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STUDIES ON THE GROWTH AND DEVELOPMENT OF EXCISED EMBRYOS OF DIFFERENT VARIETIES OF RICE[1]

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Abstract

Embryos excised from *Oryza sativa*, Japonica type (Taichung 46, Taichung 65, Tainan 5), Indica type (Taichung 1, Taichung 2) and a wild type of *O. latifolia*, were cultured *in vitro* to determine their growth and development in various conditions.

Embryos of *O. sativa* Tainan 5 excised at 5 days after pollination (0.687 mm in length) could grow and develop into whole plants on White's medium added with 2% glucose. While embryos younger than 5 days failed to grow.

Embryos excised from the seeds during the imbibition period of less than 2 hr did not grow on White's medium. When the imbibition period of seed was taken longer than 2 hr, the excised embryo grew better.

As mentioned above three types of six excised embryos were cultured on White's medium added with 2% glucose, 4% sucrose or 2% soluble starch and the no carbohydrate added (control). The result showed that the growth of each embryo was significantly different between types under different carbohydrates added and that the cultivated rices (Japonica type and Indica type) grew better in 2% glucose and 4% sucrose but failed to grow in 2% soluble starch. However, the results of wild type *O. latifolia* were altogether different as it was cultured under these medium mentioned.

Introduction

As early as 1904, the embryos of *Raphanus* and *Cochlearia* could be successfully cultured in a medium containing proper nutrient (Hanning, 1904). The techniques of embryo culture have been extensively used to produce hybrids between species of many plants (Tukey, 1933; Brink *et al.*, 1944; Kravtsov and Kasyanova, 1968). However, a successful culture of embryo depended not only on the materials used but also on the suitable culture methods employed

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including physical factors (Overbeek *et al.*, 1942; Overbeek *et al.*, 1944; Blakeslee and Satina, 1944; Norstog, 1956, 1961; Abraham and Thomas, 1962) and nutritional conditions (Wardlaw, 1955). Although some of these have found solutions, many other problems yet remain.

The germination of rice is nourished by the enzymatic breakdown of the reserved starch in endosperm tissues (Varner, 1964; Paleg, 1966; Murata *et al.*, 1968; Nomura *et al.*, 1969). Sucrose is the most commonly used carbohydrate in embryo, tissue and cell cultures *in vitro*. Murata *et al.* (1968) reported that sucrose was consumed for active respiration in the early stages of rice germination. In barley, Abdul-Baki (1969) found that the respiratory activity increased several folds during the early stages of seeds germination.

Recent works indicated that different varieites of rice showed a marked difference in several enzyme levels during germination (Ozaki and Horiguchi, 1965; Murata *et al.*, 1968; Horiguchi and Kitagishi, 1969). Akazawa *et al.* (1964) found that rice varieites differed in the starch synthesis during grain ripening. They reported that the activity of starch synthetase in Japonica type was lower than that in Indica type. Therefore, the conditions for growth of embryos *in vitro* from different varieties of rice may not be identical. The nutritional requirements for the culture of excised embryo of *O. sativa* Taichung 65 has been reported (Yie and Yang, 1973).

This work was aimed at an understanding of the growth and development of the embryo excised from different ages after pollination and excised from different seed imbibition period. A comparison study of the growth and development of the excised embryos derived from different varieties of rice and wild type grown on the White's basic nutrient added with either glucose, sucrose or starch was investigated.

Materials and Methods

Materials

Five varieties of rice were used as the experimental materials, such as a wild rice of *Oryza latifolia;* three Japonica rice varieties of *O. sativa* Taichung 46, Tainan 5 and Taichung 65; and two Indica rice of *O. sativa* Taichung 1 and Taichung 2.

Determination of growth of rice embryo isolated from different ages after pollination

Seeds of *O. sativa* Tainan 5 (TN 5) were sterilized with 70% alcohol for 1 minute and then immersed in 0.1% mercuric chloride solution for 10 minutes and rinsed with sterile distilled water. The embryos from 2 to 30 days after pollination were carefully excised every day and cultured on White's medium

added with 2% glucose for 14 days. Initial embryo size was then measured with a micrometer.

