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SYNERGISTIC INTERACTION OF GIBBERELLIC ACID AND TRIAZINONE DERIVATIVES IN THE PROMOTION OF RICE SHOOT GROWTH*

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Key Word Index—*Oryza sativa*; Gramineae; rice shoot elongation; gibberellic acid synergists; triazinones.

Abstract—Forty-five 1,3,5-triazine-2,6-dione derivatives were synthesized and their plant growth-promoting activities examined by the rice (*Oryza sativa*) seedling test in the presence or absence of gibberellic acid (GA). For high activity in promoting the growth of rice seedlings and acting as active GA-synergists, a *para*-substituted or a 2,4-disubstituted phenyl group, a hydrogen atom and an alkoxy group were required in the 1-, 3- and 4-positions of the 1,3,5-triazine-2,6-dione molecule. 4-Ethoxy-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione [TA], one of the most potent triazinones, synergized the effect of GA on the shoot elongation of different varieties of rice including normal type, dwarf mutants and chlorophyll-mutants. TA synergistically increased the growth-promoting activity of GA by both a simultaneous treatment at the same sites and separate treatments at separate sites of rice seedlings.

INTRODUCTION

Our discovery of alkoxycarbonylisoureas as growth stimulants and GA-synergists has led to a new class of plant growth modifiers, the triazinones [1, 2]. We suggested that the action of triazinones was intimately related to that of alkoxycarbonylisoureas in rice seedlings. 4-Ethoxy-1-(*p*-tolyl)-*s*-triazine-2,6(1H, 3H)-dione [TA], one of triazinones was shown to be produced biologically, as well as chemically, from 2-ethyl-3-methoxycarbonyl-1-(*p*-tolylcarbamoyl)isourea, one of alkoxycarbonylisoureas [2]. Both compounds promoted the growth of rice seedlings, and also synergistically enhanced the elongation of rice shoots induced by GA. If the alkoxycarbonylisoureas are generally converted into their counterpart triazinones by intramolecular cyclization in plant tissues, the structure–activity relationships for the alkoxycarbonylisoureas previously reported [3] will be valid for the triazinones as well. GA-synergists have been shown to be of potential value in studies of the mechanism of GA action [4]. Therefore, further study on the physiological properties of the triazinone derivatives may provide new insight into the role of GA in plant growth and development.

In this paper we report the effects of a large number of triazinone derivatives on the growth of rice seedlings in the presence and absence of GA, and to assess their structure–activity relationships. This paper also describes the synergistic interaction of GA and TA, one of the most potent triazinone compounds, in promotion of rice shoot growth.

* Part 4 in the series 'Plant growth-regulating activities of isourea derivatives and related compounds'. For Part 3 see ref. [2].

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RESULTS AND DISCUSSION

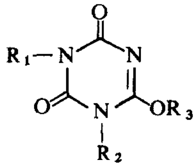
Structure–activity relationships

Forty-five triazinone derivatives were synthesized, and the substituents R_1, R_2 and R_3 in the structure were varied systematically, as shown in Table 1. Evaluation of the plant growth-promoting activity was conducted by testing their abilities to promote the growth of rice seedlings and to enhance the effect of GA on rice shoot elongation. It is clear from Table 1 that large variations in activity resulted from relatively small structural changes in the substituents. The principal structural requirements for high biological activity were the same in both growth promotion and GA-synergism tests.

As to R_1 in the 4-ethoxy-*s*-triazine-2,6(1H, 3H)-dione series, higher activities were obtained when R_1 was a phenyl group substituted with a suitable atom(s) or group(s). Substitution with a chlorine atom at the *para*-position of the benzene ring (compound **8**) produced the most active structure. The replacement of the chlorine atom by other halogen atoms such as fluorine, bromine and iodine in the *para*-position (**27–29**) maintained the high biological activity. Derivatives substituted with a methyl (**18**), an ethyl (**25**) or a methoxy (**30**) group in the *para*-position were also highly active. When a nitro group (**31**) was introduced at the *para*-position, the biological activity decreased slightly. The introduction of a *n*-propyl group in the *para*-position (**26**), however, resulted in the disappearance of the biological activity. These results suggest that it makes no difference whether these substituents at the *para*-position are electron withdrawing or electron donating groups. The biological activity seems to be associated with the size of the substituents.

