Japan. Jour. Crop Sci. 55(1) : 41—46 (1986)

# Further Studies on the Variability of Plant Regeneration from Young Embryo Callus Cultures of Rice Plants (*Oryza sativa* L.)

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Received June 28, 1985

In recent years, reports have shown that high frequencies of plant regeneration were obtained in callus of young cereal embryos, such as maize[8], oat[4], sorghum[6], wheat[1,7,14,19], barley[5], and pearl millet[20]. Such callus can be used for further cell suspension culture and protoplast culture[20,21].

The authors have previously studied rice callus and suspension culture from young embryos. They found that embryos inoculated with the scutellar surface up (i.e. the plumule-radicle axis side placed in contact with medium) gave better results in callus formation and plant regeneration than those inoculated with the scutellar surface down[12,13]. This was consistent among the 17 rice cultivars tested[13], however, the frequency of plant regeneration varied much among the cultivars. Whilst root and shoot differentiation ability of japonica type cultivars was poor, that of indica type was good. A remarkably high frequency (up to 80%—100%) of shoot differentiation was obtained from the indica type cultivar, Ai-nan-tsao 39[13].

Although many tissue culture studies have shown varying culture results among different cultivars[1,3,4,5,8,14,15,19], so far there is no explanation as to why this should be so. To approach this problem, it should first be established whether there is any relationship between regeneration ability of culture explants, and the outer morphology of donor plants. An understanding of this can be helpful both in the choice of plant materials and in further studies on plant regeneration.

In this experiment, indica type rice cultivars with characters of dwarfism and different maturation period, similar to those of Ai-nan-tsao 39, were selected. Whether plant regeneration ability is correlated with these physiological and outer morphological characters was examined.

## Materials and Methods

Seventeen dwarf, different maturation period indica rice cultivars were grown in a phytotron at 30℃ (day)/25℃ (night) during the autumn of 1981. The agronomic characters of plant height and duration of growth to both flowering and maturation, of these cultivars are shown in Table 1. Spikelets were marked on the day of anthesis. They were dehusked, surface-sterilized in 1% NaOCl for 20 min. and washed four times with sterile distilled water. Embryos were then excised and transferred, scutellar side up, onto agar culture medium, as in previous experiments[12,13]. Two basal culture media were used, one consisted of the mineral salts of MURASHIGE and SKOOG (MS) medium, the other was N6 medium. Both media contained 30 g/*l* sucrose (Sigma), 1 mg/*l* thiamin-HCl (Gibco), 1 mg/*l* pyridoxin-HCl (Gibco), 10 mg/*l* nicotinic acid (Gibco) and 8 g/*l* agar-agar (Merck). The pH value was adjusted to 5.8 prior to autoclaving (5 min. at 120°C). Both media, with 5 $\mu$M 2, 4-dichlorophenoxyacetic acid (2,4-D, Gibco) supplements, gave callus formation. Subculturing of callus was carried out only for MS medium derived callus, and only on MS medium; this was at approximately 3 to 4 week intervals.

Plant regeneration was attempted on all cultivars at the first passage of callus, and on three selected cultivars during eight successive passages in culture. For regeneration, each callus was transferred to MS basal medium without 2,4-D, but supplemented with 10 $\mu$M kinetin (Gibco) and 5 $\mu$M naphthaleneacetic acid (NAA, Gibco). All cultures were incubated at 26℃—28℃, and kept under continuous fluorescent light with an intensity of approximately 2,000 lux.

Table 1. Agronomic characters of 17 rice cultivars obtained from Taiwan Agriculture Research Institute (TARI).

| Cultivars | Plant height at maturation (cm) | Days from transplanting to | |
|---|---|---|---|
| | | flowering | maturation |
| Ai-nan-tsao 39 | 64.4 | 47 | 83 |
| Kwan-chang-ai | 68.4 | 62 | 94 |
| Ai-nan-tsao | 59.4 | 45 | 81 |
| Chen-shan 97 | 76.5 | 59 | 91 |
| Aichio-nan-te | 85.3 | 63 | 94 |
| Ai-nan-tsao 1 | 63.3 | 45 | 81 |
| Chao-yang-tsao18 | 72.2 | 66 | 99 |
| Chin-chang-ai 39 | 82.7 | 76 | 113 |
| Chao-yang 1 | 58.6 | 44 | 80 |
| Kuei-lu-ai 8 | 66.2 | 59 | 91 |
| Kwang-lu-ai 4 | 75.3 | 57 | 94 |
| Chiu-ku-ai 2 | 73.8 | 65 | 99 |
| Nan-tsao 32 | 74.0 | 63 | 97 |
| 29 Lu-1 | 66.2 | 42 | 76 |
| Huang-ko-tsao 20 | 82.2 | 49 | 87 |
| Hsin-kwang-ai | 80.4 | 69 | 100 |
| Nan-erh-ai 5 | 82.2 | 70 | 101 |

