling head permeability test. The unsaturated hydraulic conductivity was measured using One–step method (Doering 1955). The unsaturated hydraulic conductivity of the low moisture area could not be measured by One–step method so that the capillary tube model (Jury and Horton (2004)) was used for estimation (eq. 1).

$$K(\theta_s - \Delta\theta) = \frac{\tau\sigma^2\Delta\theta}{2\eta\rho_w g}\sum_{J=i+1}^{M}\frac{1}{h_J^2} \qquad \text{(eq.1)}$$

Where, τ: tortuosity, σ: surface tension, ρ_w: density of water, g: acceleration of gravity, η: viscosity, θ: volumetric water content, h: potential head.

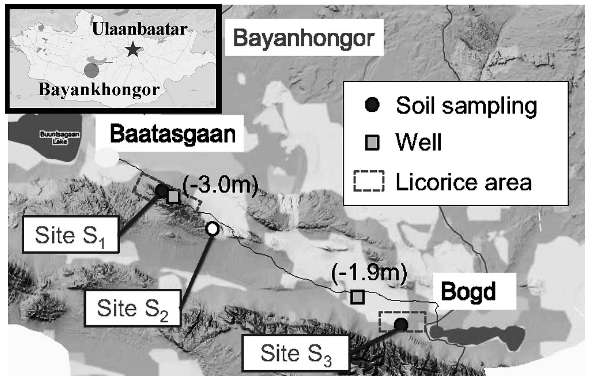

Fig. 1. Map of investigation area in Mongolia.

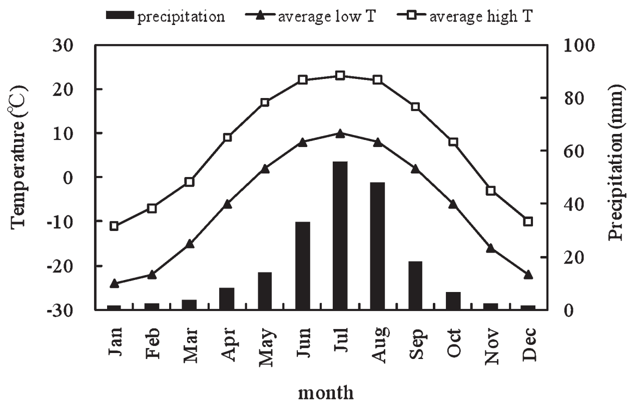

Fig. 2. Average high and low temperature for 6 years in Bayanhongor (cf. weather underground).

The soil moisture characteristic curve was measured using suction plate method and centrifuging method. Soil chemical properties, exchangeable Na, Ca, Mg, K and NO_3–N, were also analyzed.

Cultivation experiment applying environmental stress

The plastic tube cultivation experiments to evaluate the increasing effects of GL in Licorice by applying Ca^{2+} ion and low temperature as an environmental stress were conducted in Phytotron at Biotron Institute, Kyushu University. Silica sand (size 7) was set in a plastic tube made of polyvinyl chloride (ϕ=10.74 cm, h=50 cm) and was put in condition of bulk density 1.4 (g/cm^3) equally. Environmental stresses were applied after Licorice was cultivated in the tube at the Phytotron for 3 month. The air conditions of Phytotron were two types, A1 (temperature: 30 ˚C, Humidity: 70%) and A2 (temperature: 20 ˚C, Humidity: 70%). Ca^{2+} ion was applied to 2 tubes at Phytotron A1 for 7 days. Ca^{2+} concentration of the soil was controlled 100 mg/100 g which amount was equal to Mongolian soil. The 50 ml of water which contained 1.0 g of 14% $Ca(NO_3)_2$ and 0.3 g of 12% $(NH_4)_2SO_4$ irrigated to the cultivation tubes 3 days a once.

To expose two cultivation tubes to the air outside, Licorice was put out from Phytotron A2 for 7 days. The outside air temperature and Relative humidity in rainproof greenhouse was shown in Fig. 5. The 50 ml of two thousandth of liquid fertilizer 'Hyonex' (N: P: K = 5: 10: 6) was irritated to cultivation tube a once.