The substitution at the *ortho*- or *meta*-position of the ring by a chlorine atom (**6** and **7**) or methyl group (**16** and **17**) caused loss of activity. Of the di- or tri-substituted

Table 1. Plant growth-regulating activities of *s*-triazine-2,6(1H, 3H)-dione derivatives



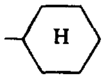
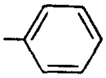
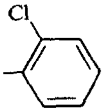
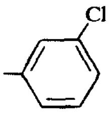
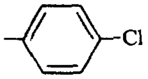
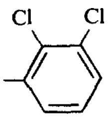
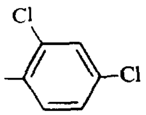
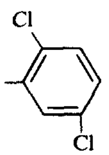
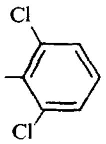
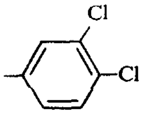
No.	R_1	R_2	R_3	Mp(°)	Conc (ppm)	Activity: Growth-promoting activity*	Activity: Synergistic action with GA†
1	Me	H	C_2H_5	204–206	1	0	0
					10	0	0
					50	+	+
2	C_2H_5	H	C_2H_5	183–186	1	0	0
					10	0	+
					50	0	+
3	*iso*-C_3H_7	H	C_2H_5	202–203	1	0	0
					10	+	+
					50	+	+ +
4		H	C_2H_5	205–208	1	0	0
					10	0	0
					50	0	0
5		H	C_2H_5	257–258	1	0	0
					10	0	0
					50	+	+
6		H	C_2H_5	214–215	1	0	0
					10	0	0
					50	+	+
7		H	C_2H_5	240–241	1	0	0
					10	0	0
					50	0	0
8		H	C_2H_5	254–257	1	0	+
					10	+	+ +
					50	+	+ + + +
9		H	C_2H_5	206–210	1	0	0
					10	0	0
					50	+	+
10		H	C_2H_5	105–110	1	0	+
					10	+	+ +
					50	+ +	+ + + +
11		H	C_2H_5	199–201	1	0	0
					10	0	0
					50	+	+
12		H	C_2H_5	217–220	1	0	0
					10	0	0
					50	0	+
13		H	C_2H_5	267–268	1	0	0
					10	0	0
					50	0	+

Table 1—*continued*

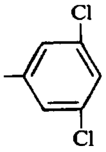
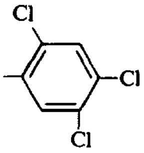
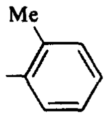
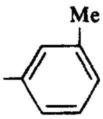
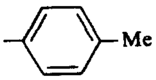
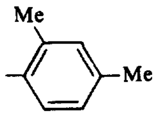
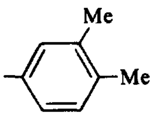
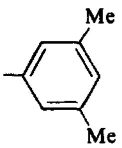
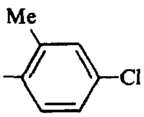
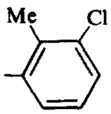
No.	R_1	R_2	R_3	Mp(°)	Conc (ppm)	Activity: Growth-promoting activity*	Activity: Synergistic action with GA†
14		H	C_2H_5	245–246	1 10 50	0 0 +	0 0 +
15		H	C_2H_5	217–222	1 10 50	0 + 0	0 + –
16		H	C_2H_5	178–180	1 10 50	0 0 +	0 0 +
17		H	C_2H_5	221–223	1 10 50	0 0 +	0 0 +
18		H	C_2H_5	260–262	1 10 50	0 + + +	+ + + + + + +
19		H	C_2H_5	201–203	1 10 50	+ + –	+ + + + –
20		H	C_2H_5	273–275	1 10 50	0 0 +	0 + + +
21		H	C_2H_5	217–219	1 10 50	0 0 0	0 0 0
22		H	C_2H_5	177–180	1 10 50	0 + +	+ + + + + +
23		H	C_2H_5	248–250	1 10 50	0 0 +	0 0 +