The effect of imbibition stage of seeds on the growth of excised rice embryos

The dehulled seeds of *O. sativa* TN 5 were also used for this experiment. The sterilized seeds were rinsed with sterile distilled water 5 times and transferred to moistened sterile petri dishes. Germination was carried out at 28°C in the dark for various lengths of time up to 48 hours. Embryos were then excised at intervals and cultured on White's medium added with 2% glucose. The seedling growth was measured after 14 days.

The seeds with different germination time were also sectioned 8–10 μ thick, and stained with safranin-fast green. Permanent slides were made for the observation of embryos.

Comparison of the growth rate of excised embryos from different rice in the medium containing different carbohydrates

Seeds of six different rices were sterilized as above and incubated at 28°C for 2 days. Embryos were excised and transferred to the White's basic medium supplemented with (1) 2% glucose, (2) 4% sucrose, (3) 2% soluble starch and (4) none (control). Results were recorded after 14 days of culture.

The growth chamber was equipped with 12,000 lux light bulb and with photoperiod of 14 hours at a temperature of 32°C. Each experiment was set in pentaplicate and repeated for three times. The data presented was the average of fifteen treatments.

Results

The growth of rice embryos isolated from different ages after pollination

The length of embryos of *O. sativa* TN 5 excised from different ages after pollination is recorded in Table 1. It was found that the length of embryos was increased linearly with the age of embryo treated.

Table 1. *The length of embryos of* ***O. sativa*** *TN 5 at different ages after pollination*

Age of embryo (days)	2	3	4	5	6	7	9	15	30
Length of embryo (mm)	0.238	0.523	0.619	0.687	0.924	1.12	1.52	1.94	2.35

The growth of excised embryos cultured on White's medium added with 2% glucose for 14 days is shown in Table 2. The results indicated that an embryo excised from the age younger than 5 days after pollination failed to

grow and develop into a whole plant. There was no significant difference in the growth and development of the embryos excised from the ages of 5 days to 15 days. The growth of mature embryos, 30 days after pollination, was much better than others.

Table 2. *The growth of rice embryos of* ***O. sativa*** *TN 5 excised from different ages after pollination*

Age of embryo (days)	Width of leaves (mm)	No. of leaves	No. of roots	Shoot height (cm)	Root length (cm)	Dry weight (mg)
5	1.4	3	3	11.17	6.84	2.11
6	1.4	3	3	12.21	6.92	2.39
8	1.75	3	4	14.64	7.93	3.50
10	1.80	3	4	14.80	9.88	4.36
12	1.79	3	4	14.76	9.17	4.33
15	2.20	4	4	14.83	10.64	5.41
30	2.22	4	6	23.20	18.41	10.45

The effect of imbibition stage of seeds on the growth of excised rice embryos

Histological changes of the embryo consisted of shoot, root and scutellum at different stages of imbibition, from 0 to 48 hours, were shown in Fig. 1. The differentiation of the shoot and root was not clear at 0, 1 and 2 hours imbibition as compared to others (3, 6, 12, 24, 48 hours). The mature embryo could be seen after 24 hours of seed imbibition (Fig. 1).

Table 3. *Growth of the embryos of* ***O. sativa*** *TN 5 excised from different stages of imbibition seeds*

Growth was measured at 14 days after culture on White's medium

Stage of embryo (hr)	Width of leaves (mm)	No. of leaves	No. of roots	Shoot height (cm)	Root length (cm)	Dry weight (mg)
0	1.0	3	2.1	3.04	4.27	0.97
1	1.46	3	4.4	9.42	9.76	3.66
2	1.8	4	5.0	19.5	11.03	6.66
3	1.8	4	5.25	20.5	11.5	6.65
4	1.95	4	6.13	21.5	13.66	8.16
6	1.96	4	6.1	22.14	14.1	8.36
8	2.0	4.25	6.25	23.80	15.4	10.20
12	2.0	4.33	6.44	24.30	15.3	10.45
16	2.03	4.33	6.33	24.50	15.8	10.33
24	2.03	4.33	6.25	24.50	16.43	10.6
32	2.1	4.33	7.00	24.50	16.83	11.03
48	2.1	4.60	6.9	24.90	20.3	13.1

