（様式５）(Style5)

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：
Name **Md. Tazul Islam Chowdhury**

学位論文題目：
Title of Dissertation **Synthesis of Rare Sugar Derivatives and Their Biological Activity on Plant Growth**

（希少糖誘導体の合成と植物生長に対する生物活性）

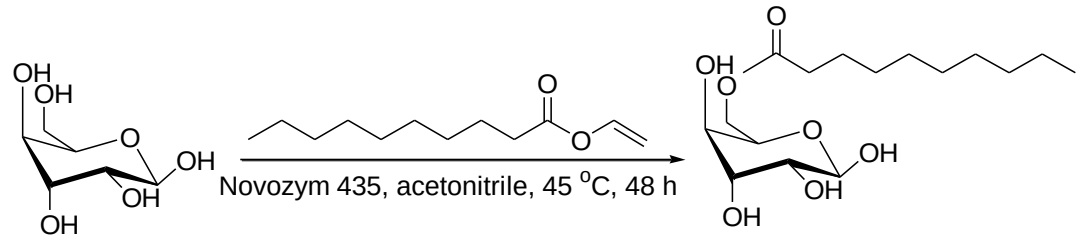
学位論文要約：
Dissertation Summary

Agriculture in the 21st century faces multiple challenges; many of today's food production systems are unsustainable. In appropriate use of synthetic chemicals causes hazardous problem to flora and fauna (Pell et al. 1998; Aktar et al. 2009; Dreistadt et al.1994). To avoid the detrimental effects of synthetic chemicals, research on novel natural products have moved from the fringe to the mainstream for the development of ecologically acceptable, environment friendly and relatively safe natural plant growth retardants. Synthetic growth retardants are becoming very popular in the farmers field but due to toxicological concerns, it has declined markedly in recent years, particularly for edible crops (Rademacher 2000). Therefore, to make environmentally friendly growth retardants, scientists are giving more concentration on natural products and their derivatives. In this regards, rare sugar derivative could replace as natural growth retardants, which is biodegradable and safe for the environment.

Rare sugars are monosaccharides that exist in nature in a limited amount. Izumori et al. developed a great idea for the mass production of rare sugar using recombinant enzymes from bacteria (Izumori 2002, 2006). Despite their limited presence in nature, rare sugars have various biological functions and vast applications in pharmaceutical, cosmetics, food, and flavor industries. The biological activities of rare sugars such as D-psicose (Takeshita et al. 2000), D-tagatose (Levin et al.1995) and D-allose (Bhuiyan et al. 1998; Sui et al. 2005; Gao et al. 2011; Hossain et al. 2000) has been extensively studied. Based on enzymatic synthesis, my research group already reported the synthesis of 6-*O*-acyl-D-allose from rare sugar, D-allose, via regioselective lipase catalyzed transesterification and its biological activity against plant growth (Afach et al. 2005, 2006). D-Allose fatty acid esters showed 2-20 times greater inhibitory activity than D-allose itself because the hydrophobic carbon chains of the D-allose derivatives

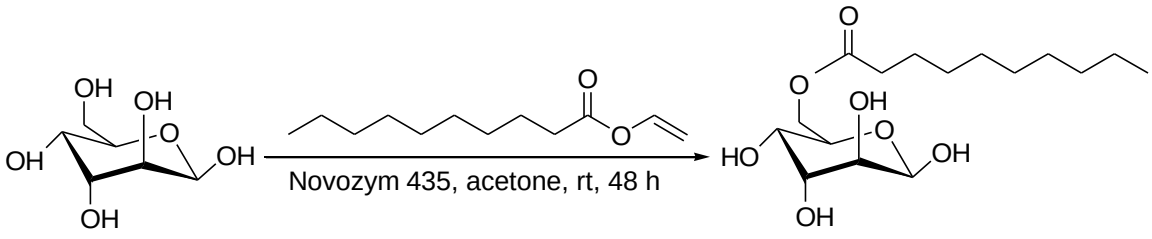
could enhance the surface activity and membrane permeability. Furthermore, they described that α-hydroxy group at C-3 might play an important role for the inhibitory activity because the 6-*O*-dodecanoate of D-mannose (C-2 epimer of D-glucose) and D-galactose (C-4 epimer of D-glucose) showed weaker effect on rice growth than that of D-allose (Kobayashi et al. 2010). Nowadays rare sugar research is important for further evaluation and application of rare sugar research. Therefore, considering the biodegradable plant growth retardants, the intention of the first study was to investigate the synthetic procedure of 6-*O*-decanoyl-D-altrose from D-altrose and 6-*O*-decanoyl-D-gulose from D-gulose.

The reactions of D-gulose and D-altrose with vinyl decanoate using *Candida antarctica* lipase in different organic solvents were carried out (Scheme 1 and 2) to obtain new monosacharride derivatives, Gul-C10 and Alt-C10 respectively. Then, the biological activity of Gul-C10 and Alt-C10 was evaluated against different plant species. Both of them showed similar inhibitory activity against cress, Italian ryegrass and rice seedlings but exhibited weaker activity against of lettuce.



D-gulose 6-*O*-decanoyl-D-gulose

Scheme 1 Synthesis of 6-*O*-decanoyl-D-gulose



D-altrose 6-*O*-decanoyl-D-altrose

Scheme 2 Synthesis of 6-*O*-decanoyl-D-altrose

Bioassay results showed that lettuce seedlings were not significantly inhibited by the D-altrose and D-gulose (Fig. 1). On the other hand, D-altrose C10 and D-gulose C10 significantly inhibited lettuce seedlings (Fig. 1) in a higher concentration (3 mM). However, these rare sugar derivatives showed

weaker inhibitory activity on the growth of lettuce shoot and root.

