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Full Length Research Paper

Influence of prohexadione-calcium, trinexapac-ethyl and hexaconazole on lodging characteristic and gibberellin biosynthesis of rice (*Oryza sativa* L.)

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We investigated the influence of prohexadione-calcium (Pro-Ca), trinexapac-ethyl (TNE) and hexaconazole (HX) on lodging and gibberellin (GA) biosynthesis pathway of rice cultivar, Hwayeongbyeo. It was observed that these novel synthetic growth retardants suppressed lodging of rice under field conditions through blocking GA biosynthesis pathway. These growth retarding chemicals were applied at basic (20 uM) and elevated (40 uM) rates either 10 days before heading (10 DBH) or 5 days before heading (5 DBH). We found that Pro-Ca, TNE and their combined application (Pro-Ca + TNE) were most effective in decreasing rice length and lodging index, when applied at 10 DBH. Similarly, the endogenous bioactive GA_1 contents of rice significantly declined with application of Pro-Ca, TNE and Pro-Ca + TNE, while they were less effected by basic and elevated rates of HX as compared to the control. The growth retardants were more effective in decreasing rice lodging and blocking GA biosynthesis when applied in elevated rates. The levels of the endogenous gibberellins in rice shoots were measured by GC/MS-SIM using $^{2}H_2$-labeled gibberellins as internal standards. Effect of these synthetic chemicals on growth and GA inhibition were stronger initially but eroded rapidly under field conditions. It was thus concluded that Pro-Ca and TNE were most effective in reducing plant length and suppressing lodging of rice crop under field conditions, where lodging is a major constraint to higher productivity.

Key words: Growth retardants, plant growth, gibberellin biosynthesis, lodging index, rice.

INTRODUCTION

Rice is one of the major cereals and provides food to bulk of the world population. Rice yield is often affected by lodging, which results from plant and environmental factors. Lodging makes machine harvesting difficult and reduce crop yield, owing to decreased canopy photo-synthesis as a result of self-shading. It also deteriorates grain quality, due to an increased coloring of brown rice and/or decreased flavor (Yoshinaga, 2005). Over the last years, scientists have been trying to reduce the yield losses caused by lodging by introducing new rice cultivars and certain chemicals that are meant to reduce and strengthen rice stem. Plant growth retardants are now widely used in plant management to suppress shoot growth. They effectively check increase in plant length by blocking gibberellin (GA) biosynthesis in plants. GAs are cyclic, diterpenoid hormones with an essential role in plant growth and development. They control a variety of growth responses in higher plants, including stem elongation, fruit set, flower induction, seed germination, and mobilization of seed reserves (Hooley, 1994; Swain

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Abbreviations: Pro-Ca, Prohexadione-calcium; **TNE,** trinexapac-ethyl; **HX,** hexaconazole; **GA,** gibberellin; **DBH,** days before heading.

and Olszewski, 1996).

Recently, compounds like, daminozide, ethephon and paclobutrazol were used for the same purpose but they provided a minor practical leverage, because of residue toxicity, yield decrease, contamination of underground water and a possibility of causing cancer (Petracek et al., 2003). However, in this study, we tested prohexadione calcium, trinexapac-ethyl and hexaconazole. Prohexadione calcium (Pro-Ca) (calcium 3,5-dioxo-4-propionylcyclohexanecarboxylate) is a structural mimics of 2-oxoglutaric acid, which is the co-substrate of dioxygenases that catalyze late steps of GA biosynthesis. Pro-Ca blocks particularly 3ß-hydroxylation, thereby inhibiting the formation of highly active GAs from inactive precursors (Rademacher, 2000). Similarly, trinexapac-ethyl (TNE) blocks the final step in the biosynthesis of the biologically active forms of gibberellins, resulting in slower shoot growth (King et al., 1997), while hexaconazole (HX) inhibit cytochrome P-450 mediated oxidative demethylation reaction, which is necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellin biosynthetic pathway and can affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Sankar et al., 2007).

Plant growth retardants are often applied in agronomic and horticultural crops to achieve the required agronomic traits without lowering crop productivity. Most growth retardants act as GA biosynthesis inhibitors. To date, four different types of such GA synthesis inhibitors are known: (a) onium compounds, such as chlormequat chloride, MC, chlorphonium and AMO-1618; (b) N-containing heterocycle compounds, ancymidol, flurprimidol, tetcyclasis, paclobutrazol, uniconazole-P and inabenfide; (c) acylcyclohexanediones, prohexadione-calcium and TNE, and daminozide; and (d) 16, 17-dihydro-GA_5 and related structures (Rademacher, 2000). Due to their ability to reduce plant stem length, these chemicals are widely used in agricultural and horticultural practices. In this study, we investigated the effect of pro-Ca, TNE and HX alone and in different combinations on rice lodging under field conditions. Furthermore, we also investigated the degree of inhibition induced by these plant growth retardants on early C-13 hydroxylation pathway of GA biosynthesis in rice.

MATERIALS AND METHODS

A field experiment with complete randomized block design was conducted at Gyeongbuk Agricultural Research and Extension Services, South Korea.

