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Effect of Plant Growth Regulators on Grain Fill *in vitro* Culture of Rice Panicle

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ABSTRACT: In vitro culture of panicle has been the method to accumulate starch and protein in immature grains by providing nutrients after florets crossed between remote genotypes artificially. Grain filling means embryo development and sucrose translocation from photosynthetic source, and starch manufacture in endosperm. The concentrations of sucrose used to culture immature rice panicle were 5, 10, 15, 20% and glutamine was 20 mM. When immature rice panicles at 5 days after flowering were cultured in distilled water with different concentrations of sucrose, glutamine 20 mM and MS medium with different concentrations of sucrose, glutamine 20 mM for 30 days the later was effective on grain filling. The optimal concentration of sucrose on grain filling in vitro culture of rice panicle was 10% and the weight of grain cultured was 10.14 mg that was corresponded to 57% of intact plant. In the method of treating plant growth regulators, the culture of immature rice panicle adding in MS medium with Kinetin, IAA, GA_3 were effective on grain filling than the culturing of immature rice panicle after submerging in solutions of Kinetin, IAA, GA_3 for 1day. When immature rice panicle was cultured in MS medium with sucrose 10% and Kinetin 46.47 μM it was effective on grain filling, respectively. The weight of grain cultured was 13.1 mg that was corresponded to 75% of intact and germination rate was 51%. When immature rice panicles were cultured in medium with different concentrations combined with Kinetin 4.65, 46.47, 464.7 μM, IAA 5.71, 57.08, 570.80 μM for 30 days and in medium with IAA 5.71, 57.08, 570.80 μM for 15 days after culturing in medium with Kinetin 4.65, 46.47, 464.70 μM for 15 days the effect on grain filling was similar.

Keywords: grain filling, sucrose, glutamine, MS medium, Kinetin, IAA, GA_3, germination rate

The mature grain of rice is mostly composed of starch that was assimilated by photosynthesis during grain filling. The other method of assimilating starch without photosynthesis in leaf is in vitro culture of panicle (Barlow et al., 1983).

The object of panicle culture was to culture immature floret to normal grain by providing with nutrients to accumulate starch and protein in immature grain. The physiological research of grain filling has been carried out by culturing spikes or panicles in vitro in wheat (Barlow et al., 1983; Cervantes *et al.*, 1989), barley (Corke & Atsmon, 1988), rice (Wei & Sung, 1993; Lee *et al.*, 2000; Lee & Lee, 2000), sorghum (Cai *et al.*, 1994).

The immature florets cultured in vitro have been studied to compare genotypes of varieties about protein synthesis in barley (Corke & Atsmon, 1988), to test heritability on plant regeneration of various varieties in rice (Chu & Croughan, 1990), and to investigate culture medium and conditions in vitro culture of rice panicle (Lee *et al.*, 2000).

The optimum concentration of sucrose used in vitro culture of wheat spike was 4% (Barlow *et al.*, 1983), barley spike was 4% (Corke & Atsmon, 1988), rice panicle was 175 mM (Lee *et al.*, 2000), and sorghum was 3% (Cai *et al.*, 1994). When immature wheat spike was cultured in medium with sucrose 36.5, 73, 164, 292 mM the grain weight cultured was 31.4, 34.1, 35.8, 38.3 mg (Cervantes *et al.*, 1989).

Plant growth regulators have a significant role to stimulate grain growth and to translocate sucrose into immature floret during grain filling. Cytokinin induced the cell division of proembryo after fertilization, such as the initial stage of grain maturing while gibberellin and auxin took an important role after initial stage, and the GA_3 content of barley increased rapidly at 12 days after pollination (Mounla & Michael, 1973).

This study was carried out to determine the concentration of sucrose to effect on grain filling in optimal medium, the effective concentration of Kinetin, IAA, 2,4-D, GA_3 on grain filling, and the combined effect of Kinetin and IAA on grain filling in vitro culture of rice panicle.

MATERIALS AND METHODS

The rice variety used for this research was Hwaseongbyeo belong to the Japonica group. Rice plants were grown in 1/5000a pots inside container with 50 L hydroponic nutrient solution in the greenhouse. Hydroponic nutrient solution was aerated with air pump and changed in two weeks intervals. The composition of hydroponic nutrient solution was same as the previous research (Lee & Lee, 2000). Rice pani-

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cles at 5days after anthesis were sampled, and sterilized by immersing in 70% (v/v) ethanol for 1min. then in 20% (v/v) of NaOCl (Sodium Hypochlorite solution) for 5min. and rinsed twice in sterile distilled water in clean bench.