After harvesting, root diameter was measured and GL in Licorice was analyzed by HPLC.

RESULTS AND DISCUSSIONS

Soil properties

Fig. 3 shows the soil moisture characteristic curves of each Site and Table 1 shows the volumetric water content at sampling time. The graph of Site S_1 and Site S_3 were similar, and Site S_3 and silica sand were similar above 130 cm H_2O. The readily available moisture of Site S_1 (60 cm), Site S_1 (80 cm), Site S_3 were 8.4%, 7.9% and 13.0% respectively. These values were low comparing with farmland. The metric potential at the sampling time was 1300~10000 cm H_2O more than wilting point (1000 cm H_2O). It indicated the possibility that Licorice could grow at severe water condition or the root grown to the deep layer where the root could use groundwater.

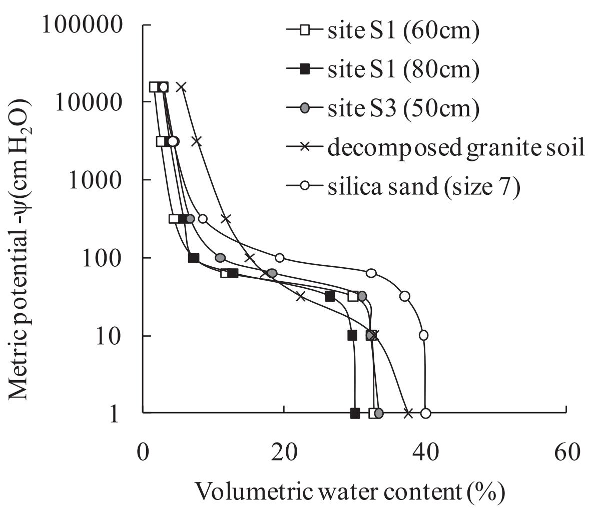

Fig. 3. Soil water characteristic curves of each soil.

Table 1. Volumetric water content at sampling time

sample	Volumetric water content (%)
Site S_1 (60 cm)	0.99
Site S_1 (80 cm)	1.64
Site S_3 (50 cm)	3.59

Table 2. Saturated hydraulic conductivities of each soil

sample	saturated hydraulic conductivity (cm/s)
Site S_1 (60 cm)	9.5×10^{-3}
Site S_1 (80 cm)	7.6×10^{-3}
Site S_3 (50 cm)	9.5×10^{-3}
decomposed granite soil	8.7×10^{-3}
silica sand (7 size)	1.8×10^{-3}

Table 2 shows the saturated hydraulic conductivity. The results of saturated hydraulic conductivity showed well drainage at all sample. Fig. 4 showed the unsaturated hydraulic conductivity. The measurement by One–step method and estimation by Capillary tube model were fit well. Site S_1 and S_3 showed same variation and well drainage different from the decomposed granite soil. The variation of Site S_3 was similar to silica sand comparatively.

The soil textures of all sample showed Sand according to the standard of International Society of Soil Science (Table 3). Table 4 shows soil chemical properties. It showed that Mongolian site soil were contained Ca and No_3–N very much than other soil. But, the value of No_3–N might be temporary because amount of No_3–N was easily to change such as rainfall or animal excretion. There were no significant difference in the amount of Na, K and Mg. As the results, the soil growing wild Licorice was well drainage and a little water retention, it resembled to silica sand.

Environmental stress

Fig. 6 shows the GL content rate analyzed by HPLC and root diameters average of two Licorices after experiment. It showed that the high Ca concentration of soil made GL of Licorice increased a little. Air condition 20 ˚C made GL of Licorice increased more than 30 ˚C of it. The Licorice applied the low temperature at outside increased GL more than anything else, although the root diameter was almost equal. It is considered that environmental stresses especially low temperature were effective to increase GL in Licorice though GL content was not satisfied the JP XV standard in a short time.