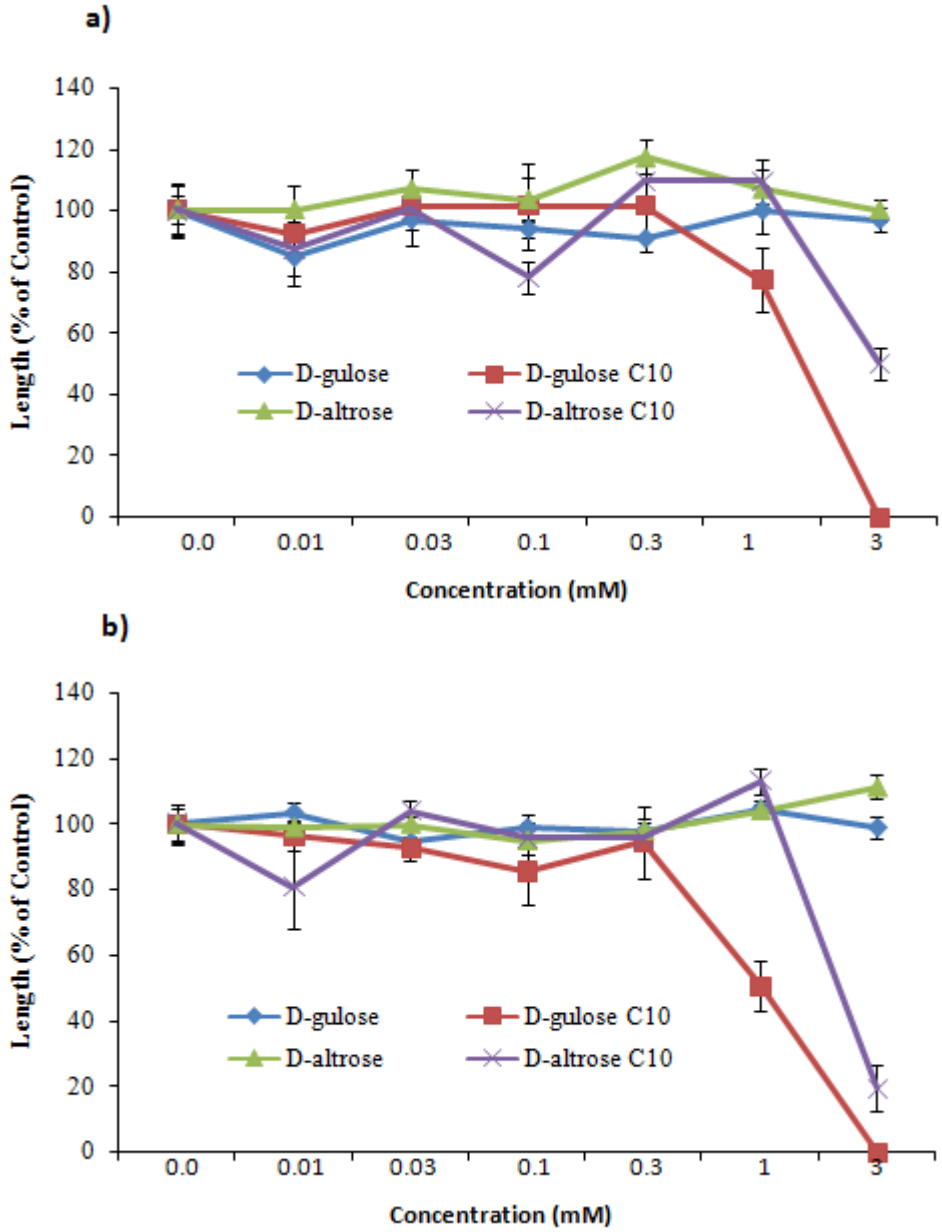


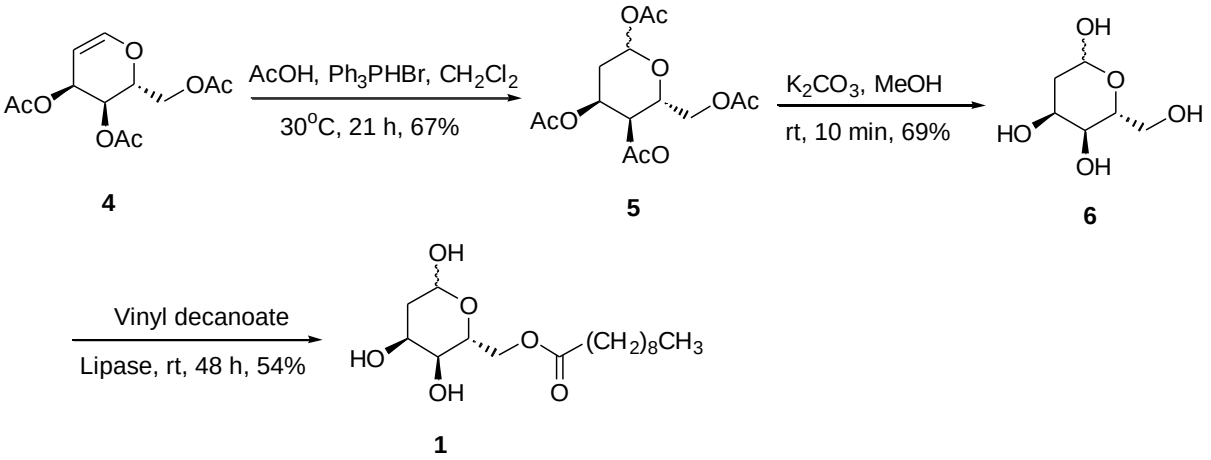
Fig. 1 Biological activity of rare sugars and their derivatives on **a)** shoot and **b)** root of lettuce seedlings. Means ± SEM from 3 independent experiments with 10 plants for each determination are shown

