Reduced allelopathic inhibition of lettuce (*Lactuca sativa*) growth caused by velvet bean (*Mucuna pruriens*) under 3D-clinorotation

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Abstract Allelopathy between *Mucuna pruriens* (velvet bean) and *Lactuca sativa* (lettuce) was studied under 3D-clinorotation. Growth of both roots and shoots of lettuce seedlings was suppressed by the presence of velvet bean. The degree of suppression was less on the clinostat compared to the normal static earth gravity. L-DOPA (L-3, 4-dihydroxyphenylalanine) is known to be a major substance in allelopathy of velvet bean. Amount of L-DOPA diffused out from a sintered filter paper into agar medium was compared between clinorotation and control group, and found no significant difference. It was concluded that some factors related to release, transport, and sensing phenomena of allelopathic substances may be responsible to the new findings in this study.

Key words: allelopathy, L-DOPA, velvet bean (*Mucuna*), lettuce, gravity, 3D-clinostat

Introduction

Interactions between organisms and species have been known in many aspects since old time. Allelopathy is a phenomenon observed in many plants that release specific chemicals acting on other organisms, including plants, animals and microorganisms, in either inhibitory or excitatory ways (Rice 1984, Fujii 1999). Even allelopathy has been known since 2000 years ago, there has been no study on the allelopathy under reduced gravitational stimulus other than normal gravity on the earth. If biosynthesis, emission, transport and sensing mechanism of allelopathic substances would be affected by gravity, many organisms and ecological system might show different behaviors based on the inter-organisms and – species interactions, when they are exposed to microgravity. Fujii *et al.* (1999) found that velvet bean plant shows strong allelopathic interactions against other plants especially lettuce seedlings after their studies for over fifteen years surveying interactions among many species of plant. L-DOPA, L-3,4-dihydroxyphenylalanine, was reported as one candidate of allelopathic substances in velvet bean plant (Fujii *et al.* 1991). In this study, allelopathy between velvet bean and lettuce under different gravitational environment was studied with 3D-clinorotation. This is the first paper to report allelopathy under exotic gravitational stimulus applied on plants.

Materials and Methods:

Plant materials

Mucuna pruriens (velvet bean) and *Lactuca sativa* (lettuce) were selected as a pair of plant species to examine allelopathy between them. Seeds of velvet bean were collected in Vietnam during the summer of 2002. Lettuce seeds were purchased from Joyo-Nosan Co. (Ibaraki, Japan) and stored under dark at 5°C until the experiment. The rate of the seed germination was over 95 %.

Intercropped incubation

Velvet bean plant was pre-incubated for two weeks before it was transplanted in Agripot® (Kilin, Tokyo) for the intercropped incubation shown in Fig.1. Velvet bean seeds were sterilized with 0.1% sodium hypochlorite for 10 min and washed with distilled water three times and kept in room for 2 hours before it was planted in Agripot® (Kilin, Tokyo) for the intercropped incubation. The medium for incubation was 0.8 % of agar with gelling temperature at 30-31 °C (Nacali tesque, Kyoto). The root part of young velvet bean plant was placed in an agar solution at 35 ± 2°C, and fixed in a gel after being cooled down to room temperature.

Another set of incubation was made with sowing seeds of two species nearby. Three seeds of velvet bean were placed on agar in Agripot® and they were fixed by adding 5 ml of new agar solution at 35 ± 2°C. When they were embedded in the agar, their hila were aligned upwards.

In both set-up, lettuce seeds were planted under the surface of agar around velvet bean plant or seeds at a distance of *ca* 20 mm or *ca* 10 mm, respectively. The embryo of the lettuce seeds was aligned upwards (turned up) when they were sown in the agar as shown in Fig. 1. Two groups were prepared for each case. One was for the experimental group, being 3D-clinorotated (Yamada *et al.* 1993, Yamashita *et al.* 1997), in order to expose them to

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Fig.1. Intcropped incubation of velvet bean and lettuce in Agripot®.

Table 1 Group for studying gravitational effects on allelopathy

	Velvet bean and lettuce intercrop	Lettuce singly planted
3D-clinorotation	3D+M	3D-M
Normal ground gravity	GC+M	GC-M

pseudo-microgravity environment. The other was incubated under the normal earth condition as the control group. Those two groups were incubated under the dark (plant-seed case) or light (seed-seed case) at 25 °C for three days. It is known that callus of a species close to velvet bean produces DOPA, and its production is higher under the light compared to that in the dark (Saito *et al.* 1982, Teramoto 1998). In this experiment, light was irradiated by a fluorescent tube (27W, National Twin 2 Parallel) from the top of Agripot® at a distance of 30 cm. The growth of lettuce seedlings was quantitatively evaluated from photographs of the plants taken after the termination of the incubation.

In addition to the intercropped incubation, lettuce seeds were singly planted without velvet bean plant. The experiment group of 3D-clinorotated plants was compared to the control under normal gravity, and the group of intercrop incubation. Summing up those groups, lettuce seedlings of the four groups are characterized with two indexes; allelopathic effects given by velvet bean plant, and 3D-clinorotation, as shown in Table 1.

Transmission of L-DOPA in agar analyzed by HPLC.

A 100 mg of L-DOPA was doped into a filter paper, which was placed on the center of agar medium. After the period similar to the protocol of the incubation, a defined volume (0.2 ml) of agar was sampled by a cutter from a position below the filter paper. Quantity of L-DOPA released into the agar medium from the filter paper was analyzed by High Performance Liquid Chromatography (HPLC) for the experiment group of 3D-clinorotation and the ground control. The sampled agar was lyophilized, before small amount (1 ml) of water was added to extracted water soluble chemicals from it. The extracted solution was fed through Mol-Cut® (Millipore, LGC, Yonezawa) in order to remove chemicals with higher molecular weight. Low molecule fraction was filtered through a filter unit of which typical pore size was 0.45 µm (Maesyoridisk W-13-5, Tosoh, Tokyo) and centrifuged for one minute to remove precipitates. Sample solution (0.1 ml) was applied into a

Fig. 2. Velvet bean and lettuce plants after three days intercropping incubation. Left: Group 3D+M, Right: Group GC+M.

