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Effects of Seed-Soaked GA_3 and Inorganic Salts on Mesocotyl and Coleoptile Elongation in Rice

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The elongation of mesocotyl and coleoptile plays important roles in the seedling emergence and stand establishment of dry direct-seeded rice. Experiments were carried out to elucidate the effects of seed-presoaking treatments of GA_3 and some inorganic salts on the mesocotyl and coleoptile elongation of rice. Seed-soaked GA_3 promoted the elongation of mesocotyl, but little effect on the coleoptile elongation. The stimulation effects of GA_3 were found to be enhanced by addition of $CaCl_2$. However, the sole treatment of $CaCl_2$ showed no stimulating effect on the mesocotyl and coleoptile elongation. Mesocotyl elongation was most prominent in the combined treatments of 50 ppm GA_3 with 100 mM $CaCl_2$. The synergistic effects of GA_3 and $CaCl_2$ on mesocotyl elongation varied with varietal groups. The stimulating effects of GA_3 were enhanced significantly by the addition of $CaCl_2$ in japonica varieties, Dongjinbyeo, Ilpumbyeo and Milyang 95, and tall indica variety, Labelle, but not in semidwarf Tongil type varieties, Tongilbyeo, Milyang 23, and Nampungbyeo, and semidwarf indica, Short Labelle. The promoting effects of GA_3 on the mesocotyl elongation were decreased in proportion to the lowered osmotic potential by PEG 6000 on the contrary to $CaCl_2$. This implies that the synergistic effects of $CaCl_2$ with GA_3 on mesocotyl elongation was not caused by osmotic potential lowered by $CaCl_2$ addition but by the salt itself. Salts such as $Ca(NO_3)_2$, $MgCl_2$, $BaCl_2$, NaCl, KCl and KNO_3 showed the synergistic effects with GA_3 on mesocotyl elongation as well. The degree of synergistic effects showed no differences among salts tested, implying that there is no specificity of ions constituting the salts.

Key words: rice, mesocotyl, coleoptile, GA_3, inorganic salts, synergistic effect

The elongation of mesocotyl and coleoptile is a crucial factor to determine the seedling emergence of dry direct-seeded rice under adverse conditions such as deep sowing, soil crusting, and soil compaction (Lee & Myung, 1995). Mesocotyl and coleoptile elongation of rice during emergence was varied not only with varieties (Inouye & Ito, 1969; Lee *et al.*, 1993), but also with environmental conditions (Ohwaki, 1967; Takahashi, 1978; Turner *et al.*, 1981, 1982). The elongation of coleoptile was not generally different between indica and japonica cultivars (Takahashi, 1978), while the elongation of mesocotyl was much higher in indica cultivars than that in japonica cultivars (Inouye & Ito, 1968; 1969; Kim *et al.*, 1989). Among the Korean varieties, Tongil type varieties have showed greater mesocotyl elongation than those of japonica varieties (Kim *et al.*, 1989). With high temperature pretreatment of 40°C for 10 days in the dark, the husked seed of a japonica cultivar, Hoyoku, was reported to elongate mesocotyl and coleoptile longer mainly due to increasing the cell division rather than increasing cell elongation (Inouye *et al.*, 1970). In addition, effects on mesocotyl and coleoptile elongation of rice have been approved with seed maturity, storage condition, light and air composition. Light inhibits mesocotyl and coleoptile elongation (Chang *et al.*, 1965). Takahashi (1978) found that CO_2 removal during rice seedling growth decreased both coleoptile and mesocotyl elongation. Ethylene removal reduced mesocotyl elongation but not coleoptile elongation. Low O_2 concentration enhances the coleoptile elongation appreciably (Turner *et al.*, 1981). However, mesocotyl elongation does not occur in hypoxic or anoxic condition (Ota, 1969). Seed pretreatments with GA_3 (Ogawa & Kitamura, 1980; Ota & Nogaki, 1967; Suge, 1971), ABA (Takahashi, 1973; Takahashi & Sato, 1972), and ethylene (Suge, 1971; 1972) promote the mesocotyl and the coleoptile elongation. ABA has the most prominent effects on mesocotyl elongation, while it has the defects to inhibit the growth of shoot and seminal roots (Terao *et al.*, 1984). GA_3 promotes the elongation of mesocotyl and coleoptile (Takahashi, 1973), but its effect on mesocotyl elongation was not as great as ABA (Terao *et al.*, 1984). GA_3 also increases the enhancement effect of ethylene and ABA on mesocotyl elongation (Suge, 1971; Takahashi, 1973). Plant growth resoponse mediated by phytohormones is stimulated or inhibited in the presence of inorganic salts (Poovaiah and Leopold, 1974).