## Results

*Effect of culture medium on regeneration*

Embryos of all tested 17 cultivars produced callus from the scutellum when placed on induction medium. Callus was yellow to yellow-white. After 4 weeks, the scutellar calli were transferred to regeneration medium. Variability in the percentage of cultures developing shoots and roots was observed, both among cultivars and between basal media (Table 2). The results show that shoots often cannot regenerate from callus on N6 medium. There were, however, two exceptions to this rule : Ai-nan-tsao 39 and Chao-yang-tsao 18, which showed shoot regeneration of 23% and 20% respectively. Another trend evident from the data is that root regeneration is usually better from callus on MS than from those on N6 medium. Here too were some exceptions (see Table 2). This substantiates the idea that growth and differentiation of callus are affected by the mineral salts on the growth medium.

*Comparisons of regeneration ability between cultivars*

A previous study[13] showed that the regeneration ability of a dwarf, early maturing, indica type rice cultivar, Ai-nan-tsao 39, was particularly good. In this experiment, the relationship between regeneration ability and the dwarf, early maturation trait was examined using Ai-nan-tsao 39 and 16 other indica type cultivars with these same two characters. Regeneration ability differed between all cultivars (Table 2). Ai-nan-tsao 39 again showed a high regeneration percentage, but only one (Kwan-chang-ai) of the other 16 did so too, indeed, four cultivars showed no shoot regeneration at all. It seems, then, that the dwarf, early maturation trait is not related to regeneration ability. It must be concluded that some other genetic character of the Ai-nan-tsao system is responsible for its good regeneration ; this relationship merits further investigation.

*Effect of subculture on regeneration*

Three cultivars, Ai-nan-tsao 39, Chao-yang 1 and Chen-shan 97, were selected to examine their regeneration ability after subculture. Calli were subcultured for 8 passages with a 3 to 4 week interval for one passage. The results in two cultivars show that shoot regeneration fell sharply after the 1st passage, and did not recover to its initial level during the successive passages (Table 3). For example, shoot regeneration of Ai-nan-tsao 39 was 68% for the 1st passage, it decreased to 17% for the 2nd passage, and then kept at about 20% until the 8th passage. A different case was found in Chao-yang 1, for which shoot regeneration was only 10% for the 1st passage, it increased after the 2nd passage, then reached 33% in the 6th passage and, after falling to 3% in the 7th passage, ended at 23% in the 8th passage. Reasons which may have caused these changes are discussed later. Regeneration of roots, unlike that of shoots, showed no sharp decrease of regeneration percentage after subculture.

## Discussion

Variation of morphogenetic response among cultivars has been reported for embryo derived cultures of other cereals, including maize[8], oat[4], barley[5] and wheat[1,14,19]. It is uncertain whether this is due to variation in tolerance to 2,4-D[19], or to the endogenous

Table 2. Organ regeneration of young embryo callus derived on two different media for 17 rice cultivars.