Table 1—*continued*

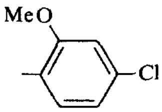
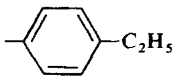
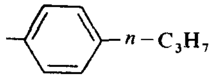
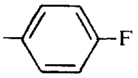
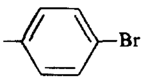
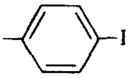
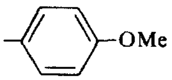
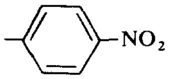
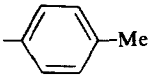
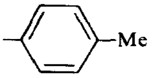
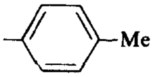
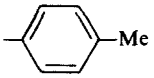
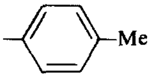
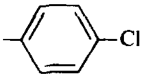
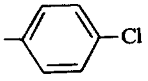
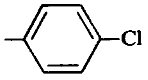
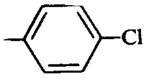
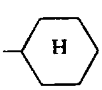
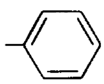
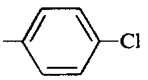
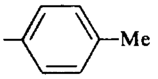
No.	R_1	R_2	R_3	Mp(°)	Conc (ppm)	Activity: Growth-promoting activity*	Activity: Synergistic action with GA†
24		H	C_2H_5	213–215	1	0	0
					10	0	0
					50	0	+
25		H	C_2H_5	227–230	1	0	+
					10	0	+ +
					50	+	+ + +
26		H	C_2H_5	221–223	1	0	0
					10	0	+
					50	0	+
27		H	C_2H_5	274–275	1	0	+
					10	+	+ +
					50	+ +	+ + + +
28		H	C_2H_5	265–267	1	0	0
					10	+	+ + +
					50	+ +	+ + +
29		H	C_2H_5	239–242	1	0	+
					10	+	+ + +
					50	+ +	+ + + +
30		H	C_2H_5	248–249	1	0	+
					10	+	+ +
					50	+	+ + +
31		H	C_2H_5	262–263	1	0	0
					10	0	+
					50	0	+ + +
32		H	Me	221–223	1	0	0
					10	0	+ + +
					50	+ +	+ + + +
33		H	n-C_3H_7	242–244	1	0	+
					10	+	+ + +
					50	+ +	+ + +
34		H	*iso*-C_3H_7	218–219	1	0	+ +
					10	+	+ + +
					50	+	+ +
35		H	$CH_2—CH=CH_2$	189–191	1	0	0
					10	+	+ +
					50	+ +	+ + +
36		H	n-C_4H_9	226–227	1	0	0
					10	0	+ +
					50	0	+
37		H	Me	246–247	1	0	0
					10	0	+ +
					50	+ +	+ + + +

Table 1—continued

No.	R_1	R_2	R_3	Mp(°)	Conc (ppm)	Activity: Growth-promoting activity*	Activity: Synergistic action with GA†
38		H	n-C_3H_7	238–240	1 10 50	0 + + +	+ + + + + +
39		H	iso-C_3H_7	220–222	1 10 50	0 + +	0 + + +
40		H	n-C_4H_9	227–228	1 10 50	0 0 +	0 + + +
41	—Me	Me	C_2H_5	79–81	1 10 50	0 0 0	0 0 −
42		Me	C_2H_5	62–66	1 10 50	0 0 0	0 − −
43		Me	C_2H_5	147–148	1 10 50	0 0 0	0 0 −
44		Me	C_2H_5	132–135	1 10 50	0 0 −	0 0 0
45		Me	C_2H_5	166–169	1 10 50	0 0 0	0 0 0

* Growth-promoting activity was measured as a percentage of control shoot length and expressed as follows: % of control; ≤84: − ; 85–115: 0; 116–135: + ; ≥136: + +.

† Synergistic action with GA was expressed as follows: [Elongation due to (10 ppm GA + test compound)]/[Elongation due to 10 ppm GA] + [Elongation due to test compound] × 100(%), ≤84: −, 85–115: 0; 116–135: + ; 136–155: + + ; 156–175: + + + ; ≥176: + + + +. Control shoot length: with GA, 41 mm; without GA 22 mm.

compounds, 2,4-dichloro (**10**), 2,4-dimethyl (**19**) or 4-chloro-2-methyl (**22**) phenyl derivatives were also effective, but further changes in the ring abolished or greatly decreased the biological activity. Compounds having aliphatic groups as R_1 had little or no activity, although the compound having an *iso*-propyl group (**3**) was weakly active. The replacement of a hydrogen atom at the R_2 position by a methyl group (**41**–**45**) completely destroyed the biological activity.

