

SUMMARY OF Ph.D. DISSERTATION

School Fundamental Science and Technology	Student Identification Number	SURNAME, First name TOKUNAGA, Takashi
<p>Title</p> <p>Chemical Studies on the Bioactive Substances Concerning Physiological Function of <i>Dionaea muscipula</i> Ellis.</p>		
<p>Abstract</p> <p>In this dissertation, studies on chemical substances concerning unique nature of <i>D. muscipula</i> were described. This dissertation consists of two chapters. In chapter 1, it was described that mechanism of chemical defense in carnivorous plant. Chapter 2, chemical study on the substance concerning carnivorous plant movement and memory was described.</p> <p>1. Mechanism of Chemical Defense in Carnivorous Plant.</p> <p><i>Dionaea muscipula</i> can survive under nutritionally poor soil conditions by capturing insects with their trap lobes. On the other hand, this singular nature potentially exposes them to danger of feeding by herbivorous insects. If their leaves were wounded, the leaves would be seriously damaged by their own digestive enzymes. In order to prevent <i>D. muscipula</i> from preying on by predators, there should be some chemical defense mechanism. Extraction of <i>D. muscipula</i> was separated according to the cell toxicity against P388. As the result, many naphthoquinones, including a grate amount of plumbagin (0.5% of fresh weight), were obtained. Plumbagin also indicated strong antifeedant activity against herbivorous insect <i>Spodoptera litura</i>. Furthermore, it was revealed that high volatility of plumbagin made predator easy to introduce it. Then it worked as antifeedant by inhibiting respiratory chain of electron transfer system. Because other carnivorous plants also include large amount of naphthoquinone derivatives, it was suggested that naphthoquinone derivatives were universal chemical defensive substance in carnivorous plants.</p> <p>2. Chemical Study on the Substance Concerning Carnivorous Plant Movement and Memory.</p> <p>The leaf closure of <i>D. muscipula</i> is induced when sensory hairs are touched twice within thirty seconds. This phenomenon suggests that <i>D. muscipula</i> has “memory”. About 130 years ago, it was discovered that active potential induced by stimuli worked as the trigger of movement, and similarity to neuron transmitting was indicated. Since then there were no remarkable results about this phenomenon. Author assumed that the closure was induced by trigger of stepwise accumulation of secreted inner bioactive substance. Then isolation of the internal bioactive substance was examined according to the bioassay using the leaves of cloned <i>D. muscipula</i>. The bioactive substance was easily deactivated under condition of repeated column separation. This difficulty was overcome by careful separation and column selection, finally 124 µg of polysaccharide showed extremely strong activity at 2 ng/leaf was obtained. Structure elucidation of the small amount of polysaccharide was carried out measuring TOCSY spectra by 500 MHz NMR equipped with cold probe, and hydrolysis followed by constituent sugar analysis using CE. As the result, it was clarified that memory substance was a polysaccharide that consisted of 2-deoxysugar, hexose and unknown sugar. From the fact that the movement was induced only when concentration of this polysaccharide in leaves go beyond a threshold, it was suggested that the “memory” in <i>D. muscipula</i> was controlled by stepwise accumulation of the substance. Furthermore, <i>D. muscipula</i> reacted with neurotransmitter of higher animal at high concentration. This indicates the similarity of memory between carnivorous plant and higher animals. These results indicated that this polysaccharide would be neurotransmitter like substance concerning “memory” observed in higher plants.</p>		