General procedure

Seeds of rice cultivar Hwayeongbyeo were procured from Gyeongbuk Agricultural Research and Extension Services, Korea, and sown under field condition. The experiment was carried out in Gyeongbuk Agricultural Research and Extension Services, Daegu, Korea. The experiment comprised three replications and each replication had a plot size of 10 m². Hwayeongbyeo was selected due to its low lodging resistance trait under unfavorable environmental conditions. The seeds were surface sterilized with 90% ethanol for 2 min and then rinsed three times with sterilized double distilled deionized water. The seeds were soaked for 6 days to get germination. The rice plants were transplanted on 21st May, 2007. The fertilizer applied were N in the form of urea (110 kg ha^{-1}), phosphorus in the form of P_2O_5 (45 kg ha^{-1}) and potassium in the form of K_2O (57 kg ha^{-1}).

Application of plant growth retardants

Plant growth retardants, that is, Pro-Ca, TNE and HX, were applied both singly and in various combinations at 10 days before heading (10 DBH) and 5 days before heading (5 DBH). Gibberellin biosynthesis inhibitors were applied in 20 and 40 µM. Pro-Ca was purchased from Kyung Nong Co., Korea (imported from BASF Corp., Research Triangle Park, NC, USA), while HX and TNE were purchased from Syngenta Korea Co., Korea (originally imported from Syngenta, USA). A wettable granular formulation containing 1% (w / w) of Pro-Ca, was applied through Sprayer Clover (Model TH-33; Taehwan Co., South Korea). The crop was harvested on 18th October, 2007.

Extraction and quantification of endogenous gibberellins

The plants were harvested, immediately frozen in liquid nitrogen and stored at -80°C. GAs extraction of lypolized samples was carried out following the protocol of Lee et al. (1998). A 0.5 gm lyophilized sample was used for GA analysis each time. The GC (Hewlett-Packard 6890, 5973N Mass Selective Detector) with HA-1 capillary column (30 m $\times$ 0.25 mm i.d. 0.25 µm film thickness) oven temperature was programmed for 1 min at 60°C, then a rise of 15°C min^{-1} to 200°C, followed by 5°C min^{-1} to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector with an interface and source temperature of 280°C, an ionizing voltage of 70 eV and a dwell time of 100 m. Full scan mode (the first trial) and three major ions of the supplemented [$^2H_2$] GAs internal standards (the second trial) and the endogenous gibberellins were monitored simultaneously (standard GAs were purchased from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). Retention time was determined by using the hydrocarbon standards to calculate the KRI (Kovats retention indices) value (Table 1). Three replicates per treatment were used for determination of endogenous GAs during the experiment.

Analysis of rice lodging index

The growth parameters studied include plant height, third internode length, culm length, fresh weight and lodging index. All these parameters were measured at the time of crop harvest. Six replicates of 20 plants per treatment were randomly selected for calculating these parameters. The stem breaking strength was measured with a Digital Force Gauge (Model: WW-59845-04). The standard breaking position of stem is 10 cm from the ground level and the time of calculating stem breaking strength is 20 days after flowering in rice (Kim et al., 2007). The lodging index was calculated through formula:

Lodging index= Culm length × Fresh weight/breaking strength of N_3

Table 1. GC-MS analysis of HPLC fractions from acidic ethyl acetate fractions of rice shoot.

HPLC fraction	GAs	KRI[a]	Source	m/z (%, relative intensity of base peak)[b]
6 ~ 8	GA_8	2818	Sample	594(100) 448(25) 379(20)
		2818	Standard	596(100) 450(24) 381(21)
12~14	GA_1	2674	Sample	506(100) 448(20) 313(17)
		2674	Standard	508(100) 450(19) 315(14)
24~26	GA_{20}	2485	Sample	418(100) 375(45) 403(14)
		2485	Standard	420(100) 377(45) 405(13)
29~31	GA_{19}	2600	Sample	434(100) 374(59) 402(41)
		2600	Standard	436(100) 376(57) 404(40)
35~37	GA_{53}	2450	Sample	448(47) 251(30) 235(30)
		2450	Standard	450(47) 253(28) 237(28)
41~43	GA_{12}	2335	Sample	300(100) 240(31) 328(31)
		2335	Standard	302(100) 242(32) 330(29)

[a]KRI, Kovats retention indices. [b]Identified as methyl ester trimethylsilyl ether derivatives by comparison with reference spectra and KRI data (Gaskin and MacMillan, 1991).

Table 2. General agronomic conditions of the field during the experiment (2007).

Date	Average day temperature (°C)	Minimum day temperature (°C)	Maximum day temperature (°C)	Rainfall (mm)	Day wind velocity (m/sec)	Averade day humidity (%)	Day irradiancy (hr)	Average day cloud (1/10)
21st May	19.4	13.8	24.2	0	1.5	48.9	5.8	4.0
10th August	28.2	25.3	32.7	0.5	1.6	74.9	5.1	5.4
15th August	28.1	24.5	33.2	0.5	1.0	72.3	4.7	6.1
20th August	29.8	25.3	34.9	0	2.2	61.1	8.7	3.8
14th September	21.0	20.1	22.5	21.5	2.7	88.3	0	9.8
18th October	15.4	9.8	20.7	0	1.5	67.1	5.5	5.3

Environmental conditions of field

The environmental conditions were recorded at sampling times in order to get better understanding of the field conditions (Table 2).

Statistical analysis

The data was analyzed statistically for standard deviation by using sigma plot software (2004). The mean values were compared using Duncan's multiple range tests at $P< 0.05$ (ANOVA SAS release 9.1; SAS, Cary, NC, USA).