In the group of compounds possessing a *para*-methylphenyl or a *para*-chlorophenyl group as R_1 and a hydrogen atom as R_2, the number of carbon atoms of an alkyl or an alkynyl group in R_3 affected the biological activities: growth promotion and GA-synergism. The most effective substituents as R_3 were methyl (**32** and **37**) and ethyl (**18** and **8**). Increasing the number of carbon atoms decreased the biological activity (see **32**–**36** or **37**–**40**). Of the R_3-substituents examined, the n-butyl group represented the least active compounds (**36** and **40**).

Several triazine triones were synthesized and evaluated for biological activity; all were inactive. The inactivity of 1-(p-tolyl)-s-triazine-2,4,6(1H, 3H, 5H)-trione compared to the high activity of **18** indicates the importance of the presence of an $—OR_3$ group for the development of the biological activity.

These results suggest that all the substituents in the 1-, 3- and 4-positions of s-triazine-2,6(1H, 3H)-dione are required for biological activity. It is clear from our present and previous work [3] that the structure–activity relationships for the alkoxycarbonylisoureas are correlated well with those of triazinones. This is completely consistent with the results of our previous work [2].

Response of various rice varieties to TA and/or GA

The interaction between GA and TA was examined in various rice varieties including normal varieties, dwarf mutants, and chlorophyll-mutants. In normal varieties as Kinmaze, Norin No. 8, Nihonbare, Kinpa, and Honenwase the growth-promoting activity of TA applied alone or in combination with GA was observed (Fig. 1).

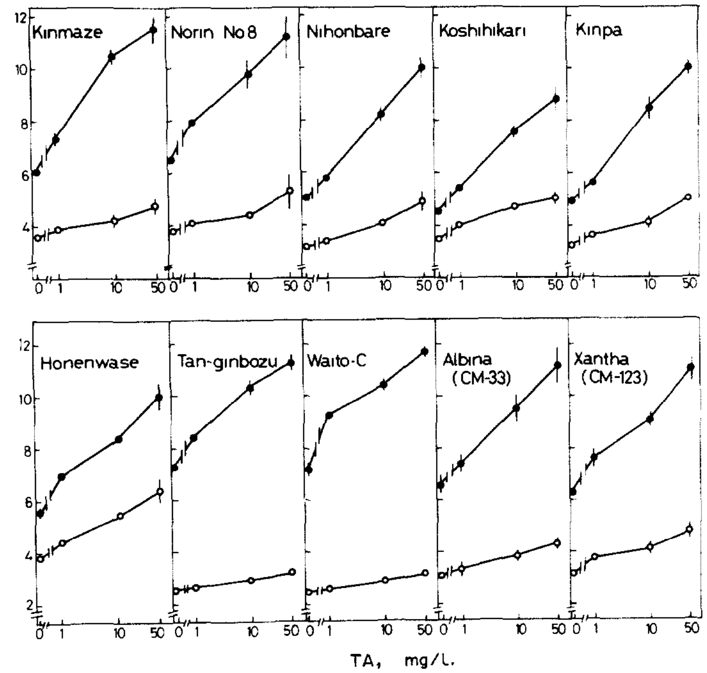


Fig 1. Effect of TA on the shoot growth of various rice varieties. —○—, without GA; —●—, with GA (10 mg/l.). Standard errors were given as vertical bars; where no bars appear, the standard errors were smaller than the symbols. Each point represents the average of three to four replications.

The synergistic interaction between GA and TA in the seedling stage (var. Kinmaze) is diagrammatically illustrated in Fig. 2. The combination of GA and TA caused the unusual and excessive elongation of the first leaf and the second leaf sheath. Note that TA promoted the root growth of rice seedlings and overcame the inhibition of root growth caused by GA. A definite synergism between GA and TA was also observed in two dwarf varieties, Tan-ginbozu and Waito-C, which are defective in the GA synthesis pathway [5]. When applied alone to these dwarf varieties, however, TA showed only a little growth-promoting activity as compared with those for normal varieties. The results suggest that TA requires endogenous or exogenous GA for its action.