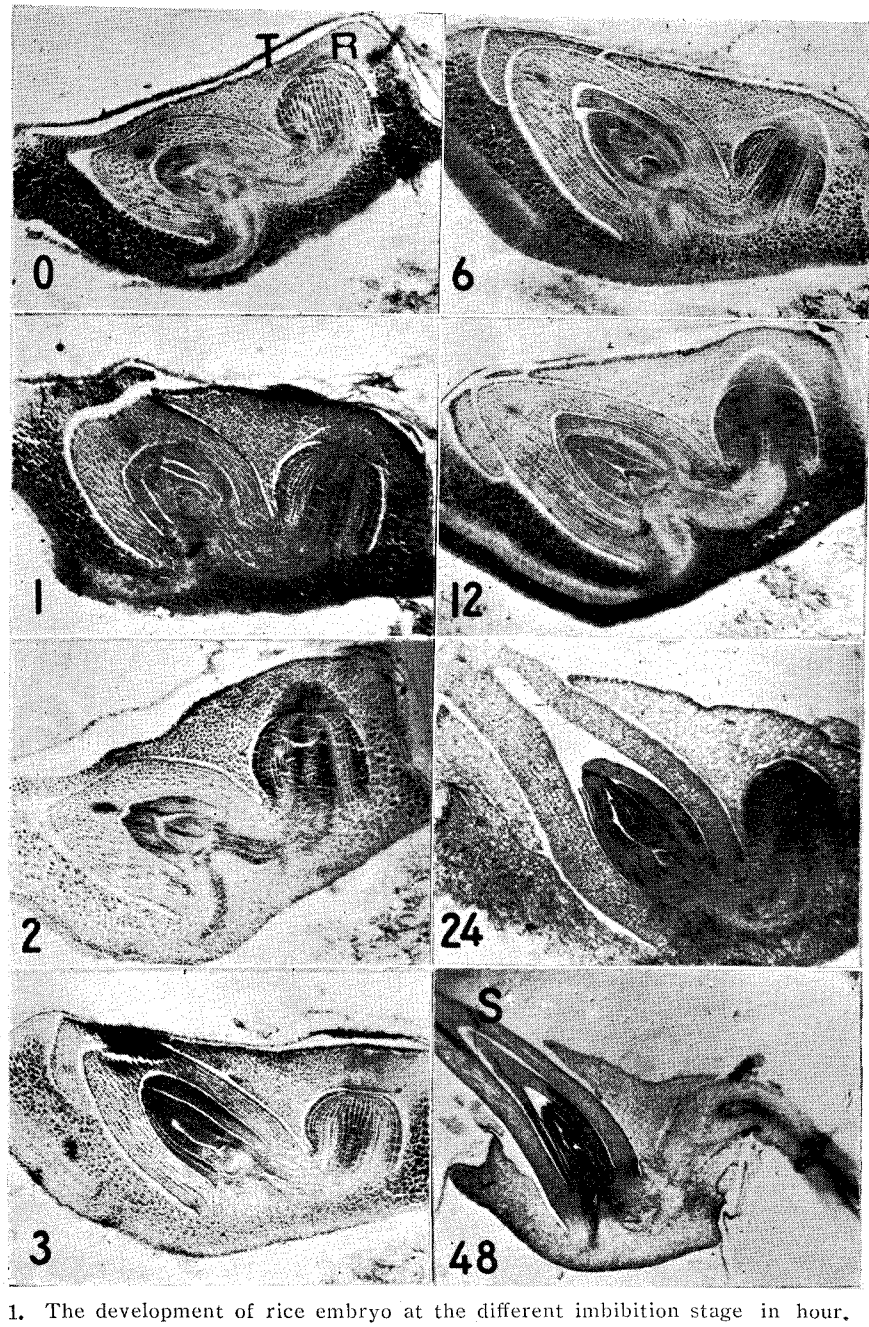

Fig. 1. The development of rice embryo at the different imbibition stage in hour. The symbols are expressed as shoot (S), root (R), and scutellum (T).