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The stimulating effect of GA_3 on rice mesocotyl elongation is increased by treating $CaCl_2$ (Leopold *et al.*, 1973). KCl treatment increases the GA_3-stimulated elongation of illuminated lettuce hypocotyl sections, but there is no effect with KCl treatment alone (Silk *et al.*, 1975). Poovaiah and Leopold (1974) reported that hypocotyl hook formation of lettuce was stimulated through a synergistic interaction of ethylene and GA with ammonium and sulfate ions in the light, but inhibited by potassium and carbonate ions. They suggested that inorganic solutes may alter the effectiveness of the hormonal regulation in part through their actions as salting-out and salting-in agents on macromolecules such as proteins.

There are not much research data relating to the interactive effects of GA_3 and inorganic salts and varietal responses on mesocotyl and coleoptile elongation of rice The objectives of this study were to (i)evaluate the interactive effects of seed-presoaking treatments of GA_3 and inorganic salts on mesocotyl and coleoptile elongation of rice and (ii) to observe the varietal difference of the responses.

MATERIALS AND METHODS

In the following four laboratory experiments, seeds were soaked in the solutions with different concentrations of GA_3 and inorganic salts for 72 hours. The solutions were maintained at 20°C until the seeding of rice. Soaked seeds were rinsed with tap water and sown at 7 cm deep in the plastic boxes filled with vermiculite. After the seeding, the plastic boxes were saturated with distilled water and drained through the perforations in the bottom. The boxes were placed in the dark incubator which was kept at 25°C. After 11 days incubation, 30 rice seedlings were sampled for measurement of their mesocotyl and coleoptile lengths. All the experiments were replicated three times.

Experiment 1

The effects of seed-soaked GA_3 and $CaCl_2$ on mesocotyl and coleoptile growth were examined for two rice cultivars, Dongjinbyeo (japonica) and Labelle (indica). 16 treatments were made by combining four concentrations of GA_3 and $CaCl_2$ each. The levels of GA_3 were 0, 10, 50, and 100 ppm, and those of $CaCl_2$ were 0, 10, 50, and 100 mM.

Experiment 2

With seed-soaking treatments of GA_3 and $CaCl_2$, varietal differences on mesocotyl and colleoptile elongation of rice were evaluated. Ten rice varieties were used for this experiment; three japonica varieties (Dongjinbyeo, Ilpumbyeo, and Milyang 95), three Tongil type varieties (Tongilbyeo, Nampungbyeo, and Milyang 23), two weedy rice varieties (Ssalshare and Galsaekshare), and two indica varieties (Labelle and Short Labelle). Four seed-soaking treatments were done by the combinations of GA_3, 0 ppm and 50 ppm, and $CaCl_2$, 0 mM and 100 mM.

Experiment 3

To determine the synergistic effects of $CaCl_2$ and GA_3 on mesocotyl and coleoptile growth, the seed-soaking solutions containing GA_3 with different osmotic potentials were made by adding $CaCl_2$ or PEG 6000. The osmotic potentials of two seed-soaking solutions containing GA_3 of 0 and 50 ppm were adjusted to 0, -0.7, -3.5, and -7.0 bar. Rice variety used in this experiment was Dongjinbyeo.

Experiment 4

This experiment was conducted to examine the interactive effect of inorganic salts other than $CaCl_2$ and GA_3 on mesocotyl and coleoptile growth. Prepared were seed-soaking solutions containing 50 ppm GA_3 and 50 ppm GA_3 plus one of the following 100 mM slats; $CaCl_2$, $Ca(NO_3)_2$, $MgCl_2$, $BaCl_2$, NaCl, KCl, and KNO_3. An indica variety, Labelle, was used for this experiment.

RESULTS AND DISCUSSION

Effects of seed-soaked GA_3 and $CaCl_2$ on mesocotyl and coleoptile elongation

Mesocotyl and coleoptile lengths in response to the seed soaking treatments of GA_3 and $CaCl_2$ are shown in Table 1. Mesocotyls were longer in Labelle (indica type) than in Dongjinbyeo (japonica type), not showing the differences with the treatments, and coleoptile were *vice versa*. Seed-soaking treatment of GA_3 showed significant effect on mesocotyl elongation at 10 ppm and above in both varieties, but no significant effect on coleoptile growth.

