

Title: Effects of Treading Stress on Changes in Morphology and *DROI* Gene Expression of Barley Roots

The traditional Japanese farming method of *mugifumi* refers to the process of treading the soil and seedlings of grain. Mugifumi causes changes such as stalk thickening and tillering promotion. Roots are important for supporting plants, but few studies have focused on the effects of treading on plants' roots. The present study investigated the effects of treading stress on the roots of barley, a crop used for beverages and food production worldwide, by comparing the length, weight, spread angle, and *DEEPER ROOTING 1 (DROI)* gene of trodden and non-trodden barley roots. I compressed barley seedlings in soil three times for the length and weight measurements. For angle measurements, barley seedlings were grown in gellan gum with bent stalks. Trodden samples showed a decrease in root length by about 50%, the mass per individual root system decreased by about 40%, and root spread was about 14° more than non-trodden samples. In light of the differences in root shape, the *DROI* gene, involved in root gravitropism and causes downward root growth in response to gravity, was evaluated. There were few studies on *DROI* in barley, so I designed primers and PCR conditions for the experiment. In the present study, Reverse Transcription PCR and Western blotting showed that treading caused a decrease in *DROI* expression in barley, indicating that it causes root morphological changes. Barley treading stimulates roots to grow horizontally to further stabilize the plants. Therefore, treading to control root shape could contribute to the cultivation of plants in diverse agricultural land.