Next, to examine the structure-activity relationship (SAR) of the hydroxy groups at C-1 and C-2 of the D-allose esters, three different analogs of deoxy-D-allose ester were synthesized: 6-*O*-decanoyl-2-deoxy-D-allose (2-DOAll-C10), lacking a hydroxy group at C-2; 6-*O*-decanoyl-1,2-dideoxy-D-allose (1,2-DOAll-C10), which is saturated at C-1 and C-2; and 6-*O*-decanoyl-1,2-didehydro-1,2-dideoxy-D-allose (1,2-DHAll-C10), having a double bond between

C-1 and C-2. Then, their biological activities on plant growth were evaluated.

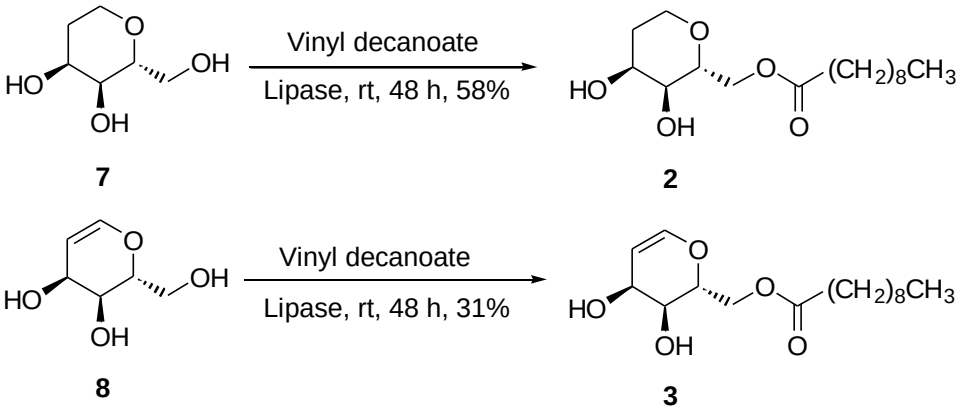
2-DOAll-C10 (**1**) was synthesized as shown in Scheme 3. First, triacetyl 1,2-DHAll (**4**) was added with AcOH (Morris and Shair 2009) to give 2-deoxy-D-allose tetraacetate (**5**) in a 67% yield because hydration with dilute H_2SO_4 afforded 5 in low yield (Haga and Tejima 1974). Then, **5** was hydrolyzed to 2-deoxy-D-allose (**6**) in a 69% yield. Finally, regioselective lipase-catalyzed transesterification with vinyl decanoate gave 2-DOAll-C10 (**1**) in a 54% yield.

6-*O*-Decanoyl-1,2-dideoxy-D-allose (**2**) and 6-*O*-decanoyl-1,2-didehydro-1,2-dideoxy-D-allose (**3**) were synthesized via regioselective lipase-catalyzed transesterification of 1,2-dideoxyallose (**7**) and 1,2-didehydro-dideoxyallose (**8**) with vinyl decanoate to give 1,2-DOAll-C10 (**2**) and 1,2-DHAll-C10 in a 58% and 31% yield, respectively (Scheme 4).



4 **5** **6** **1**

Scheme 3 Synthesis of 6-*O*-decanoyl-2-deoxy-D-allose (**1**)



7 **2** **8** **3**

Scheme 4 Synthesis of 6-*O*-decanoyl-1,2-dideoxy-D-allose (**2**) and 6-*O*-decanoyl-1,2-didehydro-1,2-dideoxy-D-allose

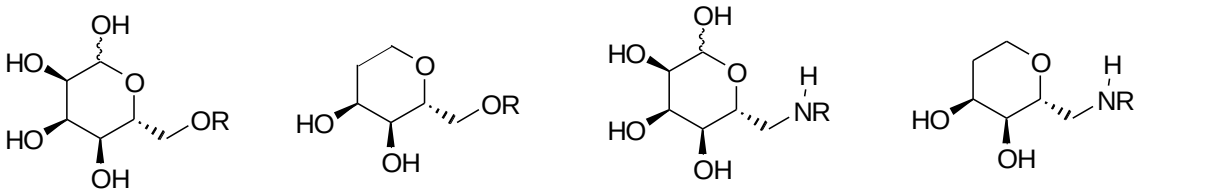
The biological activities of the deoxy alloses and their fatty acid esters were examined using lettuce, cress, Italian ryegrass, and rice seedlings. The fatty acid esters of the deoxy alloses (**1**–**3**) showed higher growth inhibitory activity on different test plants than the deoxy alloses. 2-DOAll-C10 (**1**) inhibited the growth in a concentration-dependent manner, which was a similar tendency to that of All-C10. Although 1,2-DOAll (**7**) showed growth-promoting activity, its ester, 1,2-DOAll-C10 (**2**), showed weak inhibitory activity at 3 mM. 1,2-DHAll-C10 (**3**) exhibited increased inhibitory activity to some extent compared with 1,2-DHAll (**8**). These results suggest the effectiveness of esterification of the hydroxy group with a medium fatty acid chain which could enhance surface activity and membrane permeability (Fujii 1996; Ogino 1996).