The embryos of *O. sativa* TN 5 excised from different stages of imbibition seeds were cultured on White's medium for 14 days. Results are shown in Table 3. Embryos excised from the seeds imbibition for less than 2 hours, as shown in Fig. 1, were not able to grow on the cultured medium. Imbibition of seeds for 2 hours was sufficient to allow their embryos to grow and develop *in vitro* into whole seedlings. Longer period of imbibition gave better growth of excised embryos. However, there was no significant promotion on the growth of embryo excised from the seeds imbibition for longer than 12 hours

Table 4. *Comparison of the growth of excised embryos from different varieties and a wild type of rice in the medium containing different carbohydrates for 14 days*

Substrate		No. of leaves	No. of roots	Shoot height (cm)	Root length (cm)	Dry weight (mg)
Glucose (2%)	*Oryza sativa* TC 46	5	8	30.34	13.77	18.04
	Oryza sativa TC 65	5	9	30.27	14.54	17.00
	Oryza sativa TN 5	5	7	29.65	16.54	17.24
	Oryza sativa TC 1	4	10	25.80	15.70	21.80
	Oryza sativa TC 2	4	10	25.01	15.70	21.03
	O. latifolia	3	3	12.52	5.81	4.51
Sucrose (4%)	*Oryza sativa* TC 46	4	6	28.4	16.75	12.53
	Oryza sativa TC 65	4	6	27.56	19.05	12.58
	Oryza sativa TN 5	4	6	28.10	17.87	12.76
	Oryza sativa TC 1	4	9	25.46	12.71	15.51
	Oryza sativa TC 2	4	9	24.19	14.23	17.30
	O. latifolia	2	2	11.90	3.85	3.60
Soluble starch (2%)	*Oryza sativa* TC 46	3	4	12.91	5.83	2.87
	Oryza sativa TC 65	3	5	13.55	5.50	3.05
	Oryza sativa TN 5	3	4	13.20	6.92	2.94
	Oryza sativa TC 1	3	5	18.30	9.12	4.63
	Oryza sativa TC 2	3	5	19.00	9.25	5.33
	O. latifolia	2	2	4.2	1.03	0.80
Control	*Oryza sativa* TC 46	3	2	3.20	2.60	1.20
	Oryza sativa TC 65	2	2	3.02	1.38	1.13
	Oryza sativa TN 5	2	2	2.40	1.50	1.14
	Oryza sativa TC 1	3	4	3.97	4.78	1.94
	Oryza sativa TC 2	3	3	2.23	2.67	1.37
	O. latifolia	2	1	0.95	0.25	0.25

The value of significant difference (LSD) for dry weight at 5% level between varieties, carbohydrates as well as the interaction of varieties and carbohydrates is 0.31, 0.26 and 0.35, respectively.

(Fig. 1 and Table 3).

The growth of excised embryos on the medium added with carbohydrates

The excised embryos of five rice varieties and the wild type were cultured on the white's medium either added with 2% glucose, 4% sucrose, or 2% soluble starch and without carbohydrates added as the control. The results after 14-day culture are shown in Table 4. The data of dry weight of each treatment were further analyzed statistically by means of the analysis of variance. Thus the significant values of the least significant difference (LSD.) at 5% level between varieties, carbohydrates, and the interaction of variety and carbohydrate were obtained (Table 4). It was found that the growth was significantly different between each variety and the treatment of carbohydrates. The cultivated rices grew well in the medium either added with 2% glucose or added with 4% sucrose, while the wild type rice grew poorly. Additionally, Japonica type rices (TC 46 and TN 5) grew well in the medium added with 2% glucose, but failed to grow in that added with 2% starch solution. The Indica type rice (TC 1 and TC 2) grew well in the medium added with 2% glucose and with 4% sucrose, but failed to grow in that added with 2% starch solution and in that without carbohydrate added.

