Korean J. Crop Sci. 45(1): 32~37(2000)

Composition of Culture Medium and Culture Conditions for *In vitro* Culture of Rice Panicle

Jin-Ho Lee* and Ho-Jin Lee†**

**Pacific Corporation/R & D Center, Yongin-Si, Kyounggi-do, Korea 449-900*
***Dept. of Agronomy, Seoul Nationul Univ., Suwon, Korea 441-744*
Received February 16, 2000

The *in vitro* culture of rice panicles is a culturing technique only panicle without other organs in culture solution containing organic substance, so that would be useful to study how assimilate supply affects grain development and maturation. To find the optimum stage for *in vitro* culture, rice panicles grown in greenhouse were sampled periodically after anthesis and cultured in nutrient medium. The panicles older than 1 weeks after anthesis had produced normal grains. Grain-filling was apparently dependent upon sucrose concentration (8~12%) in medium, but not affected by nitrogen concentration supplied with glutamine. As far as rice panicle was supplied with sucrose and N in nutrient medium, grains continued accumulation of dry matter and maturation regardless to light condition. Considerably, grain-filling was improved when panicles were positioned horizontally inside flask, so that each grain was partially submerged to nutrient medium.

Keywords : *in vitro* culture of rice panicle, grain-filling, sucrose, glutamine, development stage after anthesis, horizontal positioning.

Rice, one of the important edible farm products, is essential as foodstuff of human beings. Even though rice grains are the most final product in rice farming, *in vitro* culture technique of panicle unlike other organs or tissues, is not yet established and not applied to *in vitro* food production or cereal manufacture.

In vitro culture of rice panicle is a technique of culturing panicle detached from mother plant after anthesis, without any organs like stem, root, branches, and leaves. Unlikely other hydroponic culture, culture solution is supplied in an organic nutrient forms which are the same mobile substances in phloem. Therefore, it appears that rice seed in early stage of development can absorb this solution in similar mechanism used for translocation of photosynthate and convert it to storage carbohydrates.

As a panicle is detached from stem, the nutrient supply to panicle is cut off, differentiation of ovule tissue slows down, and starch accumulation in endosperm is discontinued. Under these circumstances, induction of ovule development and starch accumulation to the endosperm require exogenously supplied appropriate nutrients for the panicle development, that is called the technique of *in vitro* culture of rice panicle.

There were some reports of success in *in vitro* culture of panicle in wheat, barley and maize (Singh & Jenner, 1983; Barlow *et al*, 1983; Singletary & Below, 1989), but not in rice (Singh *et al*, 1978; Villareal & Juliano, 1987). Therefore, an establishment of successful culturing technique for rice panicle will effectively be applied in studying nutrient transport within the panicle, composition of the assimilates, and aging physiology of the panicle. In addition, it will contribute to detailed and systematic studies of filling physiology seeking for higher grain yield.

Therefore, this study was carried out in order to establish *in vitro* culture of rice panicle, by examining the effects of sampling time of panicle, position-setting method, nutrient composition and culture conditions on the development of grain and germinating ability of the seed.

MATERIALS AND METHODS

Panicle sampling and sterilization

Rice plants were grown by solution culture in green house. After panicle emerged, they were tagged by the dates of anthesis and sampling was done by cutting the panicle neck under water so that they could be prevented a cut-off of water flow to the grain. Panicle samples were cut into suitable sizes under water with a sterilized blade and were surface-sterilized 5~10 min. with 2% NaOCl solution. Surface-sterilized panicles were set position inside flask under an aseptic condition of clean bench and were transferred to growth chamber.

