Effects of a New Plant Growth Regulator Prohexadione Calcium (BX-112) on Shoot Elongation Caused by Exogenously Applied Gibberellins in Rice (Oryza sativa L.) Seedlings

Article in Plant and Cell Physiology · January 1990
DOI: 10.1093/oxfordjournals.pcp.a077892 · Source: OAI

CITATIONS
79

READS
316

6 authors, including:

Yuji Kamiya
RIKEN
375 PUBLICATIONS 22,151 CITATIONS

Akira Sakurai
192 PUBLICATIONS 5,045 CITATIONS

Some of the authors of this publication are also working on these related projects:

Chemical biology View project

Leaf growth regulation in maize View project
Effects of a New Plant Growth Regulator Prohexadione Calcium (BX-112) on Shoot Elongation Caused by Exogenously Applied Gibberellins in Rice (Oryza sativa L.) Seedlings

Ishizue Nakayama¹, Takeshige Miyazawa¹, Masatomo Kobayashi², Yuji Kamiya², Hiroshi Abe¹ and Akira Sakurai²

¹ Life Science Research Institute, Kumiai Chemical Industry Co., Ltd., Kikugawa-cho, Ogasa-gun, Shizuoka, 439 Japan
² The Institute of Physical and Chemical Research, Wako-shi, Saitama, 351-01 Japan

The effects of a novel plant growth regulator (PGR) prohexadione calcium (BX-112; calcium 3,5-dioxo-4-propionylcyclohexanecarboxylate) on shoot elongation caused by exogenously applied GA₉, GA₃, GA₄, GA₁₉ and GA₃₀ were investigated in rice (Oryza sativa L. cv. Nihonbare and cv. Tan-ginbozu) seedling test. Depending on the dose, BX-112 reduced shoot elongation in both cultivars caused by GA₉ and GA₃₀, but not by GA₃. When a high dose of BX-112 (e.g. 250 ng/plant and over) was applied with GA₉ or GA₄, shoot elongation was even promoted. This promotive effect, however, was not observed with GA₃. These results suggest that BX-112 inhibits gibberellin (GA) biosynthesis in the rice plant at the 3β- and 2β-hydroxylation of GAs, namely steps of activation and inactivation, respectively.

Key words: Gibberellin biosynthesis inhibitor — Oryza sativa L. — Plant growth regulator — Prohexadione calcium (BX-112) — Rice seedling test.

A number of plant growth retardants inhibit GA biosynthesis of plants. Quaternary ammonium compounds such as AMO-1618 [(2-isopropyl-5-methyl-4-trimethylammoniumchloride)-phenyl-1-piperidinium-carboxylate] and its analogues inhibit ent-kaurene synthesis in cell-free systems of plants (Graebe 1987 recent review). Ancymidol [α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrimidine methyl alcohol] inhibits three oxidation steps from ent-kaurene to ent-kaurenoic acid in cell-free systems (Coolbaugh et al. 1978). Recently, new growth retardants such as tetcyclacis [5-(4-chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-5,4,1,0²⁶,0⁸>l0-dodeca-3,9-diene], uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol], paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentane-3-ol] and inabenfide [4-chloro-2′-(α-hydroxybenzyl)isonicotinamidide], have been developed, and these compounds inhibit GA biosynthesis at the same oxidation steps as ancymidol (Rademacher et al. 1984, Izumi et al. 1985, Hedden and Graebe 1985, Miki et al. 1990). The inhibitory sites in GA biosynthesis by all of these growth retardants are in the pathway before the ent-gibberellane skeleton is formed. The dwarfing caused by these retardants is alleviated by exogenously applied GA₃ and other bioactive GAs (Izumi et al. 1984, Rademacher et al. 1987), indicating that the inhibition of GA biosynthesis is the primary action of these retardants.

BX-112, prohexadione calcium (calcium 3,5-dioxo-4-propionylcyclohexanecarboxylate) (Fig. 1) is a novel PGR, and it, along with its derivatives will reduce shoot elongation of a number of plants (Motojima et al. 1984, 1985). BX-112 has promising characteristics for an agricultural chemical, such as quick action by foliage treatment and no residual activity on rotational crops. In our preliminary experiments the growth retardation in the rice plant caused by BX-112 was fully alleviated by exogenously applied GA₃, suggesting that BX-112 inhibits GA biosynthesis.