| Cultivars | Medium | CallusNo. | With root | With shoot | Shoots / Callus |
|---|---|---|---|---|---|
| Ai-nan-tsao 39 | MS | 40 | 28 (70)* | 27 (68) | 3.9 |
| | N6 | 30 | 20 (67) | 7 (23) | 1.4 |
| Kwan-chang-ai | MS | 39 | 35 (90) | 21 (54) | 3.6 |
| Ai-nan-tsao | MS | 30 | 13 (43) | 10 (33) | 1.8 |
| | N6 | 30 | 14 (46) | 0 | 0 |
| Chen-shan 97 | MS | 40 | 30 (75) | 11 (28) | 3.3 |
| | N6 | 30 | 15 (50) | 0 | 0 |
| Ai-chio-nan-te | MS | 30 | 24 (80) | 6 (20) | 2.3 |
| | N6 | 30 | 14 (47) | 0 | 0 |
| Ai-nan-tsao 1 | MS | 30 | 20 (67) | 5 (17) | 2.0 |
| | N6 | 30 | 16 (53) | 0 | 0 |
| Chao-yang-tsao 18 | MS | 30 | 23 (77) | 0 | 0 |
| | N6 | 30 | 19 (63) | 4 (13) | 2.7 |
| Chin-chang-ai 39 | MS | 30 | 26 (87) | 4 (13) | 4.7 |
| Chao-yang 1 | MS | 30 | 24 (80) | 3 (10) | 2.7 |
| | N6 | 10 | 6 (60) | 0 | 0 |
| Kuei-lu-ai 8 | MS | 20 | 0 | 2 (10) | 1.5 |
| | N6 | 30 | 0 | 0 | 0 |
| Kwang-lu-ai 4 | MS | 26 | 17 (65) | 2 (8) | 3.5 |
| | N6 | 30 | 20 (67) | 0 | 0 |
| Chiu-ku-ai 2 | MS | 30 | 0 | 2 (7) | 1.0 |
| | N6 | 20 | 0 | 0 | 0 |
| Nan-tsao 32 | MS | 30 | 20 (67) | 1 (3) | 5.0 |
| | N6 | 28 | 17 (61) | 0 | 0 |
| 29 Lu-1 | MS | 28 | 0 | 0 | 0 |
| Huang-ko-tsao 20 | MS | 48 | 0 | 0 | 0 |
| Hsin-kwang-ai | MS | 30 | 24 (80) | 0 | 0 |
| Nan-erh-ai 5 | MS | 30 | 14 (47) | 0 | 0 |

* The number in parenthesis is percentage.
Regeneration was tested directly after callus induced from young embryo.

phytohormonal content of explants[1]. Both reasons are possible for young embryo derived cultures of rice.

Some studies have shown that the chemical compositions of grain, in respect of proteins[22], cytokinins[18], ABA[17] etc., change during rice grain ripening. Cytokinins, as well as auxins, are important growth regulators in that they control organ differentiation. The effects of ABA on both callus growth and organ differentiation have also been reported recently[2,9,11,16]. It is possible that these growth regulators and other unknown compounds in young embryos will affect callus induction and subsequently organ differentiation. However, because for most rice cultivars tested, regeneration ability fell sharply after the first passage, then it seems that this chemical effect is only a short term one, and is probably weakened by subculturing. Additionally,

Table 3. Organ regeneration of young rice embryo derived callus after subcultured.

| Passage | | Ai-nan-tsao 39 | Chao-yang 1 | Chen-shan 97 |
|---|---|---|---|---|
| I | Callus No. | 40 | 30 | 40 |
| | With root | 28(70)* | 24(80) | 30(75) |
| | With shoot | 27(68) | 3(10) | 11(28) |
| | Shoots/Callus | 3.9 | 2.7 | 3.3 |
| II | Callus No. | 30 | 30 | 30 |
| | With root | 17(57) | 26(87) | 25(83) |
| | With shoot | 5(17) | 6(20) | 1(3) |
| | Shoots/Callus | 2.8 | 2.7 | 2.0 |
| III | Callus No. | 30 | 30 | — |
| | With root | 24(80) | 30(100) | — |
| | With shoot | 2(7) | 2(7) | — |
| | Shoots/Callus | 4.0 | 2.0 | — |
| IV | Callus No. | 30 | — | 30 |
| | With root | 20(67) | — | 18(60) |
| | With shoot | 7(23) | — | 1(3) |
| | Shoots/Callus | 2.6 | — | 3.0 |
| V | Callus No. | 30 | 30 | 30 |
| | With root | 21(70) | 30(100) | 25(83) |
| | With shoot | 6(20) | 6(20) | 3(10) |
| | Shoots/Callus | 2.7 | 2.3 | 2.0 |
| VI | Callus No. | 30 | 30 | 30 |
| | With root | 25(83) | 28(92) | 14(46) |
| | With shoot | 3(10) | 10(33) | 0 |
| | Shoots/Callus | 3.7 | 2.1 | — |
| VII | Callus No. | 30 | 30 | 30 |
| | With root | 0 | 15(50) | 0 |
| | With shoot | 0 | 1(3) | 0 |
| | Shoots/Callus | — | 5.0 | — |
| VIII | Callus No. | 24 | 30 | 30 |
| | With root | 21(88) | 30(100) | 16(53) |
| | With shoot | 5(21) | 7(23) | 2(7) |
| | Shoots/Callus | 2.2 | 3.3 | 2.5 |

* The number in parenthesis is percentage.