Soil analysis

For soil analysis, representative random soil samples were taken from the experimental field, then air dried, ground, homogenized and screened (2 mm. sieve) paddy soil and analyzed for physicochemical properties (RDA, 1988). The paddy soil used in the experiment was of silt-loam texture (Table 3).

RESULTS

Influence of GA inhibitors on rice lodging

The plant length, 3rd internode length, culm length and lodging index were significantly reduced by Pro-Ca, TNE and HX, both singly and also when applied in different combinations, when applied at 10 DBH. Maximum breaking strength of 953.9 was recorded in plants treated with 40 µM of TNE at 10 DBH. It was observed that TNE and Pro-Ca were most effective in reducing the lodging index of rice than all other treatments as compared to the control (Table 4). We found that a combined application of Pro-Ca and TNE provided maximum reduction in plant length, 3rd internode length and culm length, although, the

Table 3. Chemical analysis of paddy soil used for the experiment.

pH (1:5)	O.M. (g/kg)	Av. P_2O_5 (mg/kg)	Ex. cation ($cmol^+$/kg)	Av. SiO_2 (mg/kg)	Ac.Fe (mg/kg)	Ex.Mn (mg/kg)	Zn (mg/kg)	Soil texture
5.9	29	68	0.22 /6.19 /1.23	101	794	14.9	2.4	Silt-loam

Table 4. Effect of plant growth retardants on growth attributes and lodging index when applied 10 days before heading.

Treatment	Concentration (μM)	Plant height (cm)	3rd internode length (cm)	Culm length (cm)	Breaking strength	Lodging index
Pro-Ca	20	87.6±0.94d	9.2±0.29fg	66.6±0.78d	835.6±44.90ab	110.6±5.76c
	40	81.0±0.89f	8.8±0.3g	60.1±0.52f	941.2±52.57a	101.1±5.93c
TNE	20	88.5±0.57cd	10.6±0.19cde	67.8±0.43cd	918.6±39.91a	105.4±4.97c
	40	83.8±0.99e	9.5±0.39fg	62.8±0.68e	953.9±55.13a	105.4±7.11c
HX	20	93.0±0.58b	11.8±0.26ab	71.7±0.65b	765.2±31.09b	136.6±6.50ab
	40	90.9±0.80bc	11.4±0.29bc	70.7±0.59b	771.8±33.78b	134.8±6.65ab
Pro-Ca +TNE	20	85.0±0.76e	10.1±0.26ef	64.4±0.53e	887.5±47.64ab	100.8±4.74c
	40	83.4±1.24e	9.9±0.31ef	62.8±1.09e	892.9±35.18ab	103.8±4.49c
Pro-Ca +HX	20	89.2±0.62cd	11.5±0.35bc	68.8±0.46c	828.2±37.57ab	116.2±6.59bc
	40	84.4±0.54e	10.5±0.27de	64.4±0.46e	870.1±43.34ab	116.0±6.25bc
HX +TNE	20	88.4±0.92cd	9.9±0.36ef	67.2±0.81cd	869.2±40.87ab	116.3±5.62bc
	40	87.6±0.75d	11.2±0.26bcd	67.6±0.61cd	945.7±61.00ab	121.7±8.93bc
Control		96.5±0.59a	12.6±0.21a	74.3±0.45a	831.5±49.32ab	151.3±11.87a

In a column, means followed by the same letter are not significantly different at $P < 0.05$ according to DMRT. Pro-Ca, Prohexadione-calcium; TNE, trinexapac-ethyl; HX, hexaconazole.

breaking strength for such treatment was lesser than that of TNE alone (Table 4). The growth inhibitors were more effective when applied in elevated concentration (40 μM) as compared to basic (20 μM) treatments. The lodging index decreased in plants treated with Pro-Ca, TNE and HX when applied at 10 DBH. Similarly, in 5 DBH treated plants, the reduction in stem length was more pronounced in Pro-Ca and TNE applied plants (Table 5). Pro-Ca plus TNE was more effective combination than Pro-Ca plus HX and HX plus TNE. The growth retardants provided best results at elevated concentrations (40 μM), as compared to basic (20 μM) treatments (Table 5).

Influence of growth retardants on GA biosynthesis

Endogenous GAs analysis of treatments showed that plant growth retardants markedly inhibited GA biosynthesis pathway. Plants treated with basic concentration of GA inhibitors at 10 DBH, showed that GA biosynthesis was significantly blocked by Pro-Ca and Pro-Ca plus TNE applied plants. The internal GA_1 levels of Pro-Ca treated plants was 1.82 ng/g, Pro-Ca plus TNE was 1.84 ng/g, followed by TNE treatments (2.03 ng/g), as compared to the control (3.27 ng/g) (Figure 1). Similarly, plants treated with elevated concentration of

Table 5. Effect of plant growth retardants on growth attributes and lodging index when applied 5 days before heading.