Table 1 IC_{50} values of deoxy-D-allose esters and All-C10 against four plant species.

	IC_{50} (mM)						
	Lettuce		Cress		Italian ryegrass		Rice
Compound	Shoot	Root	Shoot	Root	Shoot	Root	Secondary leaf sheath
1	1.02	0.98	0.37	0.40	0.51	0.48	0.19
2	>3.0	>3.0	>3.0	2.70	>3.0	3.0	2.56
3	2.83	2.03	0.56	0.39	1.02	0.6	0.42
All-C10	0.84	0.69	0.32	0.36	0.48	0.51	0.18

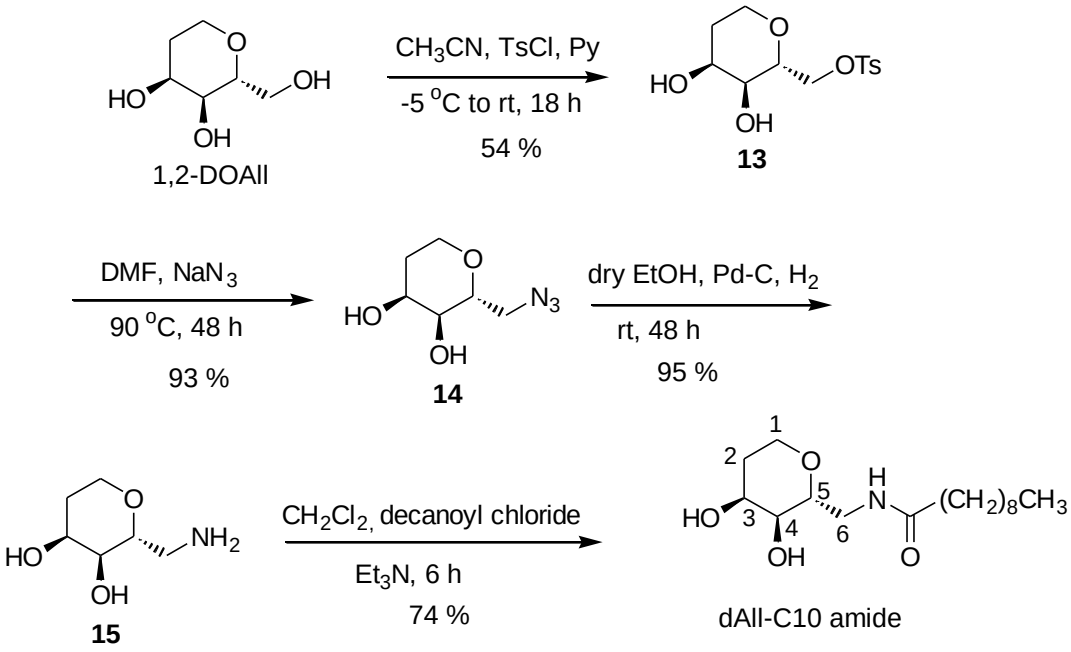
The concentrations required for 50% inhibition (IC_{50}) of lettuce, cress, Italian ryegrass, and rice seedling hypocotyls are listed in Table 1. 2-DOAll-C10 (**1**) showed inhibitory activity on four plant species similar to that of All-C10. 1,2-DHAll-C10 (**3**) showed inhibitory activity on cress, Italian ryegrass, and rice seedlings similar to that of All-C10, but lower inhibitory activity on lettuce. On the other hand, saturated 1,2-DOAll-C10 (**2**) exhibited considerably weaker inhibitory activity than the other deoxy allose esters against the four plant species. These results suggest that the hydroxy group at C-2 is not necessary for inhibitory activity and that the hydroxy group and/or hemiacetal structure at C-1 might play an important role in activity.

Then, a new deoxy derivative of All-C10 amide was synthesized and examined its inhibitory activity against plant growth to compare with deoxy All-C10 ester. 6-(Decanoylamino)-1,2,6-trideoxy-D-allose was prepared from the known compound 1,2-dideoxy-D-allose via regioselective tosylation, azidation, catalytic hydrogenation, and acylation with decanoyl chloride (Scheme 5).



9: R = $CO(CH_2)_8CH_3$ **10**: R = $CO(CH_2)_8CH_3$ **11**: R = $CO(CH_2)_8CH_3$ **12**: R = $CO(CH_2)_8CH_3$

Fig. 2 Structures of All-C10 (**9**), dAll-C10 (**10**), All-C10 amide (**11**), and dAll-C10 amide (**12**)



Scheme 5 Synthesis of 6-(decanoylamino)-1,2,6-trideoxy-D-allose (**12**)

The biological activity of 6-(decanoylamino)-1,2,6-trideoxy-D-allose was evaluated using four test species. dAll-C10 amide inhibited the growth of the lettuce seedlings in a concentration-dependent manner ranging from 0.03 to 1 mM, and interestingly, slight growth promotion (119%) was observed of the roots at 0.1 mM (Fig. 3a). The growth of the cress and Italian ryegrass seedlings was significantly inhibited by dAll-C10 amide in a concentration-dependent manner (Fig. 3b and 3c). The growth inhibitory activity of dAll-C10 amide was also evaluated using rice seedlings. The inhibitory activity increased in a concentration-dependent manner from 0.1 to 1 mM, and the growth of the rice seedlings was completely inhibited by dAll-C10 amide at a concentration greater than 1 mM (Fig. 3d).