Herein, the effect of BX-112 on shoot elongation caused by exogenously applied GAs was investigated in rice seedlings. The inhibitory sites of GA biosynthesis by BX-112 are also discussed.
Materials and Methods

Chemicals—BX-112 was synthesized at K-I Chemical Research Institute Co., Ltd. (Shizuoka, Japan) and its purity was 94%; the residual 6% is almost all inorganic compounds.

GA\textsubscript{1}, GA\textsubscript{3}, GA\textsubscript{19} and GA\textsubscript{20} (Fig. 2) were supplied by Prof. N. Takahashi, Department of Agricultural Chemistry, The University of Tokyo. GA\textsubscript{3} was purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan).

Plant materials and applications—Seeds of rice (Oryza sativa L.), normal cv. Nihonbare and dwarf cv. Tan-ginbozu, were soaked in water for two days after sterilization (Murakami 1968a). Germinated seeds were planted on 1% agar medium in 30 ml beakers and incubated for 48 h at 30°C under continuous illumination from fluorescent lamps (6,000 lux, FLR40S.W/M; Toshiba Co., Ltd., Tokyo) for cv. Nihonbare and from fluorescent and metal halide lamps (11,000 lux, FLR40S.W/M and DR400/T(L); Toshiba Co., Ltd., Tokyo) for cv. Tan-ginbozu. BX-112 and the GAs were consecutively applied (1 μl per seedling) in 50% acetone to the region between the coleoptile and the first leaf of seedling using microsyringe. Treated plants were grown under the same conditions for another three days and the length of second leaf sheath was measured.

Results

BX-112 reduced shoot elongation in both cultivars of rice relative to controls without GAs (Fig. 3, 4). The length of the second leaf sheath was reduced to 49% of control by BX-112 at a dose of 2,500 ng/plant in cv. Nihonbare and to 67% of control at a dose of 250 ng/plant in cv. Tan-ginbozu.

Effects of BX-112 on the shoot elongation caused by exogenously applied GAs were quite different between GA\textsubscript{19} or GA\textsubscript{20}, and GA\textsubscript{3}. Shoot elongation caused by GA\textsubscript{19} or GA\textsubscript{20} was reduced by BX-112 in both cultivars depending on amounts of BX-112 applied (Fig. 3, 4). When GA\textsubscript{19} or GA\textsubscript{20} was applied to rice seedlings at doses of 1 to 100 ng/plant with BX-112 at a dose of 2,500 ng/plant, the length of the second leaf sheath was shorter than that of seedlings without BX-112 treatment (Fig. 3, 4).

In cv. Nihonbare, seedlings with GA\textsubscript{1} applied at a dose of 10 ng/plant were retarded when BX-112 was applied up to a dose of 25 ng/plant. However, shoot elongation was promoted by BX-112 when it was applied at more than 250 ng/plant if GA\textsubscript{1} was also applied (Fig. 3). In cv. Tan-ginbozu, shoot elongation caused by GA\textsubscript{1} at a dose of 10 ng/plant was not reduced by BX-112 at any doses. BX-112 at high doses (250 to 2,500 ng/plant) also promoted shoot elongation when GA\textsubscript{1} was applied. When GA\textsubscript{1} was applied alone at a dose of 1 ng/plant, no shoot elongation occurred, whereas shoot elongation was stimulated up to 130% of control seedlings by an application of BX-112 (2,500 ng/plant) with GA\textsubscript{1} at a dose of 1 ng/plant (Fig. 4).

Figures 5 and 6 show the effects of BX-112 on shoot elongation caused by GA\textsubscript{3} and GA\textsubscript{4}, which are bioactive but are apparently not endogenous GAs in the rice plant shoot under vegetative conditions (Kobayashi et al. 1988). In cv. Nihonbare, shoot elongation caused by GA\textsubscript{3} was not reduced by BX-112 at any doses. BX-112 at high doses (250 to 2,500 ng/plant) also promoted shoot elongation when GA\textsubscript{3} was applied. When GA\textsubscript{3} was applied alone at a dose of 1 ng/plant, no shoot elongation occurred, whereas shoot elongation was stimulated up to 130% of control seedlings by an application of BX-112 (2,500 ng/plant) with GA\textsubscript{3} at a dose of 1 ng/plant (Fig. 4).

