Original Papers Plant Tissue Culture Letters, **6**(1), 14-18 (1989)

Morphological and Cytological Features of Plants Regenerated from Culture of an *Oryza sativa* × *O. latifolia* F_1 Hybrid

Li-Hui SHU, Hong-Yu WU and Xi-Ning ZHANG

Research Laboratory of Genetics, Wuhan University, Wuchang, Hubei, China

(Accepted June 20, 1988)

Young panicles of F_1 plants derived from *O. sativa* × *O. latifolia* were cultured *in vitro*. During several cycles of subcultures, a number of plantlets were regenerated, which were either F_1 type having $2n=36$ chromosomes and completely sterile, or new diploid types with $2n=24$ chromosomes and partly fertile. The latter types showed a range of characters, and the F_2 plants obtained from them also differed markedly from one another, suggesting that some chromosomal segments of *O. latifolia* were introduced into the *O. sativa* genome.

The use of economic traits found in wild species has been considered to be more and more important in plant breeding. In China, we have a large number of genetic stocks of wild *Oryza* species with genes for rice blast resistance, bacterial blight resistance, cold tolerance, high protein content, and so on[12,13]. With species hybrids of rice, previous workers were interested mainly in the cytogenetic aspects and discussed species relationships,[2,7] although the use of species hybrids for breeding purposes was attempted in the International Rice Research Institute. In the use of species hybrids for breeding, how to overcome the hybrid sterility is a problem of primary importance. We employed our young-panicle culturing method to reproduce F_1 plants from *O. sativa* × *O. latifolia* and finally obtained plants producing some F_2 seeds. This species hybrid was produced earlier by the Guangdong Academy of Agricultural Sciences (1958) and maintained vegetatively, but it gave no seed for 29 years[5]. Perhaps, this paper is the first report of obtaining progenies from a sterile hybrid in rice.

Materials and Methods

A cultivar of *O. sativa*, Ewan 3 ($2n=24$, with genome AA) was used as the female parent, and the male parent was a strain of *O. latifolia* ($2n=48$, with genomes C and D). An F_1 plant was obtained by means of embryo rescue, culturing fertilized overies in 1/2 MS+1.5% sucrose (**Fig. 5**). Then, the young panicles at stages 3 to 4 were sampled for culture *in vitro*. We classify the developmental stages of rice panicles as follows: 1) First bract differentiation, 2) primary branchlet differentiation, 3) secondary branchlet and spikelet primordium differentiation, 4) stamen and pistil differentiation, 5) pollen mother cell differentiation, 6) meiosis, and 7) pollen development.

The young panicles enclosed in leaf sheaths were immersed in 70% alcohol for 2 seconds and 0.1% mercuric chloride solution for 10 min for sterilization, and washed with aseptic water 3-5 times. They were then exposed in an aseptic condition and excised with a scalpel carefully, and were inoculated on the culture medium solidified with agar. A panicle was put in a test tube. The N 6 medium supplemented with 2, 4-D 2 mg/*l*, 4.5% sucrose and 0.8% agar was used for callus induction, and the MS medium supplemented with NAA 0.5 mg/*l*, KT 2 mg/*l*, 3% sucrose and 0.8% agar was used for regeneration. The test tubes were kept in a chamber at 25-30℃ under fluorescent light for 10 hr par day during the regeneration of plantlets.