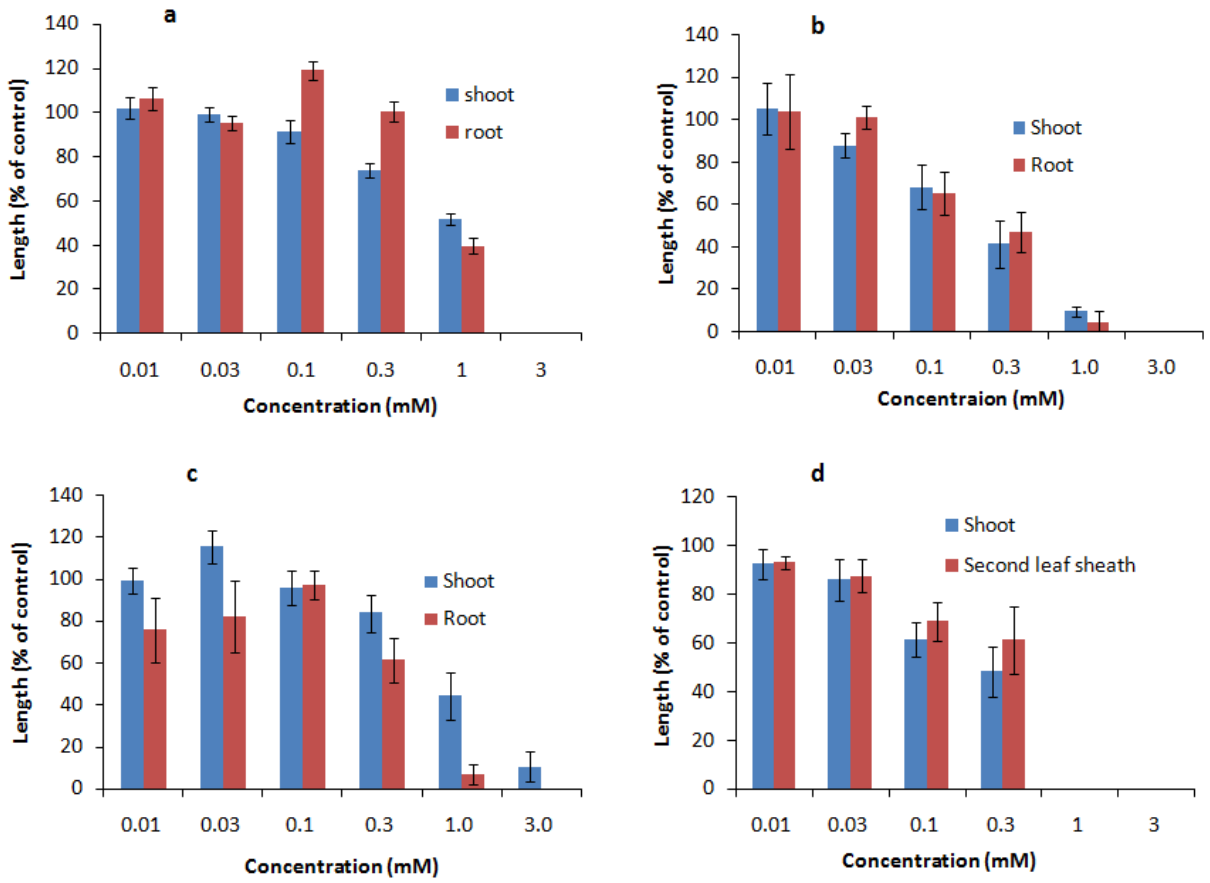


Fig. 3 Biological activity of dAll-C10 amide in a) lettuce, b) cress, c) Italian ryegrass, and d) rice seedlings. Values are mean ± SEM from three independent experiments

Comparing with those of other D-allose derivatives, the activity of dAll-C10 amide was slightly weaker than that of All-C10 but significantly higher than that of dAll-C10. These results indicate that replacement of the ester group with an amide group has opposite effects on D-allose derivatives and deoxy-D-allose derivatives. The concentrations required for 50% inhibition (defined as IC_{50}) of Alt-C10, Gul-C10, 1,2-DHAll-C10, 1,2-DOAll-C10, 2-DOAll-C10 and dAll-C10 amide (Fig. 4) against lettuce, cress, Itlian ryegrass, and rice seedling hypocotyls were displayed in Fig. 5. All-C10, Alt-C10, Gul-C10, 1,2-DHAll-C10, 2-DOAll-C10 and dAll-C10 amide showed almost similar inhibitory activity on rice, cress and Italian ryegrass seedlings. But incase of lettuce, Alt-C10, Gul-C10 and 1,2-DHAll-C10 exhibited lower inhibitory activity than All-C10, 2-DOAll-C10 and dAll-C10 amide, while 1,2-DOAll-C10 displayed very weak activity against all the test species. Among the four different test plants, rice was most sensitive to show the inhibitory activity.