Treatment	Concentration (μM)	Plant height (cm)	3rd Internode length (cm)	Culm length (cm)	Breaking strength	Lodging index
Pro-Ca	20	90.9±0.90^{de}	10.4±0.26^{fg}	69.7±0.73^{cd}	904.0±41.76^{ab}	117.5±6.47^{c}
	40	84.1±0.85^{g}	9.9±0.35^{g}	64.3±0.73^{f}	881.4±40.30^{abc}	107.2±5.76^{c}
TNE	20	88.9±0.82^{ef}	11.4±0.31^{cde}	68.3±0.67^{de}	948.5±32.75^{a}	107.4±4.98^{c}
	40	87.8±1.10^{f}	11.3±0.32^{de}	68.1±1.03^{de}	919.1±27.68^{ab}	105.8±5.10^{c}
HX	20	93.9±0.73^{bc}	12.8±0.22^{a}	73.6±0.98^{a}	758.7±42.21^{c}	140.9±7.39^{ab}
	40	91.6±0.62^{cde}	12.2±0.22^{abc}	72.6±0.53^{ab}	908.7±42.76^{ab}	128.6±8.56^{abc}
Pro-Ca +TNE	20	91.2±0.68^{de}	10.9±0.45^{ef}	71.8±1.34^{abc}	803.8±37.84^{bc}	145.8±7.08^{a}
	40	84.5±1.07^{g}	9.0±0.25^{h}	66.0±1.39^{ef}	852.3±47.62^{abc}	120.4±7.77^{bc}
Pro-Ca +HX	20	94.1±0.75^{b}	11.8±0.23^{bcd}	73.9±0.63^{a}	877.1±35.36^{abc}	138.2±8.38^{ab}
	40	90.8±0.90^{de}	11.3±0.20^{de}	70.4±0.64^{bcd}	866.3±48.46^{abc}	127.6±7.40^{abc}
HX +TNE	20	92.9±0.99^{bcd}	12.1±0.26^{abcd}	72.9±1.01^{ab}	856.6±49.64^{abc}	136.5±8.10^{ab}
	40	91.3±0.70^{de}	11.8±0.25^{bcde}	71.6±0.65^{abc}	890.0±32.62^{abc}	128.7±5.03^{abc}
Control		96.5±0.59^{a}	12.6±0.21^{ab}	74.3±0.45^{a}	831.5±49.32^{abc}	151.3±11.87^{a}

In a column, means followed by the same letter are not significantly different at $P < 0.05$ according to DMRT. Pro-Ca, Prohexadione-calcium; TNE, trinexapac-ethyl; HX, hexaconazole.

growth retardants 10 DBH showed that GA biosynthesis was significantly blocked in Pro-Ca and Pro-Ca plus TNE applied plants. The internal GA_1 levels of Pro-Ca treated plants was 0.97 ng/g, followed by Pro-Ca plus TNE treatments (1.33 ng/g), as compared to the control (3.27 ng/g) (Figure 2). However, HX alone and TNE plus HX treated plants showed least blockage of GA biosynthesis pathway at both basic and elevated concentrations. The GA_{20} contents were much higher in Pro-Ca (6.99 ng/g) and Pro-Ca plus TNE (6.92 ng/g) treated plants as compared to the control (3.68 ng/g) as shown in Figure 2.

The endogenous GA content of plants treated with growth retardants at 5 DBH were also reduced in a fashion similar to that of 10 DBH treated plants, although, GA blockage intensity was higher in these plants. Plants treated with basic concentration of growth retardants at 5 DBH showed that GA biosynthesis was significantly blocked by these chemicals. The internal GA_1 levels of Pro-Ca treated plants was 1.15 ng/g, followed by Pro-Ca plus TNE treatment (1.46 ng/g), as compared to control (3.27 ng/g) (Figure 2). However, Pro-Ca plus HX and TNE plus HX applied plants caused least blockage of GA biosynthesis pathway. The GA_{20} contents were much higher in TNE (4.65 ng/g) and Pro-Ca plus TNE (4.64 ng/g) as compared to the control (3.58 ng/g) as shown in Figure 3. Similarly, the endogenous bioactive GA_1 level of plants treated with elevated amounts of growth retardants at 5 DBH showed that GA biosynthesis was significantly reduced by these chemicals. Maximum reduction in GA content was induced by Pro-Ca (2.19 ng/g), Pro-Ca plus TNE (2.2 ng/g) and TNE (2.3 ng/g) applied plants (Figure 4). However, HX and TNE plus HX treated plants showed least blockage of GA biosynthesis pathway as compared to the control (Figure 4).

DISCUSSION

Lodging has been a major constraint in cereal production and huge quantities of rice are lost to lodging each year, especially in the areas where the crop is exposed to severe typhoons and winds. Lodging of rice decreases the grain yield by 34% at milky, 21% at dough, and 20% at yellow stage, while it decreases the grain quality, and increases the green rice contents and harvesting costs. In order to minimize lodging damage, selection of lodging resistant varieties and improvement of cultural practices such as amount and time of nitrogen application, planting density, water management, and disease and pest control methods have been studied. The annual precipitation in Korea is about 1200 mm per year while most of the precipitation is concentrated during the months of June to August. Moreover, Korea is also exposed to typhoons, as typhoon data from 1904 to 1989 revealed that months of August and September received maximum typhoons (64%), and typhoons during these months are critical for