Passage I: Regeneration was tested directly after callus induced from young embryo.

Passage II-VIII: Regeneration was tested after callus subcultured every 3 to 4 week intervals.

because the ripening rates of grains used in this experiment vary between cultivars, it is probable that the chemical compositions of explanted young embryos are different, this might then cause the varying responses to culture.

In another case (Chao-yang 1), regeneration increased after subculture. This may be due to change in endogenous phytohormonal levels during callus growth, since INOUE et al.[10] reported the ability of rice callus tissues to synthesize cytokinins. It is also possible that different cultivars themselves, at the callus growth stage, have varying abilities

for phytohormone biosynthesis. Therefore, it is proposed that the ratio of endogenous phytohormones to the exogenous 2,4-D in a medium, also corresponds to the regeneration ability of callus. Further examinations are needed to prove this.

With especial reference to the differences found between cultivars, it seems that there is no general relationship between plant regeneration and dwarf, early maturing characters of rice cultivars. Therefore, it is proposed that the ability of a young embryo derived rice callus to regenerate into a whole plant is very much dependent upon its particular genotype, it should be possible to realize which biochemical pathways are involved in rice callus regeneration. Results of such studies will be reported soon.

## Summary

Seventeen indica rice cultivars, with characters of dwarfism and different maturation period, were chosen to examine the regeneration ability of callus induced from young embryos. The inorganic salts of MS and N6 medium, with added sucrose and vitamins, were used as two basal media. Callus was induced on these two media supplemented with 5 $\mu$M 2,4-D. For regeneration, callus was transferred to MS medium without 2,4-D, but supplemented with 10 $\mu$M kinetin and 5 $\mu$M NAA. Regeneration ability among these cultivars varied considerably, however, callus induced from N6 medium had consistently lower regeneration ability. Plant regeneration ability during eight successive passages was also observed for three selected cultivars each showing the dwarf, early maturation character, here too, considerable variation between cultivars existed. Two reasons that may have caused the variation are discussed. The above results suggest that callus regeneration is correlated neither with the morphological character of dwarfism, nor with the physiological character of maturation period of these indica type rice cultivars. However, it is possible that the regeneration ability is correlated with genetic characters. It is concluded that genotypic variation in rice should provide a good opportunity to determine the mechanism of rice plant regeneration.

## Acknowledgements

The authors are grateful to Dr. C.S. HUANG in TARI for his providing the seeds and agronomic characters of rice cultivars, and to the National Science Council, Republic of China for Financial support (NSC71-0409-B002-21). Thanks are also due to Mr. Mark WRIGHT for helping with the preparation of this manuscript.