The white (albina, CM-33) and the yellow (xantha, CM-123) mutants created by irradiating a normal variety, Norin No. 8, with gamma-rays, lack pigments essential for photosynthesis [6]. TA applied alone

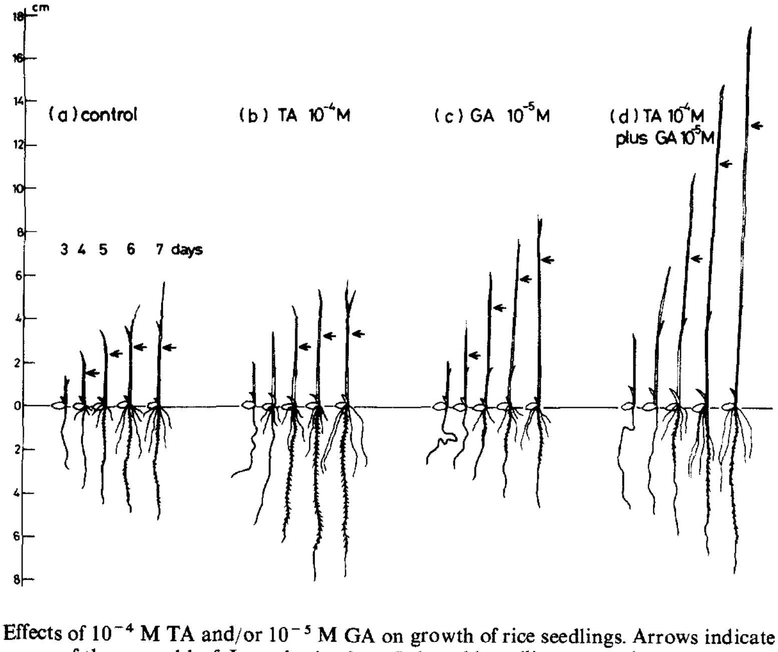


Fig. 2. Effects of 10^{-4} M TA and/or 10^{-5} M GA on growth of rice seedlings. Arrows indicate the lamination joint of the second leaf. In each plot 3- to 7-day-old seedlings were shown.

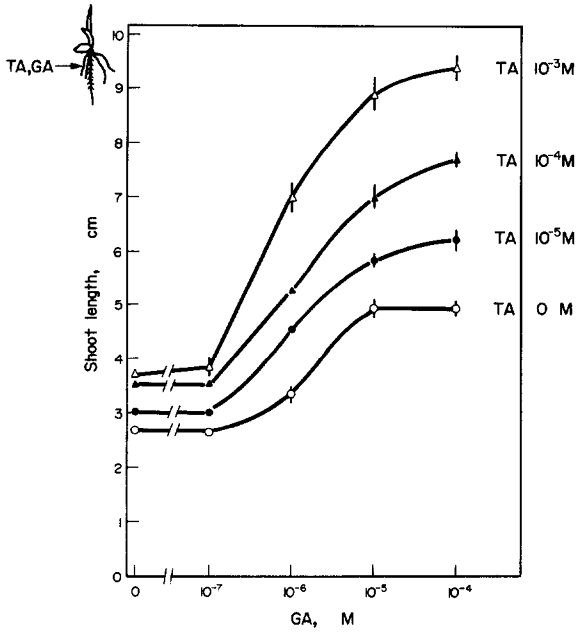


Fig. 3. Effect of TA on GA-induced elongation of rice shoots. Both TA and GA were applied to the roots. Standard errors were given as vertical bars, as in Fig. 1.

promoted shoot growth and the combined application of GA and TA was shown to be synergistic, in both chlorophyll-mutants as in the normal green varieties. There was no significant difference in the activity pattern between the chlorophyll-mutants and Norin No. 8. The results indicate that the action of TA is not associated with photosynthesis or with the chloroplast functions.