Composition of culture medium

In vitro culture medium was composed of mainly nitrogen and carbohydrate substances. Glutamine was used as the

†Corresponding author.
Phone) +82-331-290-2304
E-mail) hojinlee@snu.ac.kr

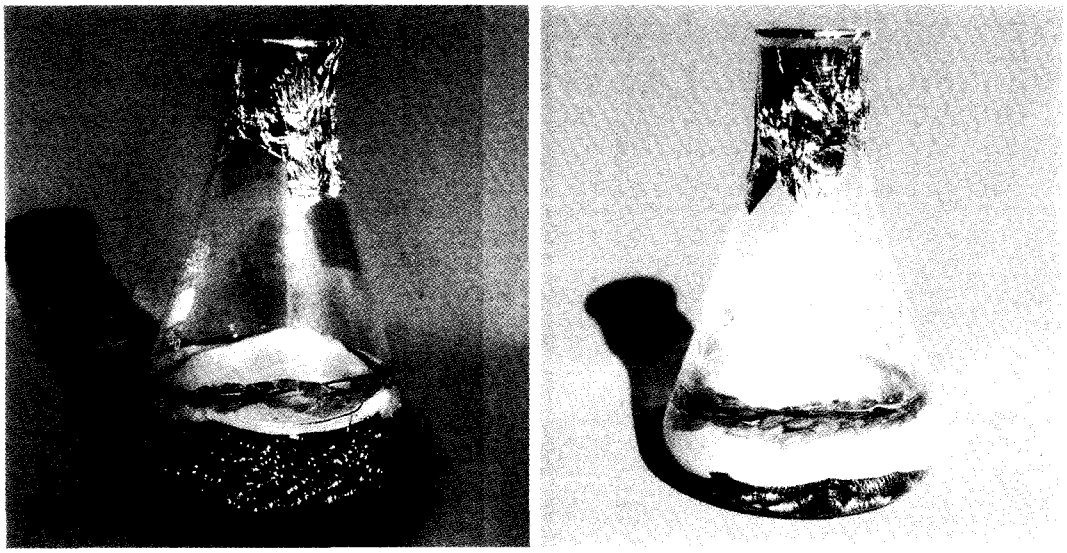

Fig. 1. Setting of panicles *in vitro* culture. a) At start of *in vitro* culture, b) After 50 days of *in vitro* culture.

nitrogen source and sucrose as the carbohydrate. The culture medium was fixed to pH 5.5~6.5 with 0.1N NaOH and sterilized for 17 minutes at 121°C. For the culture system, 100 ml of the culture medium was poured into 200 ml Erlenmeyer's flask.

Position setting of panicle inside flask

Since panicle setting inside flask determine the distance from sink to source, we tested upright and submerged setting. The panicle inside flask was supported by a stainless coil and a filtering paper at the bottom of the flask.

Every *in vitro* culture were done at 25 ± 1°C with 13 hours light in growth chamber.

After panicles were cultured *in vitro* for 50 days, the dry weight of grains was measured and the moisture content of grains was corrected to 14%. Futhermore, the matured seeds were placed in incubator to examine the rate of germination.

RESULTS AND DISCUSSION

Effects of different stage of seed development

In order to find out effect of stage of seed development on the development of final grain after the culture, rice panicles grown in greenhouse were tagged by the dates of anthesis.

Panicle samples were taken at random to find out the effect of seed development by their dates of anthesis. After rice panicles were taken at 3 rd, 6 th, 9 th, 12 th and 15 th days after anthesis among panicles in same anthesis date, the panicle was placed in culture medium in flask. As a result of *in vitro* culture, the stage of development had a great effect on rate of filling and weight of the final grain. Panicles sampled in different stages of development showed a significant difference in their final grain weights after 50 days *in vitro* culture (Table 1). In culture of the panicles that were 3 DAA sampling, grain filling was not carried out. Rice panicles had gained the ability of starch accumulation and protein storage only from 5~7 days after anthesis. Based on these results, we can conclude that the stage of development of the sample (3 DAA) was too early to store nutrients. In the samples of 6 DAA, final dry weight of the grain reached 15 mg and filling rate over 50% after 50 days *in vitro* culture. Grain filling had reached 66% in the relative grain wt., that was expressed as the percentage of grain wt. to greenhouse con-

Table 1. Effect of different stages on grain development in panicle culture of rice.

Stage of panicle development	Initial floret wt. (mg)	Final grain wt. (mg)	Filling rate (%)	Relative grain wt.* (%)
3 DAA	3.88	8.73	0	35.6
6 DAA	7.73	14.88	48.4	60.7
9 DAA	9.96	16.28	58.0	66.5
12 DAA	10.18	18.01	62.2	73.5
15 DAA	13.05	21.73	73.0	88.7
LSD		1.77**		

*Relative grain wt. was expressed the percentage relative to grain wt. in greenhouse control.
Composition of culture medium: 100 g/l sucrose and 20 mM glutamine, Duration of *in vitro* culture of rice panicle: 50 days. DDA: days after anthesis.
** Significant at the 0.01 level.

