A Novel Approach to Regulate Turfgrass Growth by Using Yucaizol, a Potent Inhibitor of Brassinosteroid Biosynthesis

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***Abstract*—Brassinosteroids are important phytohormones that affect many aspects of plant growth and development. We have shown that brassinosteroid biosynthesis inhibitor exhibits potent inhibitory activity on retardation stem elongation of *Arabidopsis* seedlings. To develop new plant growth regulators for turf management, we use Yucaizol, a potent brassinosteroid (BR) biosynthesis inhibitor developed in our laboratory, for biological evaluations. Effect of Yucaizol on retardation of pencross growth indicates that it exhibits potent activity. The Yucaizol significantly reduce the penncross growth at 100 g a.i./ha in our assay system. Although plant growth regulators that targeting on gibberellic acid (GA) biosynthesis have been widely used for golf course management, our results demonstrate the potential utility of BR biosynthesis inhibitors as new plant growth regulators.**

***Index Terms*—brassinosteroid biosynthesis inhibtors, triazole derivatives, plant growth regulators, turf management**

I. Introduction

Turfgrasses provide an enhanced environment by purifying and protecting water, soil and air on a daily basis. With the expanding urbanization, turfgrass industry has been grown rapidly for the last several decades. Management of high quality turf is challenging, in golf course maintenance, for example, to achieve desired conditions by keeping turfgrass at a height of 2-3 mm, daily mowing is required during the growing season which spent large sums of money annually worldwide.

Plant growth regulators (PGRs) are clearly a sophisticated tool for turf management. They were initially developed to slow turf growth, suppress seed heads and decrease the need to mow steep slopes, highway roadsides, airports and golf course roughs. Currently, PGRs or inhibitors are increasingly being used on golf courses. Depending upon the turfgrass and situation, PGRs may reduce mowing costs, prevent scalping, increase turf density and decrease the need to mow steep slopes.

To meet the demands for laborsaving in turf management and improve the quality of turf, we conducted a systematic search for new PGRs by using specific inhibitors of brassinosteroids (BRs) biosynthesis developed in our laboratory.

Brassinosteroids are important signal molecules that involved in plant growth and development. Molecular genetic analysis established the role of BRs as endogenous plant hormones with well-defined functions including extreme dwarfism, delayed senescence, male sterility, and constitutive photomorphogenesis in the dark [1]. Exogenous application of BR has been shown to promote cell expansion and division, regulating leaf senescence, pollen development, fruit ripening, and to modulate the plant response to numerous environmental cues [1]-[3]. BRs deficient mutants of rice, tomato and *Arabidopsis* display dwarf phenotypes [4]-[6]. In this context, manipulating BR content in plant tissues may have remarkable effect on plant growth. Overexpression of DWARF4, an enzyme catalyzes a rate limiting step in BR biosynthesis, enhances plant growth in *Arabidopsis* [7]. Similarly, transgenic rice plants over expressing a sterol C-22 hydroxylase that catalyzes a key step in BR biosynthesis increasing the seed yield [8]. Based on these observations, knockout mutations in BR biosynthesis may be useful for breeding dwarf plants for agricultural applications including rice and turf. An alternative method to manipulate the BR levels in plant tissues is the use of specific inhibitors. Our research interest is the design and synthesis of novel plant hormone biosynthesis inhibitors [9]-[14]. In the course of work, we carried out a bio-rational approach using ketoconazole as a molecular scaffold, which lead to the discovery of a new series of BR biosynthesis inhibitors. (*YCZ-series*, general chemical structure shown in Fig. 1) [13].