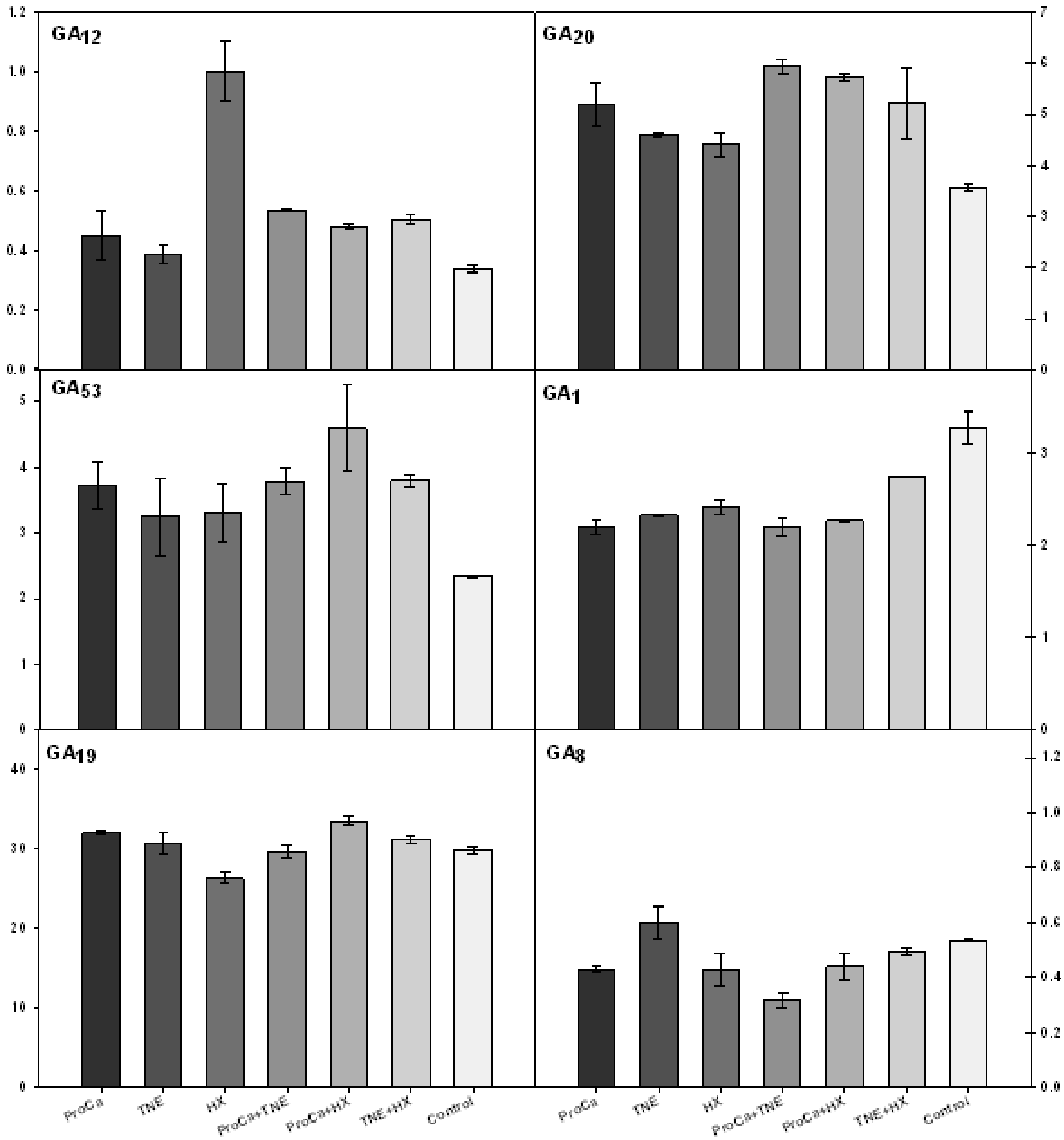

Figure 1. Endogenous GAs levels of Hwayeongbyeo as affected by different plant growth applied at 20 µM concentration. The growth retardants were applied at 10 DBH. Error bars show standard error. The quantities of GA were quantified as ng/g FW; Whereas FW stands for fresh weight.

rice growth and yield (Lee et al., 1991). Lodging of rice plants during ripening stage causes reduction in crop yield, owing to decreased canopy photosynthesis as a result of self-shading, and it also decreases the grain quality, due to increased coloring of brown rice and/or decreased flavor.

Pro-Ca, TNE and HX are novel synthetic plant growth retardants and suppresses the vegetative growth of apple, tomato, grain sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) (Byers and Yoder, 1999; Grossman et al., 1994; Lee et al., 1998; Nakayama et al., 1992; Yamaji et