Discussion

With the rice variety of *O. sativa* TN 5, the young embryos (5 days after pollination) could be successfully cultured and developed into whole plants on the White's medium added with 2% glucose. The failure of the growth of excised rice embryos obtained from the early stage, before 5 days after pollination was probably due to the unpropered medium used. A similar result was reported in the culture of cotton embryos (Lofland, 1950). Lofland found that 15-day-old embryos, 3–4 mm in length, did not grow but 27-day-old embryos were readily cultured on White's medium. Rietsma *et al.* (1953) found that different stages of embryos development of *Datura stramonium* required different minimal sucrose concentration for growth. Overbeek *et al.* (1944) and Rijven (1952) concluded that the smaller the embryo at the time of isolation the more complex nutritents were required in *Datura* and *Capsella*. Successfully embryo culture was depended on the species or genera used. In barley, for instance, the proembryo as small as 60 μ could be cultured *in vitro* and could develop into shoots and roots (Norstog, 1961).

The poor growth of embryos excised from the seeds imbibition for only one or two hours was not completely understood without detail studies in the biochemical levels. Embryos may uptake insufficient carbohydrates for their growth and development during the early stages for germination which probably

plays an important role for their subsequent growth on a culture medium. Furthermore, a short period imbibition is probably not able to produce growth factor(s) or enzyme to transfer into embryos. Such possibility has been postulated by Schander (1934) and de Ropp (1939) studied on rice and winter rye. They suggested that a growth factor was excised in the aleurone which was translocated to the embryos during early stages of seed germination.

Sucrose is the most commonly used carbohydrate in plant embryo culture. In general, sucrose was better than other sugars for growing embryos (Narayanaswami and Nortog, 1964). However, the data (Table 4) obtained from this study showed that the dry weight of each rice variety embryo cultured on White's medium added with 2% glucose was higher than on the same medium added with 4% sucrose. This result was in agreement with those reported for variety TN 5 (Yie and Yang, 1973). There was little difference between sucrose and glucose on the growth of cultured proembryos of *Pinus* (Radforth and Pecoraro, 1955) and of cultured embryos of tomato (Choudbury, 1955). Embryo culture of *Citrus* grew even better on a non-sucrose medium than on a sucrose-containing medium (Ohta and Furusato, 1957). Moreover, the dry weights were analysed by statistics. The results showed that the growth of each excised embryos were significantly different between different type under different carbohydrates test and the cultivated rices grew better with 2% glucose and 4% sucrose but failed to grow in 2% soluble starch while the wild type *O. latifolia* just reversed of the test. Thus, these rice embryos from different varieties have a genetical difference in response to the carbohydrates used in the cultured medium. Similar phenomenon was reported by Arya *et al.* (1962) that the callus derived from different species differed in their ability to utilize different carbohydrates.

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水稻切離胚在各種條件下的生長與發育

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以水稻（*Oriza sativa*）日本型（TC 46, TN 5, TC 65）；印度型（TC 1, TC 2）及野生型（*O. latifolia*）等的種子經發芽後切取胚，作組織培養，在各種條件下，測定牠的生長與發育的效果。

TN 5 變種經授粉後第五天，胚長達到0.678 mm 的時候，可移植在人工培養基中，發育成爲健全的植株。

TN 5 的種子作各種時數的浸水處理（從0時到48小時不等），即變化催芽時間，對切離胚生長發育的影響，結果種子如不浸水兩小時以上，胚很難健全生長。

用數種碳水化合物，如2％葡萄糖，4％蔗糖及2％可溶性澱粉加入 Whites 氏培養基中，測定水稻品種對碳水化合物種類之間的利用關係，切離胚經兩週培養後將乾量作生物統計分析以多種變域測驗（M. R. T.）結果顯示日本型，印度型及野生型對各種碳水化合物有顯著的差別，但同型的變種，例如 TC 1 與 TC 2 之間沒有差別顯示。

又品種間對碳水化合物的交互作用，根據最小顯著差（L. S. D.）分析結果，栽培種與野生種對碳水化合物的效應頗不相同，栽培稻對葡萄糖生長優良，而對澱粉較劣，但野生稻的試驗結果，恰相反。

又栽培稻日本型（TC 46, TN 5）對葡萄糖效果最好，澱粉最劣，但對蔗糖與對照區的反應不顯著，就印度型（TC 1, TC 2）來說對葡萄糖及蔗糖效果均最好，但對澱粉及對照區的反應均最劣，由以上結果，可知水稻品種間對培養基中碳水化合物的利用，具有遺傳上的差別似很爲顯明。