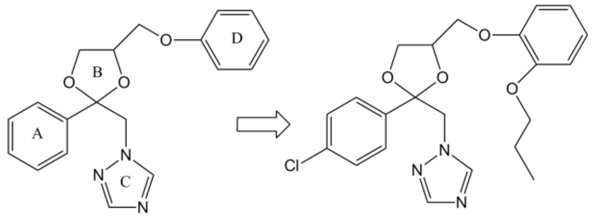

Figure 1. Chemical structures of **BR** biosynthesis inhibitors

Initial structure–activity relationship studies indicated that the introduction of a mono substitution of a chlorine atom at position 4 or a double substitution of chlorine atoms at Gernaral structure of *YCZ Yucaizol* positions 2

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and 4 on ring A (Fig. 1) exhibited potent inhibition on BR biosynthesis [14]. We also found that substitutions on ring D (as shown in Fig. 1) dramatically affected the inhibitory potency against BR biosynthesis of this synthetic series [13]. Further studies on the structure–activity relationships of this synthetic series revealed a potent and selective inhibitor, Yuzaizol (the structure is shown in Fig. 1), with an IC_{50} value approximately 50 nM on retardation stem elongation of *Arabidopsis* seedlings [15].

The objective of this study is to meet the demands for laborsaving in golf course maintenance by using chemicals that induce dwarf phenotype of turfgrasses. In the present work, we investigated the biological activity of Yucaizol, a potent inhibitor of brassinosteroid (BR) biosynthesis developed in our laboratory, on retardation penncross growth, a major turfgrass used in golf course.

II. Materials and Methods

A. General

Chemicals for synthesis were purchased from Kanto Chemicals Co., Ltd (Tokyo, Japan) and Tokyo Kasei Co., Ltd (Tokyo, Japan). Melting points (mp) were determined using a Yanako melting point apparatus. ^{1}H NMR spectra were recorded with a JEOL ECP-400 spectrometer, with chemical shifts expressed in ppm downfield from tetramethylsilane as an internal standard. High resolution mass spectra by electrospray ionization Fourier transform ion cyclotron resonance (ESI-FTICR) was taken using an Exactive MS System (Thermo Fisher Scientific, USA).

B. Plant Growth Conditions and Inhibitor Treatment

Plants from thermal *Agrostis scabra,* and cultivar Penncross of creeping bentgrass (*A. stolonifera*) were established in polyethylene bags filled with sand (10 cm in diameter and 20 cm in length) in a growth chamber.

Holes were made at the bottom of the bags for drainage. Plants were watered daily and fertilized twice a week with a nutrient solution containing 1 mM KNO_3, 0.2 mM $CaSO_4$, 0.35 mM KH_2PO_4, 2 mM $MgSO_4$, 0.2 g l^{-1} Fe-NaEDTA, and micronutrients. The plants were grown with a 14 h photoperiod, 20/15 ^{0}C day/night temperature, and a photosynthetic photon flux density of 350 mmol m^{-2} s^{-1} at canopy height. Chemicals treatment was carried out by spraying test compounds to the plants at designated concentrations. Plant heights were measured accordingly. Stock solutions of all the chemicals were dissolved in DMSO at a designed concentration.

III. Results and Discussions

The development of a synthetic route for the preparation of target Yucaizol is outlined in Fig. 2. The key transformation of *B* with *E* consisted of four steps: (1) formation of 1-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1yl) ethanone *B* [16]; (2) tosylation of isopropylideneglycerol *C*; (3) deprotection of isopropylidene ketal *D*; and (4) ketal formation to generate *F*. Compound *B* was prepared by reacting α-bromoketone *A* with triazole in DMF using a method that we described previously [15]. The tosylation of isopropylidene glycerol *C* was achieved using a standard protocol (tosyl chloride in pyridine at 0^{o}C), and hydrolysis with 1 M HCl in MeOH yielded glyceryl tosylate *E*. Ketal formation to generate *F* was carried out using 3 equiv of trifluoromethanesulfonic acid (TfOH) in toluene at room temperature for 60 h, according to a method previously described [15]. The target compound Yucaizol was prepared by reacting *F* with 2-propoxylphenol in a basic condition, as described previously [15]. All of the compounds synthesized in this work consist of four stereoisomers, and they were subjected to biological studies without further purification.