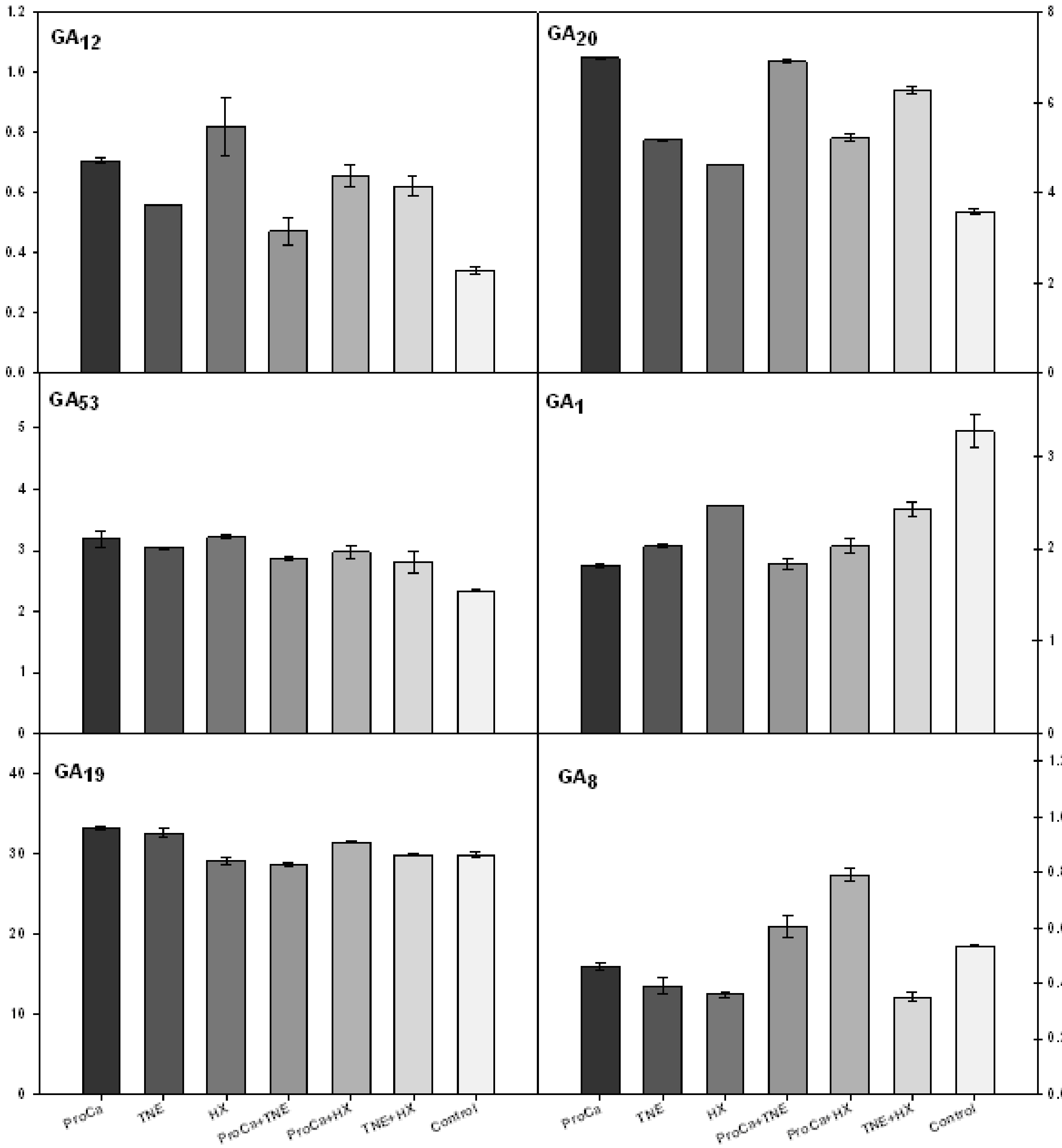

Figure 2. Endogenous GAs levels of Hwayeongbyeo as affected by different plant growth applied at 40 µM concentration. The growth retardants were applied at 10 DBH. Error bars show standard error. The quantities of GA were quantified as ng/g FW; Whereas FW stands for fresh weight.

al., 1991). These chemicals effectively block GA biosynthesis pathway, thereby reduces the level of bioactive GA_1 and GA_4 in the plant for two to three weeks following their application. However, they do not persist in the plant or affect vegetative growth the following season. Due to their short-term effect and lack of persistence, they provide a flexible tool for vegetative growth management that can be applied at a variety of timings and used to develop user-specific growth management strategies (Evans et al., 1999). We observed that application of Pro-Ca and TNE significantly reduced lodging index, when applied 10 and 5 days before heading. However, lodging index was lower in rice plants treated at 10 DBH than 5 DBH treated plants. This may be due to the fact that plant height significantly increases at this stage, which was checked with the application of