We cultured immature panicles into 100 ml liquid solution on filter paper covering coiled stainless steel in base of 250 ml Erhlenmeyer flask. The media were adjusted to pH 5.8 by 1.0N HCl or 1.0N KOH and plated in 250 ml Erhlenmeyer flask containing liquid solution autoclaved for 15min at 1.5kg/cm^2. We cultured immature rice panicles at 25°C, 100rpm, 16 hr/8 hr(day/night), in shaking incubator for 30 days.

Determination of the optimum culture medium : Rice panicles were cultured in distilled water that was included glutamine 20 mM as protein source and 5, 10, 15, 20% sucrose. At same time, MS medium that was included glutamine 20 mM as protein source and sucrose 5, 10, 15, 20% was tested in vitro culture of rice panicle for 30 days.

Effect of PGRs on grain fill : To find the effective plant growth regulators and concentration on grain filling. We have compared the treatment methods of PGR, such as 1day-pretreatment and culturing in MS medium or mixing PGR in culture solution thru 30 days-culturing period. Furthermore, the concentrations of PGRs added in culture medium were as followings; Kinetin 4.65, 46.47, 464.70, 4647.00 μM, IAA 5.71, 57.08, 570.80, 5708.00 μM, 2,4-D 4.52, 45.24, 452.40, 4524.00 μM, GA_3 2.89, 28.87, 288.70, 2887.00 μM. The culture medium was MS medium included with sucrose 10%, glutamine 20 mM.

Effect of combination of PGRs : To know effective combined concentrations of plant growth regulators on grain filling we cultured immature panicles in media with combined Kinetin 4.65, 46.47, 464.70 μM, IAA 5.71, 57.08, 570.80 μM for 30 days and in media with IAA 5.71, 57.08, 570.8 μM for 15 days after culturing with Kinetin 4.65, 46.47, 464.7 μM for 15 days.

Germination test of grains cultured in vitro : After 30 days in vitro culture, grains were dried for 72 hr at 70°C, and the rice spikelets with glumes were weighed to compare the effect of PGRs. Also, the rice grains cultured were placed on moist germination paper at 30°C to test germination rate after seven days.

RESULTS AND DISCUSSION

The determination of optimum culture medium for in vitro culture of rice panicle

MS medium has been used to culture of plant tissue for supplying necessary mineral and minor elements. We have compared the culture media composed sucrose 5~20%, glutamine in distilled water to sucrose 5~20%, glutamine in MS medium to know the effect of MS medium and the optimum concentration of sucrose used in vitro culture of immature rice panicle, 5days old after flowering. The concentration of glutamine was fixed 20mM in both basic media, which was found the optimum concentration in previous research (Lee & Lee, 2000).

The average weight of grain cultured in medium for 30days was 8.69mg in distilled water and 9.4mg in MS medium. Thus, culturing of immature rice panicle in MS medium was more effective than in distill water base on grain filling (Table 1). It turned out that the mineral composition contained in MS medium was effective to in vitro panicle culture.

The highest grain weight among all sucrose concentrations of cultured was achieved in MS medium included sucrose 10%, glutamine 20 mM that was 10.14 mg corre-

Table 1. Effects of media and sucrose concentrations on grain filling after 30 days in vitro culture of rice panicle.

Treatment	Sucrose concentration (%)	Dry weight per grain (mg)	†Index of grain weight (%)
Control	5	7.96c	44.90
	10	9.04b	50.99
	15	9.50a	53.58
	20	9.16ab	51.66
	mean	8.69	
MS medium	5	9.07b	51.16
	10	10.14a	57.19
	15	9.96a	56.18
	20	9.44b	53.24
	mean	9.40	

[1] N source : glutamine 20 mM.
[2]†Index of grain weight : grain dry weight cultured in vitro / grain dry weight cultured in the green house×100.
[3] The same letters within column mean no significant difference at 5% probability level by DMRT.

sponded to 57.19% of grain weight grown in green house at 30 days after flowering. Therefore, we used MS medium with sucrose 10%, glutamine 20 mM as optimal medium for PGRs experiment in vitro panicle culture. This result of sucrose was identical to our previous research, which the optimum composition of medium used in vitro culture of rice panicle was sucrose 10%, glutamine 20 mM (Lee & Lee, 2000).

Effect of plant growth regulators on grain fill in vitro culture of rice panicle

In grain filling stage, florets require various plant growth regulators to complete seed maturity and grain fill. There have been reported some effects of endogenous PGRs, such as auxin, gibberellin, ABA, cytokinin.

We have compared the methods of PGR treatment, such as 1day-pretreatment and culturing in MS medium or mixing PGR in culture solution thru 30days-culturing period. The PGRs tested in this experiment were Kinetin, IAA, GA_3, 2,4-D with various concentrations.

The average weight of grain after 30 days culture in medium with sucrose 10%, glutamine 20 mM after pretreatment by submerging into different concentrations of Kinetin for 1day was 11.26 mg, the average weight of grain cultured in medium mixed with different concentrations of Kinetin was 11.54 mg for 30 days. The later was effective on grain filling. The weight of grain cultured in medium with Kinetin 46.47 μM was 13.11 mg that was corresponded to 74.91% of grain weight grown in green house, respectively (Table 2).