Figures 5 and 6 show the effects of BX-112 on shoot elongation caused by GA\textsubscript{1} and GA\textsubscript{4}, which are bioactive but are apparently not endogenous GAs in the rice plant shoot under vegetative conditions (Kobayashi et al. 1988). In cv. Nihonbare, shoot elongation caused by GA\textsubscript{1} was not reduced by BX-112 at any doses. BX-112 at high doses (250 to 2,500 ng/plant) also promoted shoot elongation when GA\textsubscript{1} was applied. When GA\textsubscript{1} was applied alone at a dose of 1 ng/plant, no shoot elongation occurred, whereas shoot elongation was stimulated up to 130% of control seedlings by an application of BX-112 (2,500 ng/plant) with GA\textsubscript{1} at a dose of 1 ng/plant (Fig. 4).
Effect of BX-112 on GA-induced shoot elongation

Fig. 3 Effect of BX-112 on shoot elongation caused by exogenously applied GA19, GA20 and GA1 in normal rice cv. Nihonbare seedlings. BX-112 and GAs were simultaneously applied to seedling 48 h after planting using the micro-drop method (Murakami 1986a). The length of second leaf sheath was measured three days after the application. Vertical bars represent the standard error of the mean of five replicates. Symbols are: ••, control without GA; ○, with GA (dose of 1 ng/plant); △, with GA (dose of 10 ng/plant); •, with GA (dose of 100 ng/plant).

at doses of 250 ng/plant, or at higher doses (Fig. 5). Shoot elongation caused by GA4 at doses of 10 to 100 ng/plant was reduced by BX-112 up to 25 ng/plant but was promoted at high doses of BX-112 (250 to 2,500 ng/plant) in cv. Nihonbare. In cv. Tan-ginbozu (Fig. 6), shoot elongation caused by GA3 (1 ng/plant) was neither reduced nor promoted by BX-112 at doses of 2.5 to 250 ng/plant. Shoot elongation caused by GA4 in cv. Tan-ginbozu was not reduced by BX-112 at any dose ranging from 2.5 to 2,500 ng/plant. In fact, BX-112 at high doses (250 to 2,500 ng/plant) promoted the shoot elongation when GA4 was applied. The interaction of GA4 and BX-112 was similar to the interaction of GA1 and BX-112 for shoot growth.

Discussion

In our preliminary experiments the growth retardation caused by prohexadione (free acid of BX-112) was fully reversed by application of GA3 to the rice plant. However, using a dipping method (cv. Tan-ginbozu), shoot elongation caused by steviol (ent-13-hydroxykaurenoic acid) at a concentration of $6.2 \times 10^{-4}$ M was fully reduced by prohexadione at a concentration of $1 \times 10^{-5}$ M (data not shown).

Fig. 4 Effect of BX-112 on shoot elongation caused by exogenously applied GA19, GA20 and GA1 in dwarf rice cv. Tan-ginbozu seedlings. Application was the same as in Fig. 3. Vertical bars represent the standard error of the mean of five replicates. Symbols are: ••, control without GA; ○, with GA (dose of 1 ng/plant); △, with GA (dose of 10 ng/plant); •, with GA (dose of 100 ng/plant).
When ancymidol was applied at a concentration of $1 \times 10^{-4}$ M instead of prohexadione, the shoot elongation caused by steviol was not reduced. Since steviol may be metabolized to an active GA in rice (Murakami 1968b), the result suggested that prohexadione inhibits GA biosynthesis at some step(s) between ent-kaurenoic acid and an active GA.

Both prohexadione and BX-112 have the same general properties as PGRs, but BX-112 was developed as the more active PGR for agricultural use, hence its use in these experiments.