## References

1. AHLOOWALIS B.S. 1982. Plant regeneration from callus culture in wheat. Crop Sci. **22** : 405—410.
2. ALTMAN, A. and R. GOREN. 1974. Interrelationship of abscisic acid and gibberellic acid in the promotion of callus formation in the abscission zone of citrus bud cultures. Physiol. Plant. **32** : 55—61.
3. CHEN, C.C. and M.H. LIN. 1976. Induction of rice plantlets from anther culture. Bot. Bull. Academia Sinica **17** : 18—24.
4. CUMMINGS, D.P., C.E. GREEN and D.D. STUTHMAN. 1976. Callus induction and plant regeneration in oats. Crop Sci. **16** : 465—470.
5. DALE, P.J. and E. DEAMBROGIO. 1979. A comparison of callus induction and plant regeneration from different explants of *Hordeum vulgare*. Z. Pflanzenphysiol. **94** : 65—77.
6. GAMBORG, O.L., J.P. SHYLUK, D.S. BRAR and F. CONSTABEL. 1977. Morphogenesis and plant regeneration from callus of immature embryos of sorghum. Plant Sci. Lett. **10** : 67—74.
7. GOSCH-WACKERLE, G., L. AVIVI and E. GALUN. 1979. Induction, culture and differentiation of callus from immature rachises, seeds and embryos of *Triticum*. Z. Pflanzenphysiol. **91** : 267—278.
8. GREEN, C.E. and R.L. PHILLIPS. 1975. Plant regeneration from tissue cultures of maize. Crop Sci. **15** : 417—421.
9. INOUE, M. and E. MAEDA. 1981. Stimulation of shoot bud and plantlet formation in rice callus cultures by two-step culture method using abscisic acid and kinetin. Japan. J. Crop Sci. **50** : 318—322.
10. ———, ———, R. YOSHIDA and T. ORITANI. 1979. On the occurrence of a high content of cytokinins in rice callus tissues. Plant Cell Physiol. **20** : 917—924.
11. KOCHBA, J., P. SPIEGEL-ROY, H. NEUMANN and S. SAAD. 1978. Stimulation of embryogenesis in citrus ovular callus by ABA, ethephon, CCC and Alar and its suppression by $GA_3$. Z. Pflanzenphysiol. **89** : 427—432.
12. LAI, K.L. and L.F. LIU. 1982. Induction and plant regeneration of callus from immature embryos of rice plants (*Oryza sativa* L.). Japan. J. Crop Sci. **51** : 70—74.
13. LIU, L.F. and K.L. LAI. 1982. A comparison of

callus induction and plant regeneration from young embryos of different rice varieties. Natl. Sci. Counc. Monthly Rep. of China **10** : 135—143.
14. MADDOCK, S.E., V.A. LANCASTER, R. RISIOTT and J. FRANKLIN. 1983. Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum*). J. Exp. Bot. **34** : 915—926.
15. MAEDA, E. 1967. Varietal difference in callus formation of rice seeds under sterile culture. Proc. Crop Sci. Soc. Jap. **36** : 233—239.
16. MINOCHA, S. 1979. Abscisic acid promotion of cell division and DNA synthesis in Jerusalem artichoke tuber cultured *in Vitro*. Z. Pflanzenphysiol. **92** : 327—339.
17. ORITANI, T., T. ORITANI and R. YOSHIDA. 1975. Growth inhibitors in immature seeds of rice plant. Proc. Crop Sci. Soc. Jap. **44** : 329—334.
18. ———— and R. YOSHIDA. 1976. Studies on nitrogen metabolism in crop plants. XIV. Changes in cytokinins in rice grains during ripening. Proc. Crop Sci. Soc. Jap. **45** : 429—435.
19. SEARS, R.G. and E.L. DECKARD. 1982. Tissue culture variability in wheat : Callus induction and plant regeneration. Crop Sci. **22** : 546—550.
20. VASIL, V. and I.K. VASIL. 1980. Isolation and culture of cereal protoplasts. II. Embryogenesis and plantlet formation from protoplasts of *Pennisetum americanum*. Theor. Appl. Genet. **56** : 97—99.
21. ———— and ————. 1981. Somatic embryogenesis in cereals. BioScience **31** : 246—248.
22. YAMAGATA, H., T. SUGIMOTO, K. TANAKA and Z. KASAI. 1982. Biosynthesis of storage proteins in developing rice seeds. Pl. Physiol. **70** : 1094—1100.

〔和 文 摘 要〕

## 水稲幼胚起源カルスにおける器官再分化の特性

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矮性（株高 58.6—85.3 cm）で異なる成熟期（81～113 日）を示す印度型水稲 17 品種の幼胚からカルスを誘導し，直接あるいは異なる回数継代培養を行なった後で器官再分化の様相を観察した．

その結果，カルスから器官が分化されてくる様相は品種間で大きな差異が認められ，高いものでは根器官が 70～95%，芽が 54～68%，低いものでは根器官が 0～14%，芽が 0～10% の再分化率を示したのである．

また，継代培養の結果，器官分化の百分比が二代目で著るしく低下することが認められたが，器官分化の比率が高い品種では長期に亘る継代培養において，器官分化の比率が 20% 前後を維持していたのに対し，器官分化の比率が低い品種ではさらにその分化能力が低下したのである．

この結果，水稲カルスから器官が分化されてくる場合，品種間の遺伝的要素が矮性，成熟期などの形態的，生理的特性よりも重大な関係を持つことが示唆された．