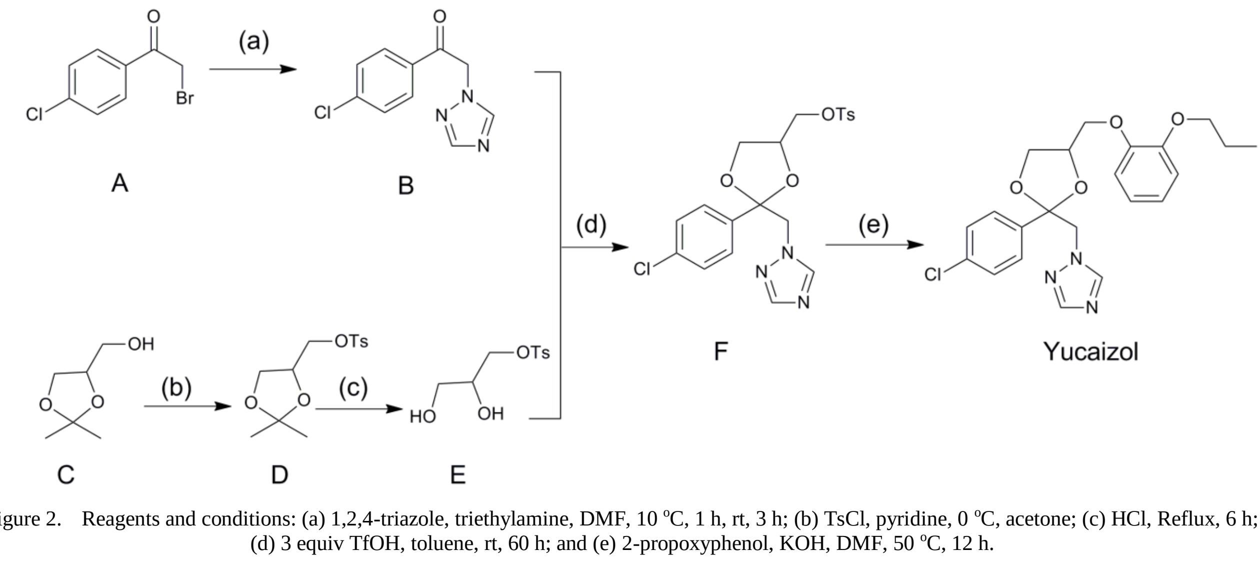

Figure 2. Reagents and conditions: (a) 1,2,4-triazole, triethylamine, DMF, 10 ^{o}C, 1 h, rt, 3 h; (b) TsCl, pyridine, 0 ^{o}C, acetone; (c) HCl, Reflux, 6 h; (d) 3 equiv TfOH, toluene, rt, 60 h; and (e) 2-propoxyphenol, KOH, DMF, 50 ^{o}C, 12 h.

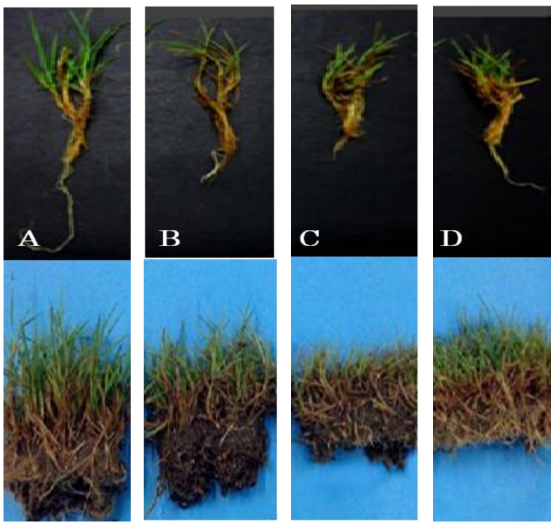

Figure 3. Effect of Yucaizol and Trinexapac-ethyl (Primo MAXX) on penncross growth. The growth condition was as described in the section of material and methods. The plants were displayed after 60 days of spraying the chemicals. A. Control; B. Yucaizol (30 g a.i./ha), C. Yucaizol (100 g a.i./ha), D. Trinexapac-ethyl (100 g a.i./ha). The upper panels are close look of the corresponding plants.