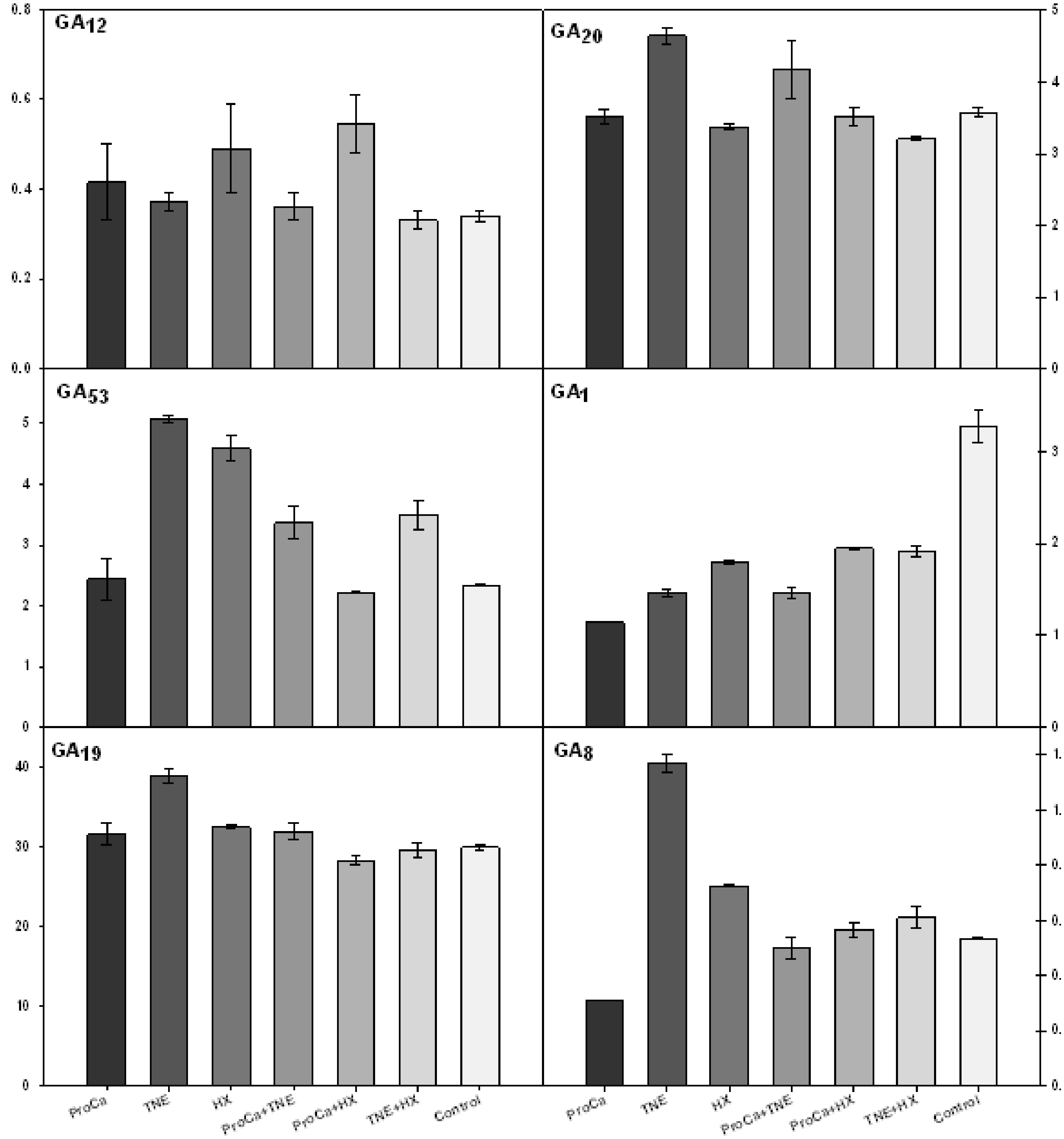

Figure 3. Endogenous GAs levels of Hwayeongbyeo as affected by different plant growth applied at 20 µM concentration. The growth retardants were applied at 5 DBH. Error bars show standard error. The quantities of GA were quantified as ng/g FW; Whereas FW stands for fresh weight.

growth retardants, and as a result over all plant length was least in 10 DBH treated plants as compared to 5 DBH treatments. The present study confirms earlier report of Kim et al. (2007), which stated that earlier application of Pro-Ca in rice was more effective in preventing lodging than its later application. The 3rd internode and culm lengths were also reduced by Pro-Ca and TNE, while an increase in the breaking strength was recorded for such treatments. This clearly indicated that Pro-Ca and TNE are more effective in the reduction of lodging index of rice crop. On the other hand, HX also decreased the lodging index as compared to control, but it was significantly less effective than Pro-Ca and TNE. The difference in effectiveness between the three com-