Of the three GAs we used, GA$_3$ and GA$_{20}$ and GA$_{30}$, GA$_3$ is considered to be the only endogenous active GA controlling shoot growth in rice (Kuroguchi et al. 1979, Suzuki et al. 1981, Kobayashi et al. 1988, 1989). GA$_{19}$ and GA$_{20}$ are logical precursors of GA$_3$ (Fig. 2), and elongation caused by GA$_{19}$ or GA$_{20}$ application is probably a result of conversion of GA$_{19}$ to GA$_{20}$ to GA$_3$ (Fig. 2). The dwarf cv. Tan-ginbozu contains little endogenous GAs in the vegetative shoot (Suzuki et al. 1981, Kobayashi et al. 1989) and Tan-ginbozu is thought to be blocked at an early step of GA biosynthesis, prior to formation of the ent-gibberellane skeleton (Murakami 1972). BX-112 has a very reduced effect on retarding growth of cv. Tan-ginbozu, undoubtedly because of reduced endogenous GA levels in this cultivar. The reducing effect of BX-112 on the shoot elongation caused by exogenously applied GA$_3$ in cv. Nihonbare (Fig. 3) is probably due to reduction of endogenous levels of GA$_3$ in the tall cultivar, an effect that is minimized for the dwarf cultivar (Fig. 4). All of the above results suggest that BX-112 is not an antagonist of GA$_3$ for shoot elongation, but rather inhibits an activation step of GA from GA$_{20}$ to GA$_3$, namely $3\beta$-hydroxylation. It is this step which is blocked in the single gene dwarf mutants of rice (dwarfing gene; $dy$), maize ($dl$) and pea ($le$) (Murakami 1972, Phinney and Spray 1982, Ingram et al. 1984). However, it is not clear from our results whether BX-112 also affects the conversion step from GA$_{19}$ to GA$_{30}$.

When a high dose of BX-112 (2,500 ng/plant) was applied with GA$_3$ (10 ng/plant), shoot elongation in both cultivars was promoted more than when GA$_3$ was applied alone (Fig. 3, 4). GA$_3$ is known to be $2\beta$-hydroxylated to GA$_8$ in seedlings of dwarf rice cv. Tan-ginbozu (Railton et al. 1973). Since GA$_8$ and other $2\beta$-hydroxylated GAs have low biological activities (cited in Reeve and Crozier 1974), $2\beta$-hydroxylation is undoubtedly an inactivation step. The promotive effect of BX-112 on the shoot elongation caused by GA$_3$ can best be explained by an inhibition of $2\beta$-hydroxylation. To attempt to confirm this, GA$_3$ and GA$_4$ were applied to rice seedlings with or without BX-112. GA$_4$ will be readily $2\beta$-hydroxylated but GA$_3$ is probably protected from $2\beta$-hydroxylation by the $\Delta^1$ bond. Shoot elongation caused by GA$_4$ was promoted by BX-112 at a high dose (2,500 ng/plant) in both cultivars, but shoot elongation caused by GA$_3$ was not affected by BX-112 (Fig. 5, 6). In seedlings of cv. Tan-ginbozu the conversion of $[^{3}H]$GA$_3$ to GA$_{19}$, GA$_2$ and GA$_{34}$, and the conversion of $[^{3}H]$GA$_3$ to GA$_8$ have been reported (Durley and Pharis 1973, Railton et al. 1973). Thus, GA$_3$ can be directly $2\beta$-hydroxylated to GA$_{34}$, or metabolized to GA$_8$ via GA$_3$. These results thus support the proposal that BX-112 may inhibit $2\beta$-hydroxylation.
A GA 3β-hydroxylase was partially purified from immature seeds of *Phaseolus vulgaris* (Kwak et al. 1988), and a GA 2β-hydroxylase was partially purified from mature seeds of *Pisum sativum* and mature and immature seeds of *P. vulgaris* by Smith and MacMillan (1984, 1986). GA 2β- and 3β-hydroxylases are classified as 2-oxoglutarate dependent dioxygenases, which require Fe^{2+} and oxygen. The similarity of the hydroxylases for requirement of 2-oxoglutarate and other cofactors also supports the hypothesis that BX-112 may inhibit both hydroxylases.

Our results are also confirmatory of the hypothesis that shoot elongation of rice is caused by GA(s) originating from GA19 and GA30. Thus, BX-112 may not only be useful as a PGR in agriculture, but also as a potent bio-reagent for determining the physiologically active GA(s) in various plant processes which are influenced by GAs. Studies on inhibitory sites of the compound in GA biosynthesis and the mode of action by using cell-free systems from plants will be reported elsewhere.

The authors wish to thank Mr. S. Kusano of K-I Chemical Research Institute Co., Ltd. for supplying us authentic BX-112. We also gratefully acknowledge the gift of GAs from Professor N. Takahashi, Department of Agricultural Chemistry, The University of Tokyo.

### References


(Received September 6, 1989; Accepted November 27, 1989)