The average weight of grain cultured in medium with dif-

Table 2. Effects of Kinetin concentrations and treatment period on grain filling after 30 days in vitro culture of rice panicle.

Treatment period	Kinetin concentration (μM)	Dry weight per grain (mg)	†Index of grain weight (%)
1 day	0	9.91c	56.63
	4.65	10.43c	59.60
	46.47	11.63b	66.46
	464.70	12.44a	71.09
	4647.00	11.66b	66.63
	mean	11.26	
30 days	0	9.91b	56.63
	4.65	12.83a	73.31
	46.47	13.11a	74.91
	464.70	10.67b	60.97
	4647.00	11.18b	63.89
	mean	11.54	

1)†Index of grain weight : grain dry weight cultured in vitro/grain dry weight cultured in the green house×100.
2) The same letters within column mean no significant difference at 5% probability level by DMRT.

Table 3. Effects of IAA concentrations and treatment period on grain filling after 30 days in vitro culture of rice panicle.

Treatment period	IAA concentration (μM)	Dry weight per grain (mg)	†Index of grain weight (%)
1 day	0	9.91b	56.63
	5.71	9.84b	56.23
	57.08	10.17b	58.11
	570.80	11.27a	64.40
	5708.00	11.11a	63.49
	mean	10.86	
30 days	0	9.91d	56.63
	5.71	12.64ab	72.23
	57.08	12.93a	73.89
	570.80	11.37bc	64.97
	8708.00	10.69cd	61.09
	mean	11.51	

1)†Index of grain weight : grain dry weight cultured in vitro/grain dry weight cultured in the green house×100.
2) The same letters within column mean no significant difference at 5% probability level by DMRT.

Table 4. Effects of 2,4-D concentrations and treatment period on grain filling after 30 days in vitro culture of rice panicle.

Treatment period	2,4-D concentration (μM)	Dry weight per grain (mg)	†Index of grain weight (%)
1 day	0	9.91b	56.63
	4.52	10.98a	62.74
	45.24	11.02a	62.97
	452.40	10.53ab	60.17
	4524.00	9.75b	55.71
	mean	10.88	
30 days	0	9.91b	56.63
	4.52	11.48a	65.60
	45.24	10.66ab	60.91
	452.40	10.32ab	58.97
	4524.00	9.93b	56.74
	mean	10.46	

[1)†]Index of grain weight : grain dry weight cultured in vitro/grain dry weight cultured in the green house×100.
[2)] The same letters within column mean no significant different at 5% probability level by DMRT.

Table 5. Effects of GA_3 concentrations and treatment period on grain filling after 30 days in vitro culture of rice panicle.

Treatment period	GA_3 concentration (μM)	Dry weight per grain (mg)	†Index of grain weight (%)
1 day	0	9.91b	56.63
	2.89	10.13b	57.89
	28.87	10.14b	57.94
	288.70	11.84a	67.66
	2887.00	10.23b	58.46
	mean	10.80	
30 dyas	0	9.91c	56.63
	2.89	11.78ab	67.31
	28.87	11.96a	68.34
	288.70	12.13a	69.31
	2887.00	10.93b	62.46
	mean	11.34	

[1)†]Index of grain weight : grain dry weight cultured in vitro/grain dry weight cultured in the green house×100.
[2)] The same letters within column mean no significant different at 5% probability level by DMRT.

ferent concentrations of IAA for 1day pretreatment was 10.86 mg, while the average weight of grain cultured in medium with different concentrations of IAA was 11.51 mg for 30 days. The later method was effective on grain filling. The weight of grain cultured in medium with IAA 57.08 μM was 12.93 mg that was corresponded to 73.89% of grain weight grown in green house, respectively (Table 3).

The 2,4-D and GA_3 were not effective in vitro panicle culture as shown the relatively lower grain weight than the other PGRs, Kinetin or IAA (Table 4, Table 5).

The Kinetin had increased cell division of endosperm at early grain development stage in barley, while Auxin and gibberellin was important on grain filling after early grain development (Mounla & Michael, 1973). IAA was needed on grain filling for 20~30 days after flowering (Bangerth et al., 1985).

Germination of the grains cultured in vitro

The germination rate of grain cultured in medium with different concentrations of plant growth regulators was ranged within 33~51%. The germination rate of grain cultured in medium with different concentrations of Kinetin, IAA, GA_3, 2,4-D was ranged within 33~51%, 33~46%, 33~43%, 32~40%. The highest germination rate was 51% in medium with Kinetin 46.47 μM, respectively (Fig. 1).