Application sites of GA and/or TA

To determine if the biological activity depended on the sites of application of GA and TA, the compounds were simultaneously applied to the roots or shoots in some experiments, and separately applied to the roots or shoots at different times in other experiments. With simultaneous application of GA and TA to the roots, TA greatly enhanced the stimulative effect of GA on the shoot growth of rice seedlings (Fig. 3). Rice seedlings treated with combination of 10^{-3} M TA and 10^{-6} M to 10^{-4} M GA grew crookedly due to the excessive elongation. Note that TA could still promote growth when optimal or supraoptimal GA concentrations were applied.

In experiments employing simultaneous application of TA and GA to the shoots, the response to GA was increased by adding TA (Fig. 4). The synergistic effect of TA increased with increased concentrations of TA. Similarly, the response of shoot elongation to GA applied to the roots was observed to be increased by TA applied to the shoots (Fig. 5). The TA effect shown in Figs 4 and 5 was a true enhancement or synergism of the GA effect since TA alone did not promote the shoot growth of rice seedlings. GA applied alone to the shoot promoted shoot growth in relation to concentration. Application of TA to the root further increased the shoot elongation induced by GA applied to the shoot (Fig. 6).

As shown in Figs 3–6, the synergistic interaction between GA and TA was observed in the shoot elongation of rice seedlings regardless of the method of application. The facile translocation of GA around most parts of various plants is well established [7]. The results reported herein strongly indicate that TA, as well as GA, is a systemic growth regulator. Since TA synergistically promoted the growth of rice seedlings when optimal or supraoptimal GA concentrations were applied and when GA was applied to a different site from that of TA application, it may be affecting some cellular metabolism(s) rather than changing the absorption or metabolism of GA.

EXPERIMENTAL

Preparation of chemicals. Compounds **1–40** in Table 1 were synthesized according to the method previously reported [2]. Compounds **41–45** were obtained by stirring compounds **1**, **4**, **5**, **8** and **18**, respectively, with MeI and K_2CO_3 at room temp. for 5 to 6 hr in Me_2CO. *s*-Triazine-2,4,6(1H, 3H, 5H)-trione derivatives were prepared by refluxing the selected *s*-triazine-2,6(1H, 3H)-diones with HBr (47%) for 1 hr in MeOH. Mps (uncorr.) are listed in Table 1. Their purities were checked by Si gel TLC, and their structures were confirmed by elemental analyses, IR and NMR spectra. GA chiefly composed of GA_3 was obtained from Wako Pure Chemical Industries, Ltd.

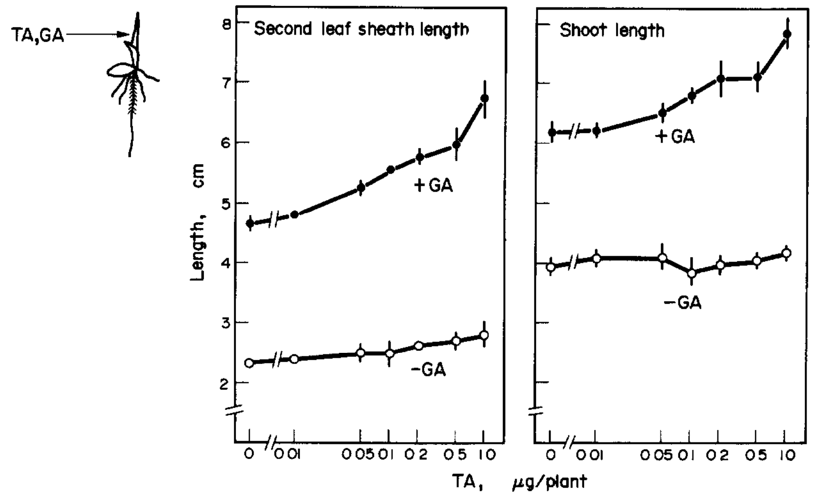


Fig. 4. Effects of TA and/or GA on rice shoot growth. Both TA and GA were applied to the shoots. —○—, without GA; —●—, with GA (0.2 µg/plant). Standard errors were given as vertical bars, as in Fig. 2.

