

The *in vitro* culture characteristics of ten selected rice (*Oryza sativa* L.) varieties

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Abstract: The *in vitro* culture characteristics of ten selected rice varieties were comparatively investigated with a model rice variety Taipei 309. It was found that addition of 0.5 mg/L BA to the induction medium with 2 mg/L 2,4-D could increase significantly the formation frequency of embryogenic callus and produce evidently more amount of embryogenic callus of Javanica rice. Although calluses of Huahang 1 and Huahang 3 could regenerate plants on medium without cytokinin like Taipei 309, the regeneration rates were very low. By incorporating 3 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to the medium with 2 mg/L BA, plant regeneration from calluses of Huahang 1, Shuijingzhan and Taiwanxiangzhan was significantly enhanced. Best result of plant regeneration was obtained for all the varieties when a two-step culture method involving a pre-differentiation culture was applied. Results of the experiments indicate that by proper modification of the culture methods, culture responses of most of the varieties can be largely improved. Among the varieties tested, Shuijingzhan and Taiwanxiangzhan showed favorable *in vitro* culture characteristics and can be the potential candidate varieties for genetic improvement by the application of biotechnology.

Key words: callus culture; plant regeneration; hormone; rice

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10 个特选水稻品种的离体培养特性

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摘要: 将 10 个特选的水稻品种的离体培养特性与模式品种台北 309 进行了比较。试验发现在培养基含有 2 mg/L 的 2,4-D 的情况下添加 0.5 mg/L 的 BA 对爪哇稻胚性愈伤组织的形成显著有利, 而对其他品种的正向作用不大或反向作用明显。虽然华航 1 号和华航 3 号的愈伤组织和台北 309 一样能够在没有细胞分裂素的培养基上再生植株, 但再生效率很低。在含有 BA 的培养基上所有品种的愈伤组织都能够再生出更多的植株, 添加铜元素对部分品种的植株再生也有促进作用。先将愈伤组织进行分化预备培养然后才进行植株再生培养能够取得最好的植株再生培养效果。本研究结果表明, 采取适当的方法能够显著改善大多数品种的培养反应。经过比较筛选出离体培养特性与模式品种相似的优质籼稻品种水晶占和台湾香占, 可以作为利用生物技术进行品种改良的材料。

关键词: 愈伤组织培养; 植株再生; 激素; 水稻

It has been well known that different varieties may have very different behavior under *in vitro* culture conditions^[1,2]. Hence, in order to apply bio-technical means for the improvement of a certain variety, the *in vitro* cul-

ture characteristics of the variety must first be clarified. The present experiments were to investigate the *in vitro* culture response of some locally important and special varieties in south China.

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Ten varieties adopted in the present experiments were determined following a recommendation by senior agronomist Zhang Jianguo at the College of Agriculture, South China Agricultural University. Among these varieties, Huahang 1 and Huahang 3 were bred by space mutation induction techniques by scientists of the University with high yielding and excellent taste quality; Jingxian 89 and Jingxian 93 are progenies of crosses between Japonica and Indica parents; Shuijingzhan, Hunansimiao and Taiwanxiangzhan are all high quality rice cultivars and have prominent application potentials both for cultivation production and for use as parents for crossing. Wushanwunuo is a local waxy variety with black color of the grains. Brazil upland rice is a variety introduced from Brazil recently with high resistance to water deficiency, and Javanica rice has characteristics between Japonica and Indica rice and is commonly used as a bridge variety for crossing between the two subspecies. Taipei 309, a Japonica variety with favorable *in vitro* culture characteristics, was used as a standard for comparison.

1 Materials and Methods

1.1 Plant materials

Mature seeds of the ten varieties were collected from the experimental farm of South China Agricultural University, while seeds of Taipei 309 were originally obtained from the China Rice Research Institute. Seeds were dehusked and used as explants for culture.

1.2 Culture media

All media contained N6 inorganic macro-elements, MS inorganic micro-elements, N6 vitamins, 3% sucrose, 0.7% agar and were adjusted to pH 5.8 ~ 6.0 before autoclaving. Callus induction and proliferation medium was supplemented with 500 mg/L proline and 2 mg/L 2,4-D, and a comparison medium was additionally supplemented with 0.5 mg/L BA; Plant regeneration media contained 1 mg/L NAA and consisted of a cytokinin-free medium, a medium added with 2 mg/L BA and the other medium added with the same amount of BA plus 3 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; Pre-differentiation culture medium contained 8 mg/L BA, 1 mg/L NAA and 3 mg/L ABA.