Table 3. Soil separate and soil texture classification

sample		Sand (%)	Silt (%)	Clay (%)	soil texture
Site S_3	0 cm	100	0	0	Sand
	30 cm	94	5	1	Sand
	60 cm	94	5	1	Sand
	100 cm	80	16	4	Sand
decomposed soil		96	3	1	Sand
silica sand (7size)		100	0	0	Sand

Cf.: ISSS (International Society of Soil Science)

Table 4. Soil chemical properties of each soil

	Southern Area in Mongolia	Decomposed granite soil	Silica sand (7 size)
Exchangeable Ca (mg/100 g)	111.0	36.0	35.0
Exchangeable Mg (mg/100 g)	6.1	36.0	1.3
Exchangeable Na (mg/100 g)	7.1	5.4	3.2
Exchangeable K (mg/100 g)	4.8	3.2	1.5
NO_3–N (mg/100 g)	1.65	0.08	0.07

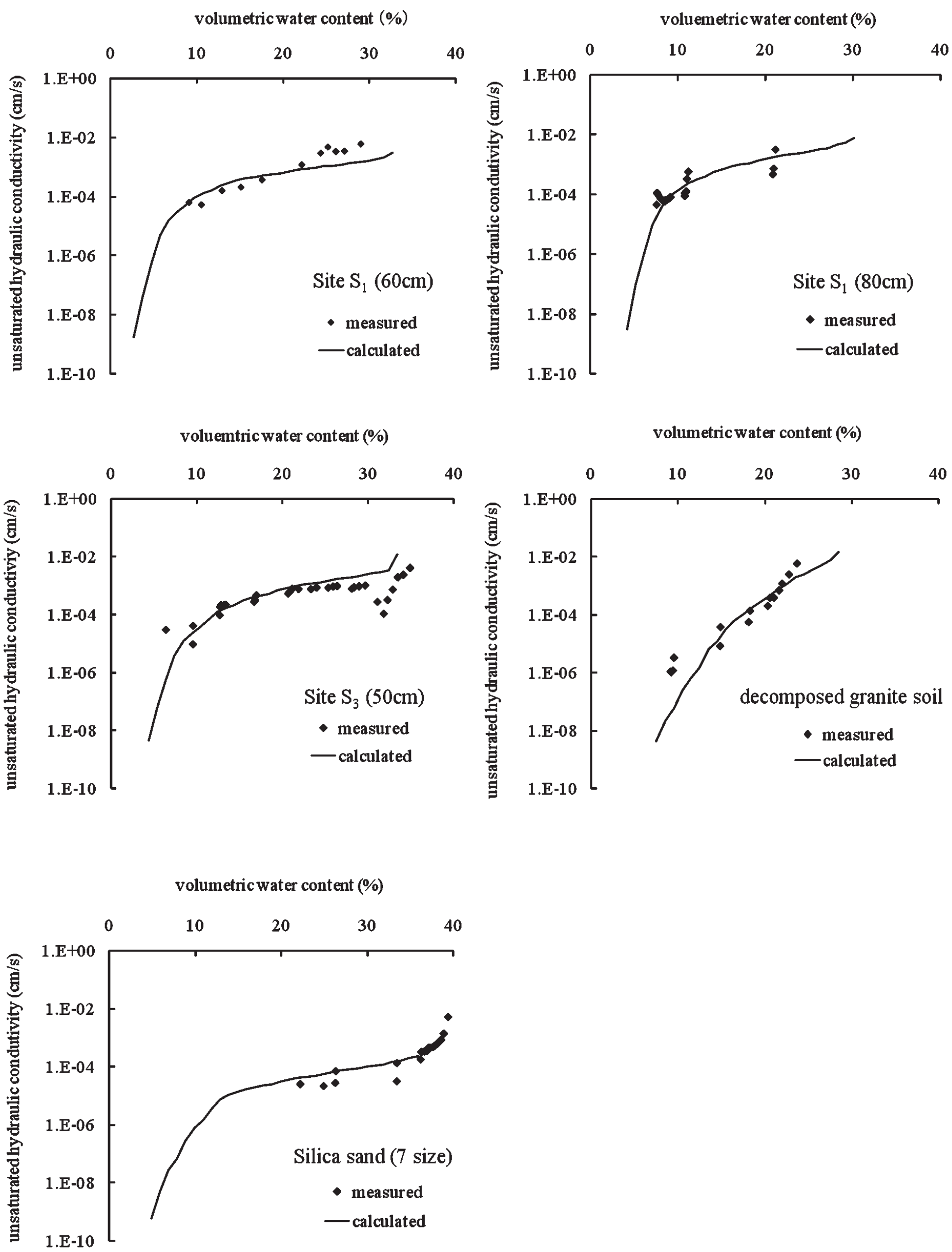

Fig. 4. Unsaturated hydraulic conductivities using One–step method and Capillary tube model of each soil.

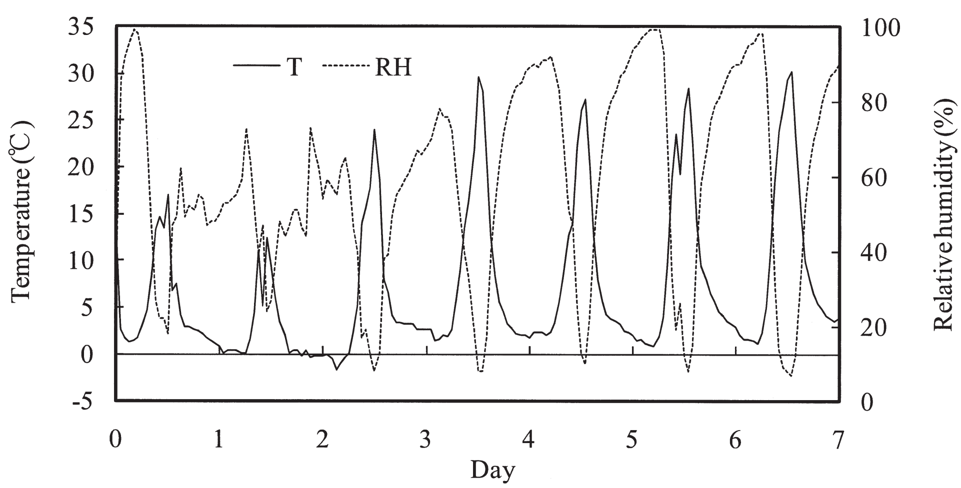

Fig. 5. Temperature and relative humidity of outside air.