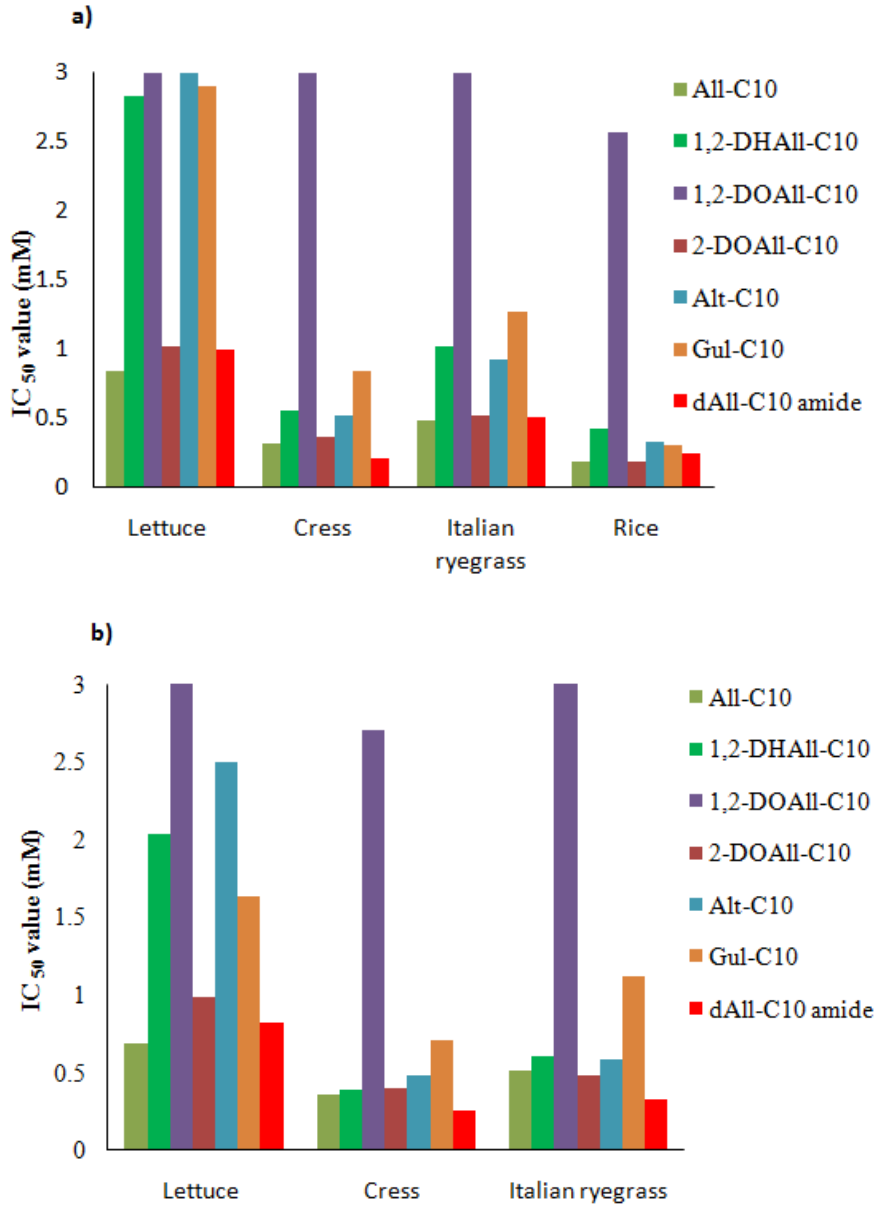


Fig. 5 IC_{50} values of the rare sugar derivatives on **a)** shoot and **b)** root of different plant species

These results suggested that the hydroxy group at C-1 and C-3 position is important to exhibit inhibitory activity on plant growth for rare sugar derivatives of 2-DOAll-C10 and All-C10. Moreover, the amide group of deoxy allose at C-6 is also important to increase the retarding activity on plant growth.

To disclose the mechanism of growth-inhibitory activity of rare sugar derivatives on plants, the effect of active gibberellin with rare sugar derivatives on rice seedlings were examined because it has also been reported that some growth inhibitors retarded the rice shoot elongation and this growth was able to be recovered by the treating with GA_3 (Rademacher et al. 1991; Graebe 1987). In these studies, the

recoveries of rice growth were also observed by the co-addition of GA_3.