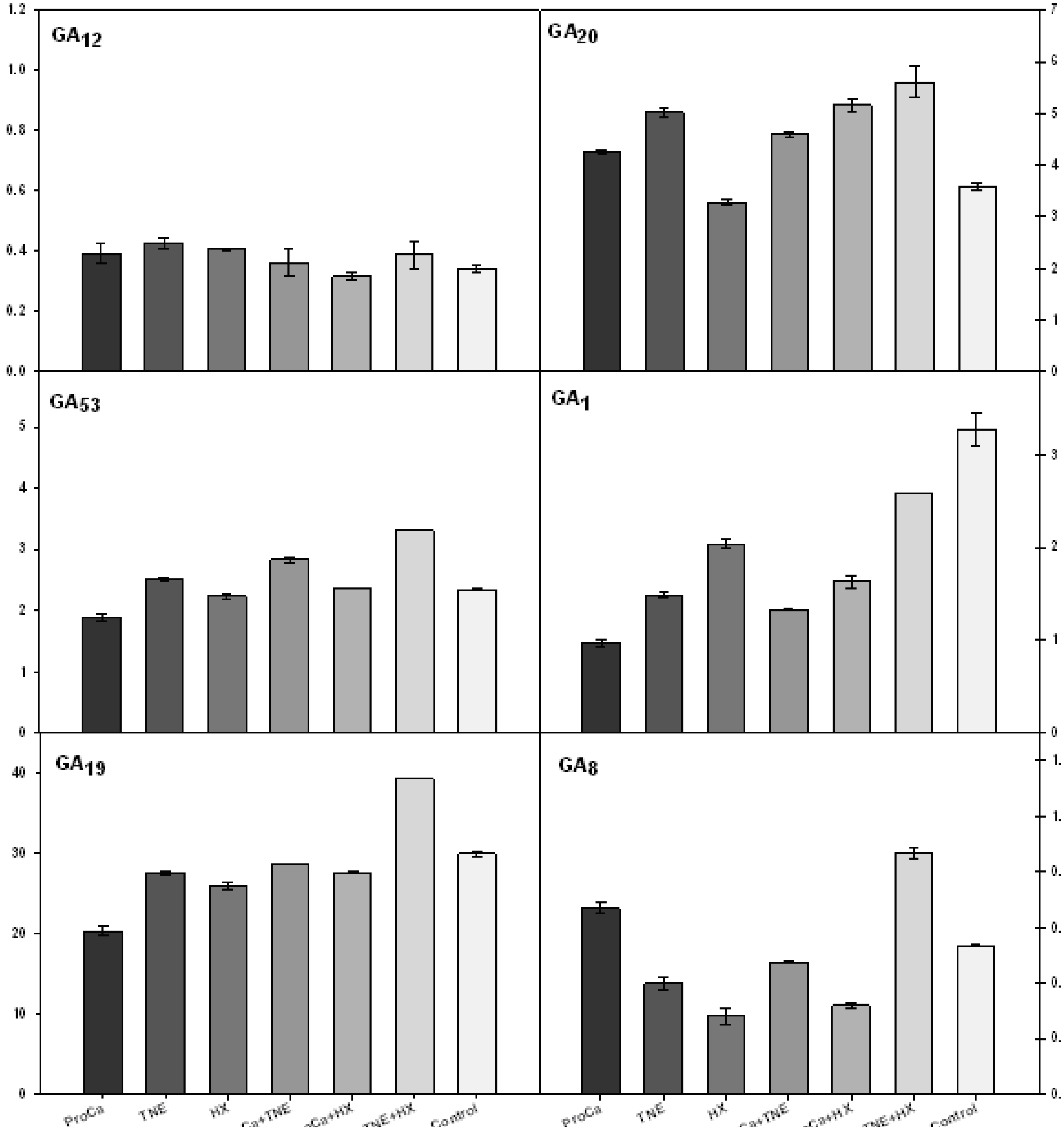

Figure 4. Endogenous GAs levels of Hwayeongbyeo as affected by different plant growth applied at 40 µM concentration. The growth retardants were applied at 5 DBH. Error bars show standard error. The quantities of GA were quantified as ng/g FW; Whereas FW stands for fresh weight.

pounds could be due to different modes of action. HX blocks an early step in the gibberellin biosynthetic pathway that is oxidation of ent-kaurene to GA_{12} (Coolbaugh and Hamilton, 1976), whereas prohexadione-Ca and trinexapac-ethyl block a late step in the pathway (3β-hydroxylation of GA_{20} to GA_1) (Brown et al., 1997).

Pro-Ca and TNE have been also shown to prevent metabolism of GA_1 to inactive GA_8 by blocking the 2β-hydroxylation (Brown et al., 1997). Application of elevated amounts of these chemicals provided better reduction than basic amounts in almost all treatments. Timing of application was also effective in reducing lodging index and suppressing plant length, as the best results were obtained when plants were treated 10 DBH as compared to 5 DBH. According to Miller (2002), timing of the spray was more important than rate in achieving early growth suppression, but rate was most important for maximum season-long growth control. Similarly, treatment of wheat and oil seed rape seedlings with increasing retardant concentrations in hydroponics, plant height and fresh weight of shoots were reduced by up to 40% (Grossmann et al., 1994). Concomitantly, the amount of bioactive gibberellins decreased, on a fresh weight basis, when compared with levels in the shoots of control plants.

The endogenous GAs analysis of rice plants showed that GA_1 contents drastically reduced with the application of Pro-Ca and TNE, while was least affected by HX as compared to the control. However, higher GA_{20} contents were observed with Pro-Ca and TNE application as conversion of GA_{20} to GA_1 was blocked by these chemicals, while rest of GAs contents remained almost unchanged to those of control. $GA_{19,}$ a precursor of GA_{20} was most abundant while GA_1 was found to be least abundant in all treatments. The GA_{19} was about 5 to 10 folds higher than GA_{20}, a precursor of bioactive $GA_{1,}$ which coincide with the results of Appleford and Lenton (1991). Present results suggest that Pro-Ca and TNE hinder gibberellin (GA) biosynthesis in the rice at the 3β- and 2β-hydroxylation stage of GAs biosynthesis, namely steps of activation and inactivation, respectively. Our results further support the hypothesis that GA_1 and not GA_{19} nor GA_{20} is active in promoting shoot elongation in rice. Almost similar results were recorded by Nakayama et al. (1992), in rice plant. An increase in GA_{20} contents after Pro-Ca and TNE application suggests that the blocking of GA biosynthesis at later stage has resulted in the accumulation of GA_{20}, which confirmed a similar previous report (Kamyia, 1991).

In summary, it was observed that Pro-Ca, TNE, HX and their combinations significantly reduced GA biosynthesis and ultimately decreased plant length and lodging index of rice. However, Pro-Ca and TNE were more effective in suppressing growth and lodging index when used alone or in combination. It was found that application at 10 DBH provided better result that 5 DBH as the effect of these growth retardants gradually declined with passing time. We also observed that elevated concentrations of Pro-Ca and TNE provided best results, while HX was more effective when applied in basic amount. It was found that combined application of Pro-Ca and TNE was more effective in the reduction of lodging index than the combinations of either Pro-Ca and HX or TNE and HX. An application of such chemicals will enhance both quality and quantity of rice yield, especially in areas where rice crop is subjected to severe winds and typhoons

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