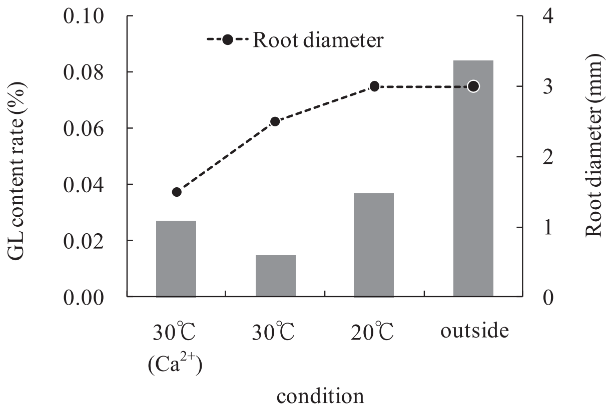

Fig. 6. Results of average GL content rate in Licorice applying environmental stress.

CONCLUSION

In this study, the soil properties of growing wild Licorice in Mongolia were investigated and the tube cultivation experiments were conducted based on results of field investigation to clear the effect of applying environmental stress to the high quality Licorice.

As the results, there were many big Licorices in South Western Mongolia where was sandy soil, the well drainage and the little available moisture. The concentrations of Ca^{2+} in south Mongolia were high comparing with silica sand (size 7) and decomposed granite soil.

GL increased to apply the low temperature and Ca^{2+} ion as environmental stress. It is considered that environmental stresses were effective to increase GL in Licorice.

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