Mesocotyl length was increased curve-linearly with raising the concentration of GA_3. The stimulating effect of GA_3 on mesocotyl growth was enhanced by the addition of $CaCl_2$. However, the treatment of $CaCl_2$ alone did not show stimulating effects on mesocotyl elongation. Mesocotyl elongation was enhanced significantly by the addition of 100 mM $CaCl_2$ to 10 ppm and 50 ppm GA_3 in Dongjinbyeo, and to 50 ppm GA_3 in Labelle. However, the addition of $CaCl_2$ showed no significant synergistic stimulation on mesocotyl growth with 100 ppm GA_3 in both varieties. $CaCl_2$ showed no consistent effect and no interactive effect with GA_3 on

Table 1. Mesocotyl and coleoptile length at 11 days after seeding as affected by seed-soaking treatment of GA_3 and $CaCl_2$ in rice varieties, Dongjinbyeo and Labelle.

Treatment		Dongjinbyeo		Labelle	
GA_3 (ppm)	$CaCl_2$ (mM)	Mesocotyl	Coleoptile	Mesocotyl	Coleoptile
		-------------- mm --------------			
0	0	0.22	44.2	23.3	22.5
	10	0.43	44.0	23.2	23.4
	50	0.21	37.7	22.5	23.9
	100	0.36	48.3	24.2	27.2
	mean	0.31	43.6	23.3	24.3
10	0	2.99	44.4	31.0	26.8
	10	3.89	42.5	32.5	26.3
	50	3.78	47.3	32.7	22.6
	100	4.49	49.2	36.9	26.8
	mean	3.80	45.9	33.3	25.6
50	0	5.43	45.8	32.1	24.1
	10	5.03	29.9	32.7	21.1
	50	5.78	47.9	39.2	25.8
	100	7.60	48.1	39.1	25.5
	mean	5.97	42.9	35.8	24.1
100	0	5.87	47.3	38.8	27.0
	10	6.40	48.0	38.9	26.9
	50	5.95	49.2	40.5	26.5
	100	5.82	47.3	42.5	23.8
	mean	6.02	47.9	40.2	26.1
$LSD_{0.05}$ between					
GA_3 treatment means		0.37	2.0	2.1	1.5
$CaCl_2$ treatment means		0.31	1.9	2.0	1.6
$CaCl_2$ levels within GA_3 level		0.74	3.9	2.1	3.1

coleoptile growth. Leopold *et al.* (1973) found that $CaCl_2$ enhanced the GA_3 stimulating effect on rice mesocotyl growth as in the present experiment. Silk *et al.* (1975) reported that KCl showed the synergistic promotion of lettuce hypocotyl segment elongation with GA_3.

Varietal difference in responses to seed-soaked GA_3 and $CaCl_2$

Ten rice varieties including three japonicas (Dongjinbyeo, Ilpumbyeo, and Milyang 95), two indicas (Labelle and Short Labelle), three Tongil types (Tongilbyeo, Nampumbyeo, and Milyang 23), and two weedy rices (Ssalshare and Galsaekshare) were evaluated for the responses of mesocotyl (Fig. 1) and coleoptile elongation (Fig. 2) to GA_3 and $CaCl_2$.

Mesocotyl lengths in control condition without GA_3 and $CaCl_2$ were significantly different among varietal groups, being shorter in japonicas and Tongil types (semidwarf) than weedy rices and indicas. Among indicas, Short Labelle, a semi-dwarf variety was shorter than Labelle, a tall variety. The genes expressing plant height and mesocotyl length are linked closely (Dilday *et al.*, 1990), and semidwarf varieties generally have shorter mesocotyl than tall varieties (Turner *et al.*, 1982; Dilday *et al.*, 1990).

GA_3 enhanced mesocotyl growth significantly in all varieties, except semidwarf varieties including Tongil types and Short Labelle. $CaCl_2$ addition stimulated mesocotyl growth synergistically with GA_3. Coleoptile lengths showed no differences among varietal groups and was not increased consistently by treating GA_3. Among ten varieties, Ilpumbyeo, Tongilbyeo, Nampungbyeo, and Short Labelle had significant increase in coleoptile length by treating GA_3. $CaCl_2$ addition had significant effect on coleoptile growth synergistically only in japonica varieties.