HPLC system (Tosoh HPLC-PD system; Column; ODS 80Ts, solvent; 5% methanol, with a flow rate; 0.8ml min⁻¹, detection wave length; 200-400nm). L-DOPA was identified and analyzed quantitatively with a calibration of the HPLC system made by an authentic substance, L-3, 4-dihydroxyphenylalanine (Tokyo-Kasei Kogyo, Tokyo).

All the experiments were duplicated more than twice in order to confirm reproducibility of the observation.

Results

Intcropped incubation

The allelopathic action of velvet bean on the growth of lettuce and effects of 3D-clinorotation were evaluated from the morphological and physical dimension of lettuce plant. Figure 2 shows velvet bean and lettuce plants after their intercropped incubation. Lettuce roots grew normally in the agar medium for both the 3D-clinostat experimental group, and the control, that was exposed to static gravity environment of the earth. Mechanical integrity of the 0.8% agar medium was kept under the 3D-clinorotation, where agar should hold its own weight by itself under the inverted configuration of the Agripot®, in respect to the earth gravity vector.

Growth of lettuce shoots and roots incubated with pre-incubated velvet bean plant at the start is compared in two groups, GC+M and 3D+M (Fig. 3). Average length of lettuce shoot and root in 3D+M are longer than that in GC+M. In the intercropped case for lettuce with pre-incubated velvet bean plant, degree of influence by 3D-clinorotation in growth was higher in root compared to shoot.

Growth of lettuce roots incubated with velvet bean seeds is also compared among four groups in Fig. 4. Suppression of growth is clearly indicated by the presence of velvet bean by comparison between GC+M and GC-M under normal gravity. The 3D-clinorotation does not show difference in root length as seen in the pair of 3D-M and

Fig. 3. Effects of clinorotation on the growth of roots and shoots of lettuce seedlings incubated together with velvet bean plant, Group 3D+A and GC+M. Velvet bean seedling was placed on agar in Agriplot® under the dark at 25 °C. Bars indicate S.E. (n=12)

GC-M. The effect of the clinorotation on the degree of allelopathic suppression is indicated by 3D+M versus GC+M. The clinorotation induced lower degree of growth suppression for lettuce roots.

Transmission of L-DOPA into agar medium

The relative ratio of L-DOPA released into agar medium from the filter paper under 3D-clinorotation and ground control was 104:100 determined by the HPLC analysis. Transmission of the major allelopathic substance from the model system to medium was not affected by 3D-clinorotation.

Discussion

Degree of the allelopathic inhibition of lettuce growth by the presence of velvet bean plant was reduced under the 3D-clinorotation. Allelopathy between velvet bean and lettuce, reported by Fujii *et al.* (1991) was reproduced with the experiment set up of this study. Major chemical substance responsible to allelopathy of velvet bean is L-DOPA (Fujii *et al.* 1991). Since transmission of this substance is made by solvated form through incubation media, the higher suppression of root growth, shown in Fig. 3, can be explained by the processes initiated by the contact to allelopathic substances primarily at root region of lettuce (Nishihara *et al.* 2002, Hachinohe and Matsumoto, 2003).

We can conclude that the 3D-clinorotation affects allelopathic phenomena of plants, and allelopathic responses are expected to be modified under microgravity environment in space. As shown in Fig. 4, the degree of growth suppression for lettuce was higher on ground, than

Fig. 4. Growth of roots and shoots of lettuce seedlings incubated together with velvet bean seeds, Group 3D+M, 3D-M, GC+M and GC-M. Three seeds of velvet bean were placed on agar in Agriplot® under the light at 25 °C. Bars indicate S.E. (n=12)

on the 3D-clinostat. Pseudo-microgravity environment or gravitational stimuli given by the 3D-clino-rotation induces the reduced allelopathic action of velvet bean against lettuce seedlings.

In the transmission of L-DOPA from the filter paper into agar in two experimental groups, amount of the release is slightly high under the clinorotation, but it did not show remarkable differences among them. These results indicate that endogenous DOPA level or some substances related to DOPA in velvet bean plant are affected by 3D-clinorotation.

Deviation in allelopathic response of plant under different environment can be attributed to the effects of the environmental factor on the elementary processes of the allelopathy. Production and release of allelopathic substances could be modified by different environment. Transmission of the substances through the media between two plant bodies might be changed. Sensitivity of the target plant body would depend on environmental factors. In order to know which part of the process; among production, release, transmission, sensing and expression, is modified by the 3D-clinorotation, further analytical studies are required. Quantitative analysis of L-DOPA released from velvet bean root at different gravitational environment is left for the future study, together with other factors associated to the transportation of L-DOPA through the media both agar and aerial path, and the unknown secondary substances that is modulated by the clinorotation.

In some plant species, it has already reported that growth differs under microgravity (Ueda *et al.* 2000, Lanfang *et al.* 2001, Hoson *et al.* 2003) and some of phenomena can

be mimicked by the clinorotation. Plant growth regulators, such as auxin, and physiological response of plants against those substances are known to respond gravity. Even mechanism that explains the allelopathic actions of L-DOPA is not known in detail yet, results of this study indicate importance of gravity on allelopathy and possible link of allelopathic response and growth regulators under different gravitational environment. The basic knowledge on allelopathy and gravity may contribute to the synthesis of ecological systems in outer space with multiple species as its member.

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