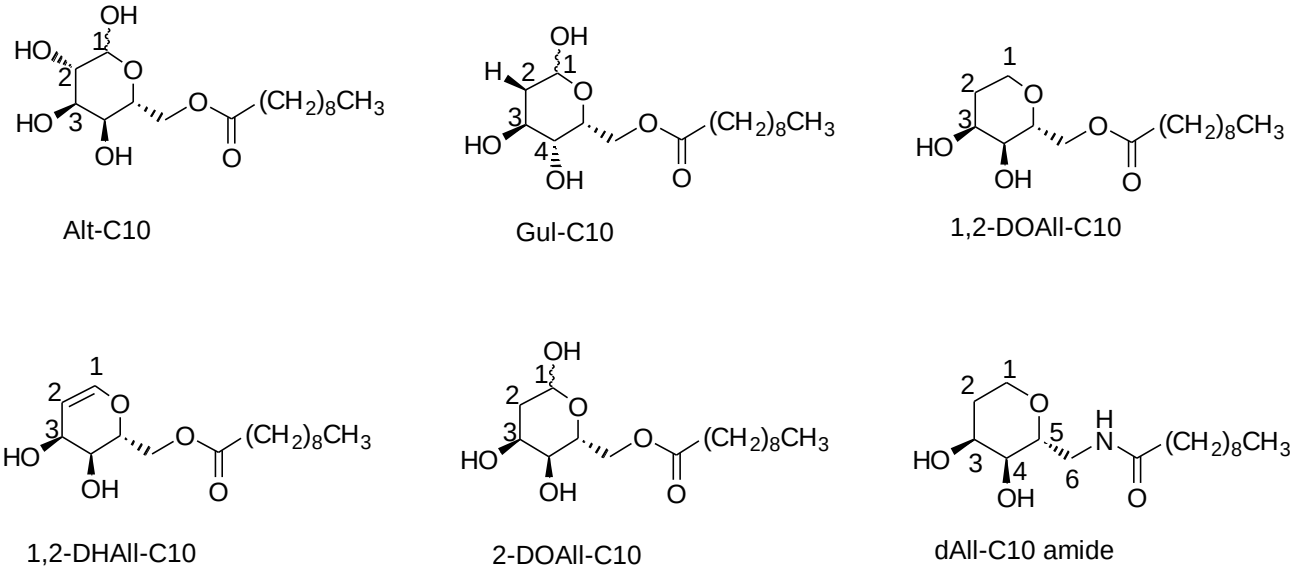


Fig. 4 Rare sugar derivatives

Moreover, daminozide was used as a positive control for growth recovery experiment. Rice growth was recovered in the similar tendency to rare sugar esters when GA_3 was co-added with a solution of daminozide (Fig. 6).

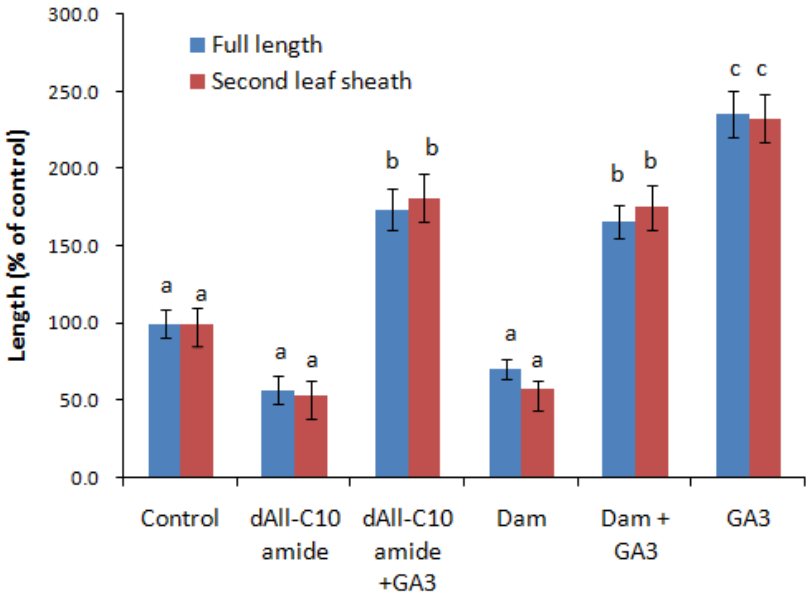


Fig. 6 Effect of dAll-C10 amide, daminozide (Dam), and GA_3 on rice seedlings. Relative lengths (% of control) of shoot and second leaf sheath of rice at 7 days after treatment. Values are mean ± SEM (n = 14), and bars with different letters indicate significant difference as determined by Tukey's honestly significant difference comparison ($p<0.05$)

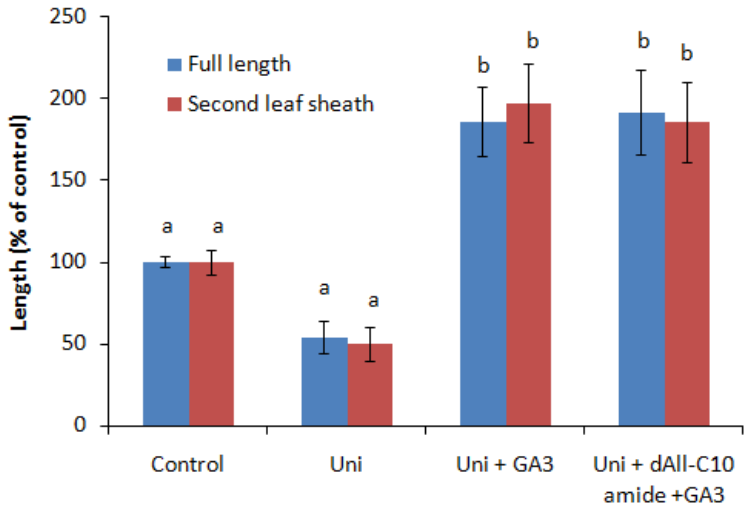


Fig. 7 Effect of uniconazole (Uni), GA_3, and dAll-C10 amide on rice seedlings. Relative lengths (% of control) of shoot and second leaf sheath of rice at 7 days after treatment. Values are mean ± SEM (*n* = 14), and bars with different letters indicate significant difference as determined by Tukey's honestly significant difference comparison ($p<0.05$)

Next, the effect of dAll-C10 amide on the GA signaling pathway in rice was examined using another known growth retardant, uniconazole, which inhibits the conversion of ent-karuene to ent-karuonic acid in an earlier stage of gibberellin biosynthesis (Rademacher 2000). Co-addition of GA_3 and dAll-C10 amide on rice seedlings pre-treated with uniconazole resulted in similar recovery (Fig. 7), suggesting that dAll-C10 amide does not inhibit GA signaling in the way D-allose does. This is the main difference with D-allose beacause it has been shown that D-allose inhibits the gibberellins signal transduction pathway; thus growth seedlings and elongation of the second leaf sheath of rice were inhibited (Fukumoto et al. 2011, 2013).