Table 2. Effect of different sucrose level in culture medium on rice grain filling.

Sucrose conc. (%)	Initial floret wt. (mg)	Final grain wt. (mg)	Filling rate (%)	Relative grain wt.* (%)
2	8.81	13.90	43.90	56.74
4	8.81	14.70	50.00	60.00
6	8.81	15.86	47.89	64.74
8	8.81	16.64	52.24	67.92
10	8.81	18.36	72.54	74.94
12	8.81	17.43	65.15	71.14
14	8.81	16.16	67.21	66.00
LSD		1.46**		

*Relative grain wt. was expressed the percentage relative to grain wt. in greenhouse control.
N level: 20 mM glutamine, Duration of *in vitro* culture of rice panicle: 50 days.
**Significant at the 0.01 level.

trol. For sample of 12 DAA, the final weight of the grain was increased to 18 mg and filling rate 62%. For sample of 15 DAA, the final weight of the grain was 21.7 mg which was almost the same as that of filled grain at mature stage and filling rate reached 73%.

As a result of the test, rice panicles suitable for *in vitro* culture were panicles at 10 days after anthesis and even though they are at milky stage, they had a perfect ability of starch and protein accumulation. Therefore, we were able to obtain filling grains similar to that of the rice panicles at mature stage after 50 days *in vitro* culture (Table 2).

Effects of sucrose concentration in culture medium

High levels of sucrose concentration in culture medium were requisite to increase the final weight of grain and this was true to *in vitro* cultures of wheat, maize and barley spikes (Singh & Jenner, 1983; Barlow *et al*, 1983; Singletary & Below, 1989). Even *in vitro* culture of the rice panicles, sucrose level in culture medium influenced the amount of dry weight of grain and starch accumulation, and it has been reported that the sucrose level affects physiological development of the filling grain (Singh *et al*, 1978; Villareal & Juliano, 1987).

Since rice grain consisted of starch granules, the concentration of sucrose being the main source of starch, is thought to have great influence on the dry weight of the grain. In order to find out sucrose concentration suitable for *in vitro* culture of rice panicle and to find out effect of sucrose concentration within the nutrient solution to nutrient accumulation of grain, rice panicles were cultured at various sucrose concentrations (Table 2). They were cultured for 50 days in a culture medium whose glutamine concentration was 20

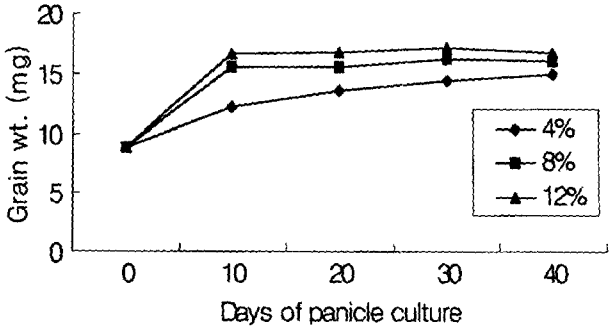

Fig. 2. Variation of grain weight on sucrose concentration after *in vitro* culture of rice panicle.
*N level was 20 mM.

mM/L in N. After the culture, the weights of final grain were significantly high (18.3 mg and 17.3 mg) at 10% and 12% of sucrose concentrations but low at 2% and 4% of sucrose concentrations. Rate of grain filling was also the highest at 10% sucrose concentration which was relatively high. The weight of rice grain increased as the concentration of sucrose increased and it was the highest at 10% concentration but decreased above this level. In previous research of *in vitro* cultures of rice panicles, the culturing was done under 1.5~2.0% sucrose concentration (Singh *et al*, 1978) with samples taken around 20 days after pollination for duration of 10 days. In this study, we were able to obtain fully filled grain from culture medium of comparatively high sucrose concentration of 8% and above, since we used younger panicle samples taken around 10 days after pollination and prolonged the duration of the cultivation to 50 days. (Table 2).