1.3 Culture methods

Dehusked seeds were surface-sterilized and inoculated onto the callus induction medium as previously de-

scribed^[3]. Compact and nodular calluses (embryogenic calluses) were selected and subcultured for further proliferation. Plant regeneration was achieved by transferring these proliferated calluses onto the plant regeneration media. Some proliferated calluses were also transferred onto the pre-differentiation medium for the enhancement of the regeneration ability of the calluses. All the experiments had at least six replicated cultures for each treatment.

1.4 Data analyses

All data are expressed as mean value and standard error and were subjected to statistical analyses using SAS (Statistical Analysis System, release 6.12 TS level 0020, Windows Version 4.10.2222) and tested for significance by Duncan's New Multiple Range Test.

2 Results and discussion

2.1 Callus induction culture

Results of callus induction culture were comparatively evaluated one month after the initiation of the culture and are summarized in Tab. 1. Two criteria were used together for the evaluation; one was the numbers of callus of different sizes, and the other was the percentage of formation of embryogenic calluses. Applying these two criteria could give more precise evaluation of the culture responses. On medium with 2,4-D only, Shuijingzhan behaved even better than the model variety Taipei 309 and was definitely the best among all the other varieties. Other varieties that showed high embryogenic callus formation percentage were Jingxian 89 and Taiwanxiangzhan, however, most of the calluses produced by Jingxian 89 were of small size and because of this, Taiwanxiangzhan was considered to be better than Jingxian 89 for *in vitro* cultures. It was also clear that supplement of BA to the callus induction medium was conducive to two varieties, Wushanwunuo and Javanica rice. The numbers of large and medium size calluses of the former were increased prominently, while both the criteria of the later were largely improved.

2.2 Plant regeneration culture

Calluses, after a dehydration treatment^[3], were transferred onto the three kinds of plant regeneration media for direct regeneration of plants, or to the pre-differentiation culture medium for enhancing plant regeneration ability and subsequently to plant regeneration medium. Results of the experiments are summarized in Tab. 2.

Tab. 1 Callus formation on two media with different hormone compositions

variety	on medium with 2 mg/L 2,4-D				on medium with 2 mg/L 2,4-D + BA 0.5 mg/L			
	size of callus (number) ¹⁾			embryogenic callus formation ²⁾ /%	size of callus (number)			embryogenic callus formation/ %
	large	medium	small		large	medium	small	
Taipei 309	11	12	9	88.89 ± 3.51a, A	14	10	7	88.89 ± 5.56a, A
Huahang 1	2	4	16	61.11 ± 8.24cde, A	1	4	21	72.22 ± 8.24bc, A
Huahang 3	3	13	13	80.56 ± 7.95ab, A	0	3	27	83.33 ± 7.45ab, A
Jingxian 89	0	3	29	88.89 ± 5.56a, A	0	0	25	69.44 ± 6.69bc, B
Jingxian 93	7	8	12	75.00 ± 3.73abcd, A	0	0	11	41.67 ± 8.83e, B
Shuijingzhan	18	13	2	91.67 ± 3.73a, A	7	14	12	91.67 ± 3.73a, A
Hunansimiao	1	3	20	66.67 ± 4.30bcde, A	0	0	5	13.88 ± 5.12f, B
Taiwanxiangzhan	4	7	19	83.33 ± 7.45ab, A	0	0	13	36.11 ± 5.12e, B
Wushanwunuo	3	9	17	80.56 ± 6.69cde, A	11	14	4	80.56 ± 6.69ab, A
Brazil upland rice	0	1	13	38.89 ± 3.51f, A	0	3	9	33.33 ± 3.51e, A
Javanica rice	2	7	13	61.11 ± 5.56cde, B	7	17	9	91.67 ± 3.73a, A

1) There were 36 explants in each treatment, but only those showed noticeable formation of callus are listed here; 2) Data are presented as mean ± SE of six cultures in each treatment; data followed by different small letters at the same column, or followed by different capital letters at the same row, are significantly different at $P \leq 0.05$ level by Duncan's test