To determine the biological activity of Yucaizol on retardation penncross growth, a GA biosynthesis inhibitor Trinexapac-ethyl (Primo MAXX) was used as a positive control. The penncross treated with and without chemicals were shown in Fig. 3. The upper panel display a close look of the plants. The chemical treatment starts at a plant height approximately 3 cm. As shown in Fig. 3, after 60 days of chemical treatment, the plant height of none chemical treated penncross (Fig. 3A) was approximately 8.5 cm. In contrast, the plant height of Yucaizol treated plant (30 g a.i./ha, Fig. 3B) was approximately 7.2 cm, indicating that Yucaizol mildly retards penncross growth approximately 20%. This retardation effect of Yucaizol was increased with the increasing of the concentration of Yucaizol. The plant hight was reduced to approximately 4.5 cm (100 g a.i./ha, Fig. 3C) which is similar to the plant height of Trinexapac-ethyl treatment.(100 g a.i./ha, Fig. 3D). The plants of both chemical treated plants are at the same height. This result clearly indicates that Yucaizol exhits potent activity on retardation penncross growth.

Next, we determine the time course of the effects of Yucaizol and Trinexapac-ethyl on penncross growth. The plant height was measured each week. The amound of of Yucaizol for plant treatment were designed at 30, 50, and 100 (g. a.i./ha). The positive control of Trinexapac-ethyl was 100 (g a.i./ha). As shown in Fig. 4, the plant height was increased along with the incubation time in none chemical treated plants (blue diamond). The plant height was increasing with the increasing the amount of Yucaizol (red square: 30 g a.i./ha; green triangle: 50 g a.i./ha; orange cycle: 100 g a.i./ha). When treated penncross with Yucaizol and Trinexapac-ethyl (blue asterisk) at 100 g a.i./ha, the plant height was almost at the same height at different incubation time. This result indicates that Yucaizol exhibits potent inhibitory activity on retardation penncross growth.

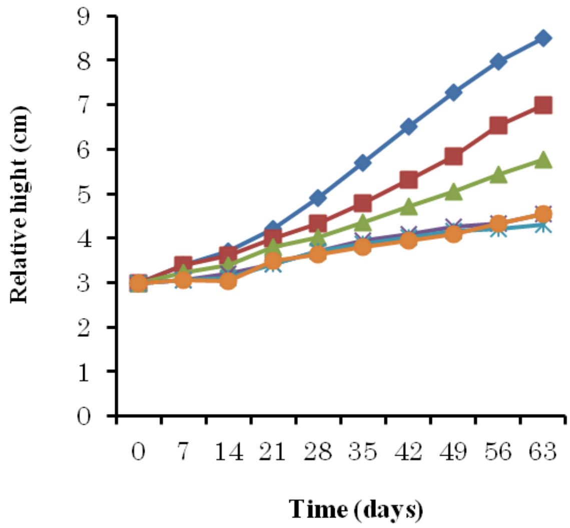

Figure 4. Time course of growth inhibition of penngrass with the treatment of Yucaizol and Trinexapac-ethyl.

IV. Conclusion

We tested novel specific inhibitors of BR biosynthesis, Yucaizol, on retardation penncross. We found that Yucaizol exhibits potent inhibitory activity on retardation penncross growth. The potency of Yucaizol is similar to turfgrass growth regulator: Trinexapac-ethyl. Our findings suggest that inhibitors of BR biosynthesis may be a potential candidate as a novel turfgrass growth regulator.

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