Effects of Serpentine on the Growth and Allocation of *Raphanus sativus* var. *radocula* Seedlings in Cultivation Medium Including Cu

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Abstract

This study investigated the effects of serpentine on the growth and morphology of *Raphanus sativus* var. *radocula* (*R. sativus*) seedlings in cultivation medium including Cu, which is toxic heavy metal or micro elemental nutrient. *R sativus* was raised on hydroponic condition using Hoagland cultivation medium including Cu of concentration levels ranging 0.005-1ppm with particles of serpentine, Mitate conglomerate or silica sand (as control) for 15 days. Length of main root significantly increased when serpentine was added to the medium. In the medium including 0.25ppm of Cu, the length of main root of half the individuals reduced half by adding serpentine, whereas the length of all the plants decreased by adding Mitate conglomerate or silica sand. Total dry-weight did not decrease up to Cu concentration of 0.25ppm, whereas leaf and root recorded maximum value. This result implies that serpentine reduced Cu stress on root of *R.sativus*, thus root and leaf growth was not affected by Cu. Therefore serpentine was expected to be effective on reducing Cu stress on plants.

Keywords: serpentine; Mitate conglomerate; copper; main root elongation; allocation of dry-weight

Introduction

Heavy metals are one of the essential factors for controlling plant growth as toxins as well as micro nutrients. Low concentration of heavy metals after weathering rocks are important as essential mineral elements in soil. Thus, chemical characters of rock are important for determining types of vegetation and growth of individual plants. Therefore it is important to estimate the role of rocks in interaction of plant and heavy metals in soil.

Serpentine contains toxic Ni or Cr for growth of plants and was used in this study for assumption that heavy metals in serpentine and soil affect plant growth reciprocally. Serpentine soil has specific chemical characters with high heavy metals contents and high base contents, and consequently it has reported that specific morphologic change of root elongation or drying tolerant of leaf appears in serpentine soil (Kuruckeberg 1984, Brooks 1987). Cu is essential for growth of plant as micro nutritional element and morphological change of plant in the medium including Cu with serpentine was investigated to estimate how rock materials influence the interaction between heavy metals and plants.

Materials and methods

(1) Rock and plant materials

Serpentine was collected on Saganoseki Peninsula in Ohita Prefecture belonging to Minamikawa belt. Mitate conglomerate was collected on Mitate formation in Miyazaki Prefecture belonging to Chichibu belt. Commercially-produced silica sand was used as control (KANTO CHEMICAL CO., INC). Collected rocks was crushed and sieved into 1-2mm diameter. After washing, they were dried at 105℃. *Raphanus sativus* var. *radocula* seed (TAKII SEEDS CO., Ltd.) was used as plant materials.

(2) Growth methods and conditions

Raphanus sativus var. *radocula* was grown on hydroponic condition using Hoagland cultivation

medium with 2g of serpentine, Mitate conglomerate or silica sand for 15 days. Cu concentration of Hoagland cultivation mediums were changed at 6-stages, 0.005, 0.02, 0.1, 0.25, 0.5, 1ppm. After sowing seeds, plants were grown in a growth chamber at constant light condition (photon flux density: 220.6±0.1 μ mol $m^{-2}s^{-1}$) and constant temperature at 20℃. Plants were raised for 15 days at the repetition of 5. The length of main root and number of lateral roots were measured in the plants for 15 days after sowing. After the measurements, plants were dried at 80℃ over 48h and weighed dry-weight of root, stem, leaf and cotyledon.

Results and discussion

The length of main root and dry-weight of root, stem, leaf and cotyledon of *R. sativus* var. *radocula*, which was grown in the medium including Cu concentration of 0.005, 0.02, 0.1, 0.25, 0.5 or 1ppm and adding serpentine, Mitate conglomerate or silica sand for 15 days are presented in Fig.1 and Fig.2. The results of two-way ANOVA on these parameters were shown in Table1. The length of main root was significantly longer by adding serpentine or Mitate conglomerate than adding silica sand. Dry-weight of leaf was also significantly higher by adding serpentine than the other treatments. The length of main root and dry-weight of root and leaf significantly different between treatments of different Cu concentration (Table1).

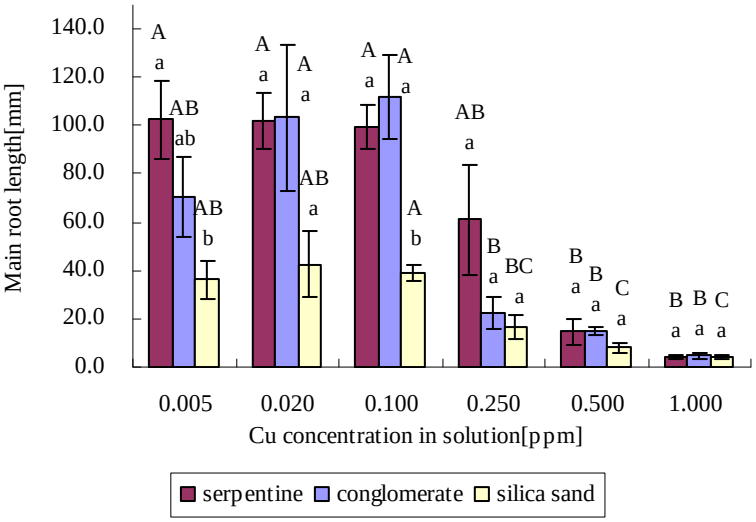

Fig.1. The length of main root of R.sativus grown in medium including Cu for 15 days.
Capital letters of the alphabet : Significant between different Cu concentration treatments ($p<0.05$), Lower letters : Significant between rocks ($p<0.05$). Sharing the same letters means not significant difference.