Effects of seed-soaked GA_3 under different osmotic potentials on mesocotyl length

The responses to GA_3 soaking treatment under different

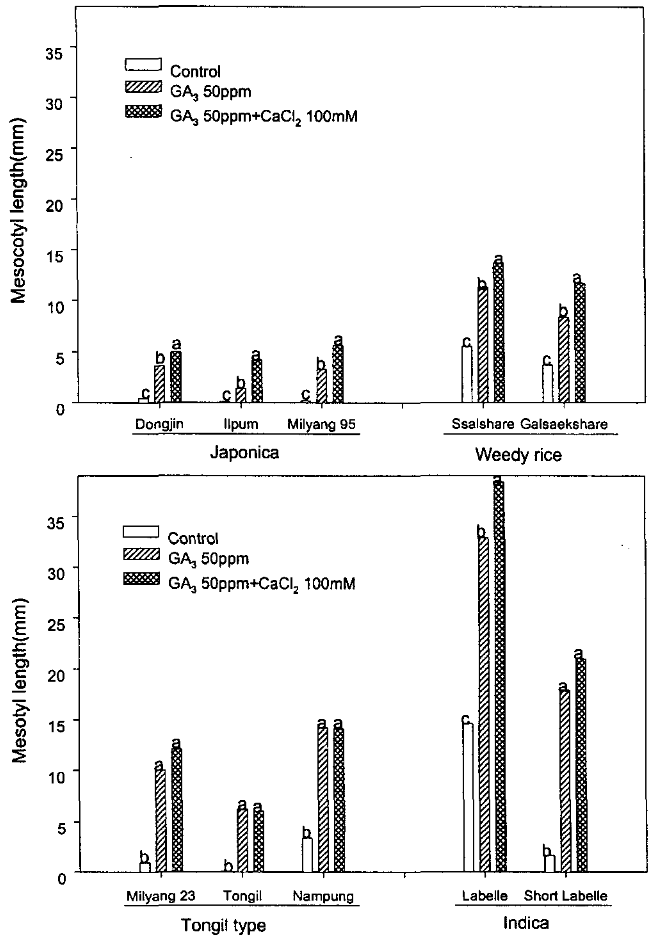

Fig 1. Mesocotyl elongation in response to seed-soaking treatments of GA_3 and $CaCl_2$ in 10 rice varieties. The same letters above bars within a variety mean no significant difference at 5% probability level by DMRT.

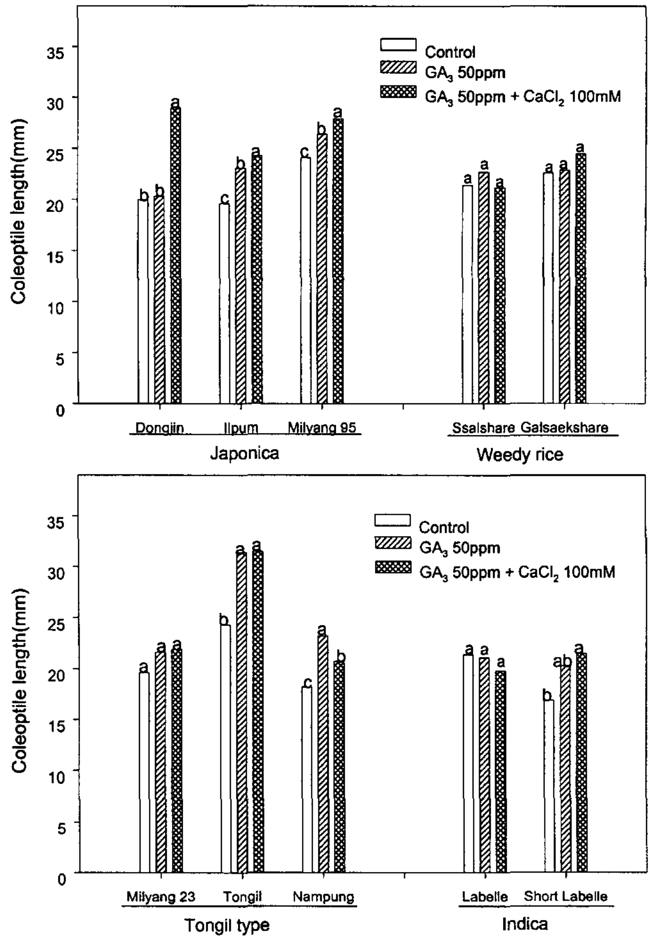

Fig 2. Coleoptile elongation in response to seed-soaking treatments of GA_3 and $CaCl_2$ in 10 rice varieties. The same letters above bars within a variety mean no significant difference at 5% probability level by DMRT.

osmotic potentials were shown in Fig. 3.