In experiment seeking for the trend of increase in dry weight as a funtion of sucrose concentrations, there was a rapid accumulation of nutrients until 10 days after position setting but the accumulation became slow afterwards (Fig. 2). In 4% and 8% of sucrose concentration, a rapid increase in grain weight for the first 10 days of culture was observed, while the increase of grain weight was slow from the beginning in sucrose concentration of 4%. There was no significant difference in the final grain weights at 8% and 12% sucrose concentrations.

Effects of nitrogen level in culture medium

In *in vitro* culture of wheat panicle, inorganic nitrogens such as ammonium or nitrate limits the grain filling and glutamine is known to be the best source of nitrogen (Singh & Jenner, 1983). For this rice panicle culture, glutamine was also used as N source. Glutamine concentration up to 10mM in culture medium did not have a significant effect on the increase of grain weight. When rice panicles were cultured

Table 3. Effect of different N level in culture medium on grain filling.

N level (mM)	Initial floret wt. (mg)	Final grain wt. (mg)	Filling rate (%)	Relative grain wt.* (%)
10	8.81	16.04	68.7	65.5
20	8.81	15.03	47.5	61.4
30	8.81	15.39	53.3	62.8
40	8.81	14.25	49.4	58.2
LSD		2.84^{NS}		

*Relative grain wt. was expressed the percentage relative to grain wt. in greenhouse control.
Sucrose level: 100 g/l sucrose, N source: glutamine.
Duration of *in vitro* culture of rice panicle: 50 days.
^{NS}not significant within same column.

for 50 days in the culture media including all combinations of 10% sucrose concentration and different levels of nitrogen (10, 20, 30 and 40 mM), there were no significant differences among different levels of nitrogen concentrations (Table 3). Since the protein content of rice grain ranges only 7~8% and starch contributes most of the rice grain, protein is believed to have less effect on the weight of the grain, with its concentration above a certain limit.

Effects of combinations of different concentrations of sucrose and nitrogen in culture medium

In order to find out the composition of culture medium suitable to induce full filling of the rice grains, *in vitro* culture of panicle was conducted using various combinations of different levels of sucrose and nitrogen concentrations (Fig. 3, and 4). Rice panicles were cultured for 50 days in combined culture medium of sucrose levels of 5, 10 and 15% and nitrogen levels of 10, 20 and 30 mM. After the culturing, culture medium of 10% sucrose concentration showed a better filling compared to that of 5% and 15% sucrose concentration but, under the same sucrose concentration, nitrogen had no significant effect on the nutrient storage of the grain (Fig. 3). The highest grain wt. was obtained in the medium 10% sucrose with 10 mM N.

Average of initial grain weight was 7.87 mg, and cultured for 50 days.

Effects on the germination of the grain in *in vitro* culture

In order to find out germination ability of the grains matured *in vitro* culture, the germination test was done in seed germinator at 30°C for 20 days. For grains cultured under 10% sucrose concentration, the rate of germination was also high, based on the complete grain filling achieved. But in 5% and 15% sucrose concentrations, the germination rates were comparatively low, since the grain fillings were poor (Fig. 4). At glutamine concentrations higher than 10 mM, there were no significant differences in germination rates according to different concentrations of glutamine. It appeared that N had no effect on germination rate of the grains.

Effects of light condition *in vitro* culture of rice panicle

It has been reported that the light affects dry matter accumulation of grains while grain filling is continued by an artificial supply of sucrose *in vitro* culture of wheat panicles (Singh & Jenner, 1983).

In order to find out whether light affects grain filling of rice *in vitro* culture, rice panicles were cultured under light

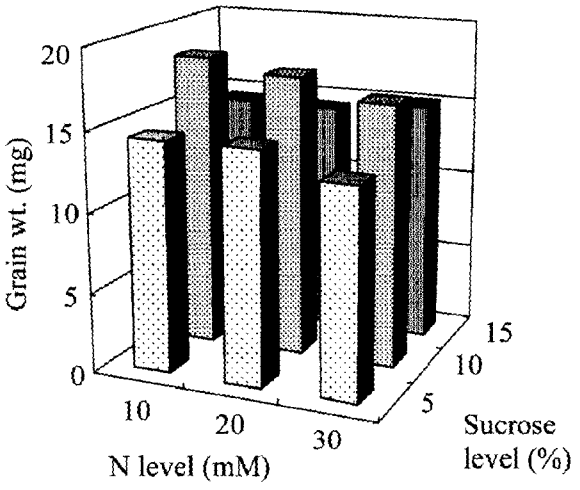

Fig. 3. Effect of combinations of sucrose and N in medium on final grain weight.