Therefore, it may conclude that rare sugar derivative might mainly block the formation of active gibbrellins in the same way to the known plant growth retardants daminozide and 16,17-dihydro-GA_5. These results suggest that rare sugar derivative could be a new class of lead compounds for plant growth regulators similar to known growth retardants and may contribute to establish a biodegradable and environmental friendly plant growth regulator.

References

Afach G, Kawanami Y, Izumori K (2005) Synthesis of D-allose fatty acid esters via lipase catalyzed regioselective transesterification. Biosci Biotechnol Biochem 69:833–835

Afach G, Kawanami Y, Kato-Noguchi H, Izumori K (2006) Practical production of 6-*O*-octanoyl-D-allose and its biological activity on plant growth. Biosci Biotechnol Biochem 70:2010–2012

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdisc Toxicol 2(1):1–12

Bhuiyan SH, Itami Y, Rokui Y, Katayama T, Izumori K (1998) D-allose production from D-psicose using immobilized L-rhamnose isomerase. J Ferment Bioeng 85:539-541

Dreistadt SH, Clark JK, Flint ML (1994) An integrated pest management guide. University of California Division of Agriculture and Natural Resources; Pests of landscape trees and shrubs. Publication 3359

Fujii T (1996) Biosurfactants. J Jpn Oil Chem Soc 45:1013–1022

Fukumoto T, Kano A, Ohtani K, Kokudo YY, Kim BG, Hosotani K, Saito M, Shirakawa C, Tajima S, Izumori K, Ohara T, Shigematsu Y, Tanaka K, Ishida Y, Nishizawa Y, Tada Y, Ichimura K, Gomi K, Akimitsu K (2011) Rare sugar D-allose suppresses gibberellin signaling through hexokinase-dependent pathway in *Oryza sativa* L. Planta 246(6):1083–1095

Fukumoto T, Kano A, Ohtani K, Inoue M, Yoshihara A, Izumori K, Tajima S, Shigematsu Y, Tanaka K, Ohkouchi T, Ishida Y, Nishizawa Y, Tada Y, Ichimura K, Gomi K, Yoo S, Sheen J, Akimitsu K (2013) Phosphorylation of D-allose by hexokinase involved in regulation of OsABF1 expression for growth inhibition in *Oryza sativa* L. Planta 237:1379–1391

Gao D, Kawai N and Tamiya T (2011) The anti-inflammatory effects of D-allose contribute to attenuation of cerebral ischemia-reperfusion injury. Med Hypotheses 76:911-13

Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465

Haga M, Tejima S (1974) Synthesis of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*ribo*-hex-1-enitol and 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*ribo*-hex-1-enitol. Carbohydrate Research 34:214–218

Hossain MA, Wakabayashi H, Goda F, Kobayashi S, Maeba T, Maeta H (2000) Effect of the immunosuppressants FK506 and D-allose on allogenic orthotopic liver transplantation in rats.

Transplant Proc 32:2021–2023

Izumori K (2002) Bioproduction strategies for rare hexose sugars. Naturwissenschaften 89:120–124

Izumori K (2006) Izumoring: a strategy for bioproduction of all hexoses. J Biotechnol 124:717–722

Kobayashi M, Ueda M, Furumoto T, Kawanami Y (2010) Retarding activity of 6-*O*-acyl-D-allose against plant growth. Biosci Biotechnol Biochem 74:216–217

Levin GV, Zehner LR, Saunders JP, Beadle JR (1995) Sugar substitutes: their energy values, bulk characteristics, and potential health benefits. Am J Clin Nutr 62:1161S-1168S

Morris WJ, Shair MD (2009) Stereoselective synthesis of 2-deoxy-ß-glycosides using anomeric o-alkylation/arylation. Org Lett 11(1):9–12

Ogino K (1996) Research and development of novel surfactants. J Jpn Oil Chem Soc 45:921–932

Pell M, Stenberg B, Torstensson L (1998) Potential denitrification and nitrification tests for evaluation of pesticide effects in soil. Ambio 27:24–28

Rademacher W (1991) Inhibitors of gibberellin biosynthesis: applications in agriculture and horticulture. In: Takashi N, Phinney BO, MacMillan J (eds) Gibberellins. Springer, New York. pp 296–310

Rademacher W (2000) Growth retardants effects on gibberellins biosynthesis and other metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol 51:501–531

Sui L, Dong Y, Watanabe Y, Yamaguchi F, Hatano N, Izumori K (2005) Growth Inhibitory Effect of D-allose on Human Ovarian Carcinoma Cells *In Vitro*. Anticancer Research 25:2639-2644

Takeshita K, Suga A, Takada G, Izumori K (2000) Mass production of D-psicose from D-fructose by a continuous bioreactor system using immobilized D-tagtose 3-epimerase. J Biosci Bioeng 90:453-455

(注)　要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。
(Note)　The Summary should be about 10％ of the entire dissertation and may include illustrations