Table1. Results of two way ANOVA for the parameters of dry-weight of root, stem, leaf, cotyledon and length of main root by factors of rock type and Cu concentration in cultivate solution; significance probability shown as *(p<0.05), **(p<0.01), ***(p<0.001)

factor	parameter	DOF	F value	significance probability	
type of rock	Root-DW	2	1.67	0.198	
	Stem-DW	2	1.73	0.187	
	Leaf-DW	2	7.86	0.001	**
	Cotyledon-DW	2	1.59	0.213	
	Main root length	2	16.32	0.000	***
Cu concentration in cultivate solution	Root-DW	5	9.85	0.000	
	Stem-DW	5	0.71	0.618	
	Leaf-DW	5	20.76	0.000	***
	Cotyledon-DW	5	10.72	0.000	***
	Main root length	5	25.08	0.000	***
type of rock ×Cu concentration in cultivate solution	Root-DW	10	2.97	0.005	**
	Stem-DW	10	4.12	0.000	***
	Leaf-DW	10	1.30	0.257	
	Cotyledon-DW	10	2.23	0.030	*
	Main root length	10	2.47	0.016	*

In the medium of Cu concentration of 0.005 and 0.1ppm, the length of main root increased by adding serpentine (Fig1). The length of main root significantly decreased with increasing Cu concentration of medium independent of rock type. Length of main root did not increase by adding serpentine when Cu concentration reached 0.5ppm. In the medium of Cu concentration of 0.25ppm, main root elongation was observed for half the number of plant by adding serpentine, whereas the length of main root was significantly lower compared with the treatment with Cu concentration <0.25ppm by adding Mitate conglomerate or silica sand. However, difference between rocks was not significant.

When silica sand was added to medium, leaf and root dry-weight was significantly lower and total dry-weight of plant was lower at higher Cu concentration. When serpentine was added in medium, dry-weight of any organ were not significantly lower than cultivated at Cu concentration <0.1ppm. When Mitate conglomerate was added in medium, dry-weight of stem and cotyledon were significantly lower than cultivated at Cu concentration 0.1ppm. When silica sand was added in medium, dry-weight of leaf and root were lower than cultivated at Cu concentration <0.1ppm. The results implied that serpentine contributed to enhance resistance in *R. sativus* to Cu toxicity in medium.

In the medium of Cu concentration of 0.25ppm, root and leaf developed when serpentine was added to the medium. Growth of leaf was increased when serpentine was added to high concentration of Cu in medium, probably because of well development of root under condition of high Cu stress. In the samples of adding serpentine, increase of number of lateral roots was observed. Lateral roots were important for absorbing nutrients by plants. Thus it was suggested that increase of number of lateral root may promote increase of leaf weight.

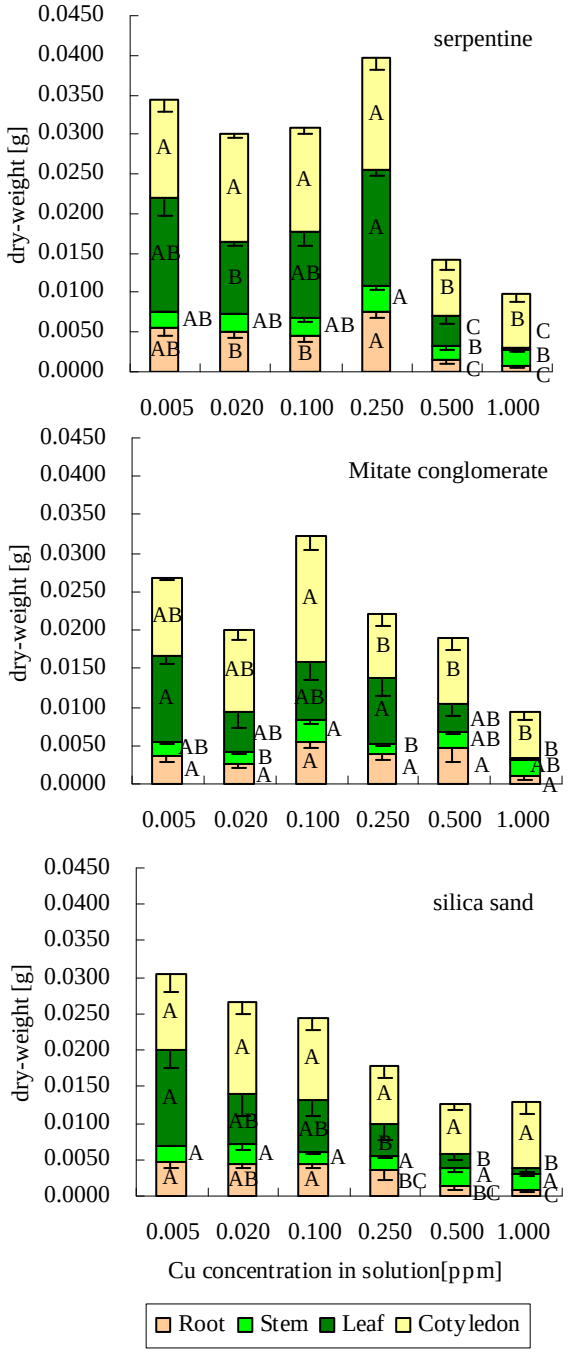

Fig.2 Dry-weight of organ cultivated in medium including Cu for 15 days. Capital letters means significant difference between Cu concentrations in medium. Sharing the same letters means not significant difference.

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