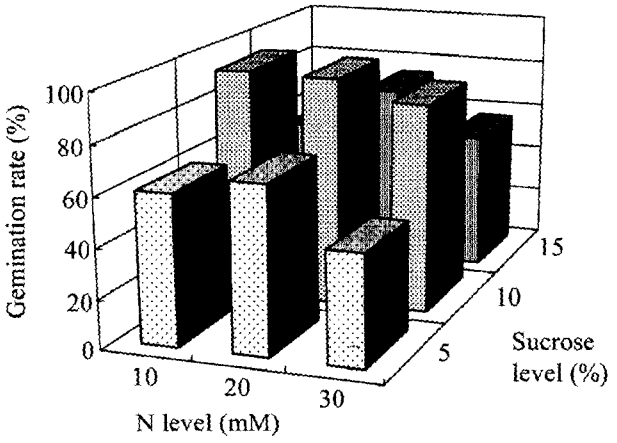

Fig. 4. Germination rate of rice grains *in vitro* cultured.
The grains were cultured in media with combinations of sucrose and N for 50 days.

Table 4. Effect of light condition on grain filling in the *in vitro* culture of rice panicle.

Light environment	Initial floret wt. (mg)	Final grain wt. (mg)	Filling rate (%)	Relative grain wt.* (%)
Light	9.05	17.28	42.5	70.5
Dark	9.05	17.96	47.3	73.3
LSD		2.45^{NS}		

*Relative grain wt. was expressed the percentage relative to grain wt. in greenhouse control.
Composition of culture medium: 100 g/l sucrose and 20 mM glutamine.
Duration of *in vitro* culture of panicle: 50 days.
^{NS}not significant within same column.

Table 5. Effect of panicle position placed *in vitro* culture on grain filling.

Position	Initial floret wt. (mg)	Final grain wt. (mg)	Filling rate (%)	relative grain wt.* (%)
Horizontal	9.02	18.62	58.4	76.0
Vertical	9.02	16.14	57.2	65.9
Vertical-open	9.02	14.65	63.3	59.8
LSD		1.73**		

*Relative grain wt. was expressed the percentage relative to grain wt. in greenhouse control.
Composition of culture medium: 100 g/l sucrose and 20 mM glutamine.
Duration of *in vitro* culture of rice panicle: 50 days.
Vertical-open: Panicle was located outside of flask and its peduncle was dipped into culture medium.
**Significant at the 0.01 level within same column.

or darkness, and their grain filling were compared (Table 4). The panicles were cultured for 50 days in culture medium composed of 100 g/l sucrose and 20 mM glutamine. As a result, the average of final grain weight cultured under light condition or under darkness was 17.28 mg or 17.69 mg, respectively. It indicated there were no significant differences in filling rate. Therefore, we can concluded that light did not affect grain filling or development of the grain *in vitro* culture of rice panicle. This may be due to the fact that the nutrients are supplied in organic forms which are same as the storage substance in grain under the condition excluding current photosynthesis of the plant.

Effects of position setting of panicle in *in vitro* culture

In the previous research on *in vitro* culture of the panicles (Singh *et al*, 1978; Villareal & Juliano, 1987; Oparka & Gates, 1981a), the rice panicle was set in vertical position, in which only the peduncle of the panicle is being dipped into the culture medium. But this vertical positioning may extend the translocation distance of nutrient solution and cut off the nutrient flow in peduncle. Futhermore, it was hard to prolong the duration of culture for more than 10 days and difficult even to obtain fully filled grains due to contamination.