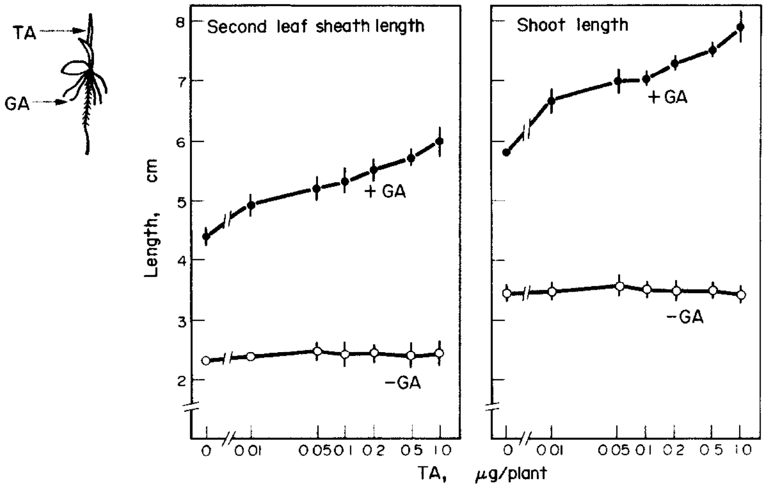


Fig. 5. Effects of TA and/or GA on rice shoot growth. TA was applied to the shoots, while GA was applied to the roots —○—, without GA; —●—, with GA (10^{-5} M). Standard errors were given as vertical bars, as in Fig 1.

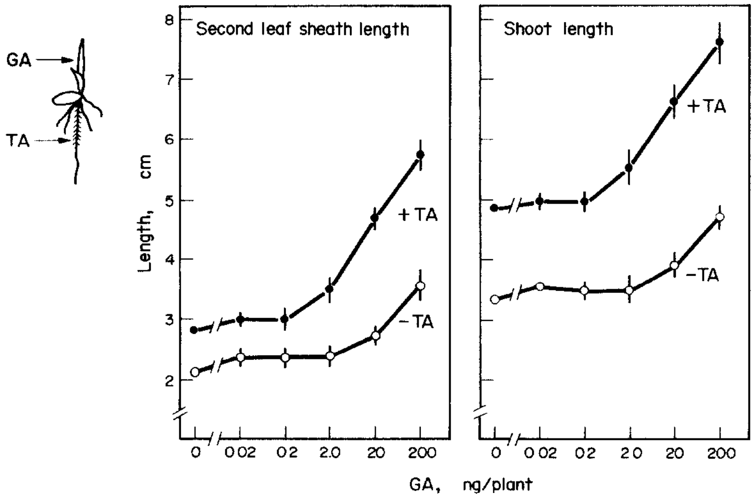


Fig. 6. Effect of TA on GA-induced elongation of rice shoots. GA was applied to the shoots, while TA was applied to the roots —○—, without TA; —●—, with TA (2×10^{-4} M). Standard errors were given as vertical bars, as in Fig 1.

Plant material and bioassay. In all experiments rice (*Oryza sativa* L.) was used, and the bioassay was carried out at 28° under continuous fluorescent illumination of 4500 lux at plant level. For an assessment of structure–activity relationships of triazinones, 5 germinated seeds of rice (var Kinmaze) were planted on 10 ml of 0.5% agar medium containing each test compound with or without 10 mg/l. GA in a test tube (2.6 cm dia. × 6 cm). Rice seedlings were incubated for 4 days and thereafter their shoot lengths were measured. In a second experiment, the interaction between GA and TA in shoot growth of various rice varieties was investigated. 25 germinated seeds were planted on 35 ml of 0.5% agar medium containing TA with or without 10 mg/l. GA, in a 7.5 cm Petri dish. Five-day-old seedlings were harvested and their shoot lengths were measured. To examine the influence of application sites of GA and TA, a third experiment was set up, using normal variety Kinmaze. Root application was conducted under the same conditions as the second experiment: rice seedlings were incubated on the agar medium containing TA and/or GA. Shoot application was carried out according to the microdrop method of ref. [8]. Rice seedlings incubated for 2 days on the agar medium were treated by applying GA and/or TA in a 1 µl drop to the coleoptile surface. In both applications, rice seedlings were allowed to grow for 5 days after planting, and their lengths of shoots or second leaf sheath were measured. This experiment consisted of four groups: (i) root application of GA plus TA; (ii) shoot application of GA plus TA; (iii) root application of GA and shoot application of TA, and (iv) root application of TA and shoot application of GA. There were three to four replications through the experiments.

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