Effect of combination of plant growth regulators on grain filling in vitro culture of rice panicle

We have compared the effect of combination with Kinetin, IAA on grain filling in vitro culture of rice panicle. We have cultured immature rice panicle in medium with differ-

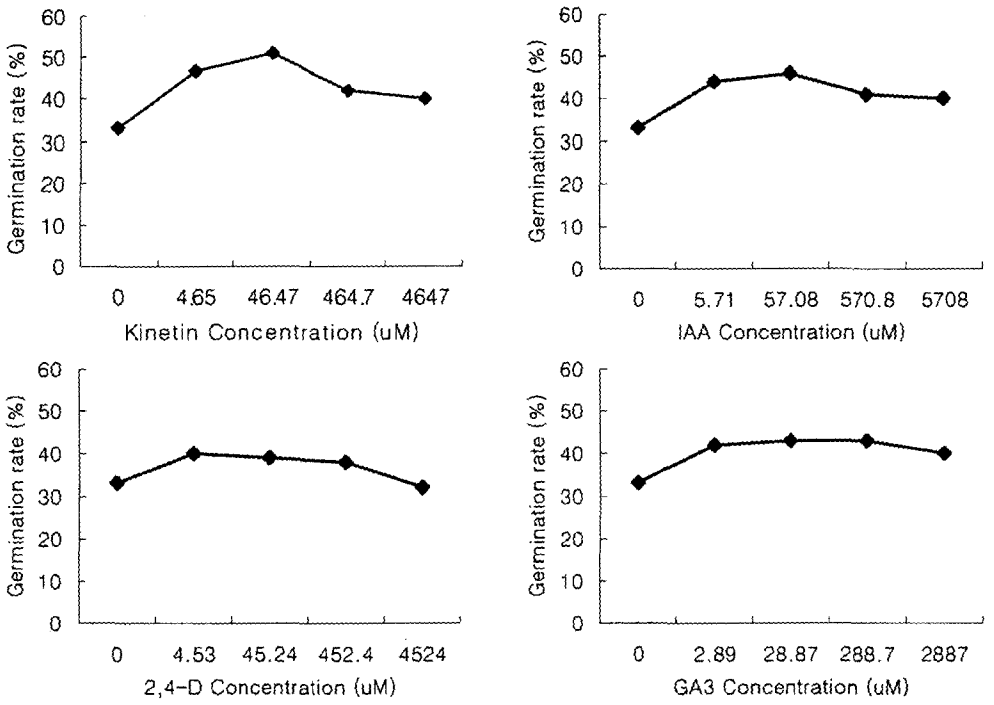

Fig. 1. *Effect of plant growth regulators with different concentrations on germination of grain cultured.*

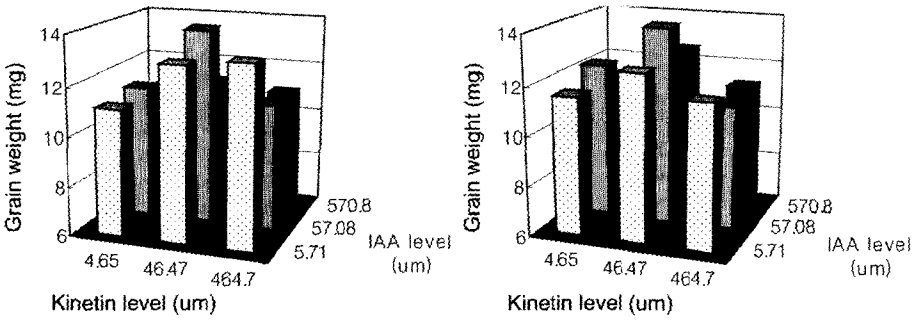

Fig. 2. Effect of combination of Kinetin, IAA on grain filling in vitro culture of rice panicle. (a) The grain weight cultured in mediu m combined different concentrations of Kinetin, IAA for 30 days. (b) The grain weight cultured in medium with different IAA concentrations for 15days after culturing with different Kinetin concentrations for 15days.

ent concentrations combined with Kinetin+IAA for 3 weeks and cultured in medium with Kinetin for 15days and IAA for next 15days.

The effect on grain filling in vitro culture of rice panicle was better than single treatment of PGRs. The Kinetin 46.47 μM+IAA 57.07 μM was best combination in grain weight among whole combinations. The weight of grain cultured in medium combined with Kinetin 46.47 μM and IAA 57.08 μM for 3 weeks was the highest, 13.92 mg, which was corresponded 79.53% compared to grain weight grown in the green house. But, there were a similar trend between treatments with Kinetin+IAA for 3 weeks and with Kinetin for 15 days and IAA for next 15days (Fig. 2).

In conclusion, MS medium with sucrose and glutamine was better medium than only medium with sucrose and glutamine. Furthermore, PGR addition in panicle culture media were effective increasing grain weight, especially, in the combined treatment of Kinetin + IAA.

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