When the osmotic potentials of seed soaking solution containing 50 ppm GA_3 were adjusted by PEG 6000, the promoting effect of GA_3 on the mesocotyl elongation was decreased in proportion to the lowering of osmotic potential. Contrasting with these, mesocotyl length was increased with decreasing the osmotic potential adjusted by $CaCl_2$. Mesocotyl growth was slightly decreased with decreasing the osmotic potentials of GA_3-free soaking solution adjusted by both $CaCl_2$ and PEG 6000. This implies that the synergistic effects of $CaCl_2$ with GA_3 on mesocotyl elongation was not caused by osmotic potential lowered by $CaCl_2$ addition but by $CaCl_2$ itself. Poovaiah and Leopold. (1974) suggested that inorganic solutes may serve to alter the plant growth response to hormone at least in part through salting-out and salting-in actions on macromolecules such as proteins. However, the mechanisms have not been elucidated yet.

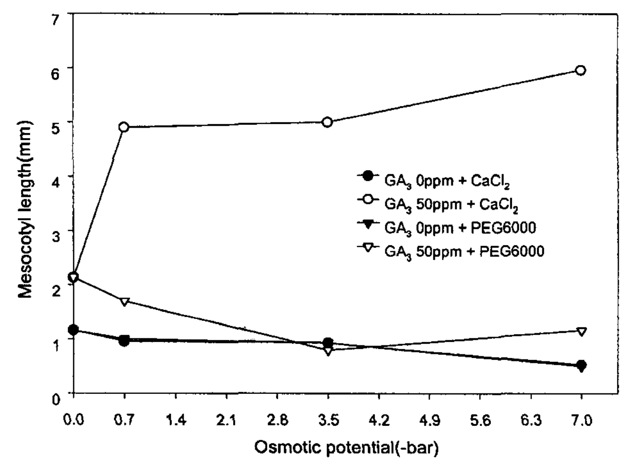

Fig. 3. Mesocotyl elongation in response to seed-soaking treatment of GA_3 under different osmotic potentials adjusted by either $CaCl_2$ or PEG 6000 in rice variety, Dongjinbyeo.

Table 2. Comparison of mesocotyl and coleoptile length under seed-soaking treatments of GA_3 (50 ppm) and different inorganic salts (100 mM) in rice variety, Labelle.

Treatment	Mesocotyl	Coleoptile
	----------- mm	-----------
GA_3	22.3^c	28.1^a
GA_3+$CaCl_2$	38.8^{ab}	26.7^a
GA_3+$Ca(NO_3)_2$	37.3^{ab}	27.2^a
GA_3+$MgCl_2$	39.8^{ab}	25.9^a
GA_3+$BaCl_2$	41.3^a	25.6^a
GA_3+NaCl	37.7^{ab}	25.5^a
GA_3+KCl	37.7^{ab}	26.9^a
GA_3+KNO_3	35.2^b	27.1^a

*The same letters within column mean no significant difference at 5% probability level by DMRT.

Effects of seed-soaked GA_3 and inorganic salts on mesocotyl and coleoptile elongation

The responses of mesocotyl and coleoptile elongation to the combined seed-soaking treatments of GA_3 and inorganic salts were presented in Table 2.

All the inorganic salts such as $Ca(NO_3)_2$, $MgCl_2$, NaCl, KCl, KNO_3 and $CaCl_2$ showed significant synergistic effects with GA_3 on mesocotyl elongation. On the contrary, any tested inorganic salts did not promoted coleoptile growth when treated with GA_3. The stimulating effect of inorganic salts on mesocotyl showed no significant difference among inorganic salts composed of different kinds of anion and cation. This implies that there is no specificity of ions constituting the salts for their synergistic effects on mesocotyl growth with GA_3.

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