Tab. 2 Regeneration of plants from callus cultured on different media¹⁾

variety	medium additives			
	1 mg/L NAA	1 mg/L NAA + 2 mg/L BA	1 mg/L NAA + 2 mg/L BA + 3 mg/L CuSO ₄ ·5H ₂ O	1 mg/L NAA + 8 mg/L BA + 3 mg/L ABA ²⁾
Taipei 309	15.67 ± 1.48aB	21.83 ± 1.97aA	24.0 ± 2.13aA	25.83 ± 1.22a
Huahang 1	0.67 ± 0.42cB	1.67 ± 0.56dB	4.83 ± 1.70dA	20.83 ± 1.40b
Huahang 3	4.0 ± 1.06bA	6.33 ± 2.02cA	4.50 ± 0.56dA	14.50 ± 0.76d
Jingxian 89	0.0 ± 0.0dB	5.67 ± 2.18cA	6.33 ± 1.76cdA	21.83 ± 1.13b
Jingxian 93	0.0 ± 0.0dB	7.83 ± 2.12bcA	9.16 ± 1.80cA	14.0 ± 0.58de
Shuijingzhan	0.0 ± 0.0dC	3.0 ± 0.52cB	5.83 ± 1.30dA	17.33 ± 0.88c
Hunansimiao	0.0 ± 0.0dB	3.17 ± 0.87cA	2.17 ± 0.60eA	21.33 ± 0.88b
Taiwanxiangzhan	0.0 ± 0.0dC	11.5 ± 2.93bB	18.17 ± 1.99bA	27.33 ± 1.28a
Wushanwunuo	0.0 ± 0.0dB	7.0 ± 3.05bcA	0.0 ± 0.0fB	14.17 ± 0.70de
Brazil upland rice	0.0 ± 0.0dC	4.17 ± 1.05cA	2.0 ± 0.0eB	13.67 ± 0.56de
Javanica rice	0.0 ± 0.0dB	3.50 ± 1.28cA	4.0 ± 1.32dA	12.67 ± 0.88e

1) Data are numbers per bottle of regenerated plants presented as mean ± SE of six cultures; those followed by different small letters in the same column, or followed by different capital letters in the same row are significantly different at $P \leq 0.05$ level by Duncan's test, with an exception of the last one in each row which consisted of two culture steps and is not suitable to be subjected to the test; 2) Regeneration of plants was achieved by a subsequent culture onto a medium containing 1 mg/L NAA; the data were obtained after the subsequent culture

On the medium containing NAA as the sole additive, plant regeneration was observed for only three varieties, namely, Taipei 309, Huahang 1 and Huahang 3. The capability of the calluses to regenerate plants on this medium reveals the present of pre-existed somatic embryos in the calluses; somatic embryos were formed on medium containing 2,4-D and would germinate when 2,4-D had been removed. Supplement of BA to the medium significantly

stimulate plant regeneration in most of the varieties, and addition of CuSO₄·5H₂O could further enhance the regeneration in three varieties. Best results of regeneration were achieved for all the varieties by culturing the calluses to the pre-differentiation medium and then to a plant regeneration medium containing 1 mg/L NAA. Among the varieties tested, Taiwanxiangzhan showed the highest plant regeneration efficiency, while Shuijingzhan, which showed

very favorable characteristics on callus induction medium, ranged the middle.

Induction of callus formation and regeneration of plants from the callus are two essential steps for the application of biotechnology for crop improvement. In order to establish a high efficient *in vitro* culture system to facilitate the biotechnical breeding, much effort has been tried and many innovations have been made^[4-7]. A few years ago, Yang et al^[8,3] found that enriching copper to the medium is a simple and efficient method for increasing the plant regeneration efficiencies of quite a number of rice varieties, and established a culture procedure for regeneration of plants from long-term cultured callus of Taipei 309, in which the strategy of the pre-differentiation culture is incorporated. By the application of this procedure, micro-propagation of a photoperiod-temperature sensitive genic male sterile rice becomes theoretically possible^[9]. It is clear that these two methods are also effective for a range of varieties tested in the present experiments.

In south China, there are many locally important rice varieties but their *in vitro* culture characteristics have not been assessed so far. Results of the present experiments showed that by modifying medium composition and by innovating culture methods, the *in vitro* culture responses of some of these varieties are amendable and the application of bio-technical means for the improvement of these varieties is feasible.

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