In this study, in order to find position setting which can extend the duration of culturing and which makes possible to obtain fully filled grains after the culturing, the comparisons were made between panicle samples placed in three different positions within the beaker, such as horizontal (Fig. 1), vertical and vertical-open position (Table 5). For vertical-open position, high moisture environment was made to prevent the occurrence of white head due to excessive evapotranspiration. The water table was composed and covering made inside 2 *l* beaker. As a result of culturing in culture medium of 100 g/l sucrose and 20 mM glutamine for 50 days, final grain weight of horizontally positioned sample was significantly higher than those of vertical or vertical-open positioned samples. This horizontal positioning was an advantageous setting with increasing contact area between panicle and solution and decreasing chances of microbial contamination. In vertical-open position, it was difficult to maintain long culturing period due to excessive contamination and evapotranspiration, and the final weight of grain was only 14.65 mg. As for vertical position, the results were not much different from that of vertical-open position. In conclusion, the horizontal positioning is believed to have better position for grain filling, because the half of panicle was submerged in culture medium and so greater chance for nutrient supply but less contamination.

REFERENCE

Barlow, E. W. R., G. R. Donovan, and J. W. Lee. 1983. Water relations and composition of wheat ears grown in liquid culture: Effect of carbon and nitrogen. Aust. J. Plant Physiol., 10:99-108.

Corke, Harold and Dan Atsmon. 1988. Effect of nitrogen nutrition on endosperm protein synthesis in wild and cultivated barley grown in spike culture. Plant Physiol. 87:523-528.

Donovan, G. R. C. F. Jenner, J. W. Lee, and P. Martin. 1983. Longitudinal transport of sucrose and amino acids in the wheat grain. Aust. J. Plant Physiol. 10:31-42.

Faisal, A. K. Sakri. 1975. Movement of ^{14}C-labeled sugars into kernels of wheat. Plant Physiol. 55:881-889.

Giese, H, B. Andersen, and H. Doll. 1983. Synthesis of the major storage protein, hordein, in barley. Planta. 159:60-65.

Haeder, Hans-Eckhard and Helmut Beringer. 1984. Long distance transport of potassium in cereals during grain filling in detached ears. Physiol. Plant. 62:433-438.

Jenner, C. F. 1982. Movement of water and mass transfer into developing grains of wheat. Aust. J. Plant Physiol. 9:69-82.

Mather, D. E. and Henriette Giese. 1984. Protein and carbohydrate

accumulation in normal and high-lysine barley in spike culture. Physiol. Plant. 60:75-80.

Oparka, K. J. and P. Gates. 1981a. Transport of assimilates in developing caryopsis of rice. The pathways of water and assimilated carbon. Planta. 152:388-396.

Oparka, K. J. and P. Gates. 1981b. Transport of assimilates in developing caryopsis of rice. Ultrastructure of the pericarp vascular bundle and its connections with the aleurone layer. Planta. 151:561-573.

Singh, B. K. and C. F. Jenner. 1982. Association between concentrations of organic nutrients in the grain, endosperm cell number and grain dry weight within the ear of wheat. Aust. J. Plant Physiol. 9:83-95.

Singh, B. K. and C. F. Jenner. 1983. Culture of detached ears of wheat in liquid culture:modification and extension of method. Aust. J. Plant Physiol. 10:227-36.

Singh, Rsngil, Consuelo M. Perez, Cynthia G. Pascual, and Bienvenido O. Juliano. 1978. Grain size, sucrose level and starch accumulation in developing rice grain. Phytochemistry. 17: 1869-1874.

Singletary, George W. and Fredrick E. Below. 1989. Growth and composition of maize kernels cultured in vitro with varying supplies of carbon and nitrogen. Plant Physiol. 89:341-346.

Villareal, Ruth M. and Bienvenido O. Juliano. 1987. Effect of Sucrose level on rice grain deposition in detached panicle system. Phytochemistry. 26(12):3121-3125.

Wang, Ning and Donald B. Fisher. 1994. The use of fluorescent tracers to characterize the post-phloem transport pathway in maternal tissues of developing wheat grains. Plant Physiol. 104:17-27.

Wang, X. T. and F. E. Below. 1995. Tillering, nutrient accumulation and yield of winter wheat as influenced by nitrogen form. Journal of plant nutrition. 18(6):1177-1189.