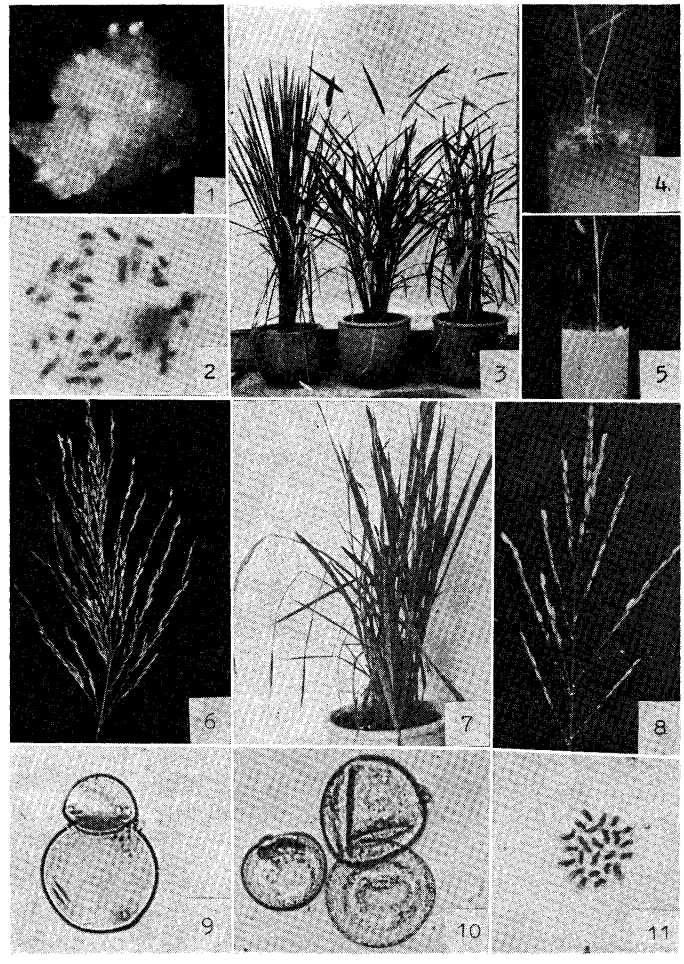
For observing chromosomes in root-tip cells, the cell-wall digestion hypotonic expansion-flame method

was employed[8,11]. For observing meiosis, pollen mother cells were stained with acetocarmine and squashed.

Results

1. Callus induction and plantlet regeneration

An observation of chromosomes in root-tip cells of the F_1 plant showed $2n=36$, indicating that it was an allotriploid with genome ACD (**Figs. 2, 3**). In outward appearance the F_1 plant was an intermediate between the parental species and completely sterile. But it grew well and produced profuse tillers. Its panicles looked like those of the wild parent, the primary branches being either dense or sparse, and had purplish-red apiculus, red stigma and red awns. The spikelets dropped immediately after flowering. Backcrosses with *O. sativa* pollen grains were unsuccessful.



Figs. 1~11.

Fig. 1. Callus developed from a young panicle. 2. Root-tip chromosomes of an allotriploid, $2n=36$. 3. Plants of cultivar Ewan 3 (left), F_1 (center) and *O. latifolia* (right). 4. A green plantlet from a young panicle of allotriploid. 5. A seedling developing from a hybrid embryo. 6. A panicle with about 500 spikelets. 7. A plant setting seeds, with $2n=24$ chromosomes. 8. A panicle with primary branches only. 9. A pollen grain with gemma. 10. Pollen grains markedly differing in size. 11. Chromosomes of a diploid plant obtained from culture, $2n=24$.

Table 1. The rate of callus induction and plantlet differentiation from cultures of young panicles at different developmental stages

No./rate (%)	Developmental stage*						
	1	2	3	4	5	6	7
Panicles cultured	10	38	38	30	39	20	10
Calluses obtained	0	10	38	30	24	8	0
% calluses induction	0	26	100	100	62	40	0
Calluses transplanted	0	10	38	30	24	8	0
Green plantlets obtained	0	2	25	15	8	0	0
% successful differentiation	0	20	66	50	33	0	0

* Described in text.

After 7–10 days of inoculation in culture medium, the young panicles showed swelling and initiation of calluses on the surface of spikelets (**Fig. 1**). The calluses were either sticky masses containing much water whose regeneration ability was very low, or compact and granulate in structure and milky white which were embryogenic. In such an embryogenic cell cluster, mature embryoids occurred as time elapsed. It was similar to that reported by Ling *et al.*[5]. Green plantlets were obtained either from callus or directly developed from an initiating spikelet (**Fig. 4**). The rate of differentiation of green plantlets was highest at the 3 rd stage (**Table 1**). The frequency of albino plantlets was 22% in total. A total of 50 green plants were obtained.

In the winter of 1983, 20 regenerated plants were grown in Hainan Island and 10 of them survived. In the summer of 1984, they were grown in the experimental field at Wuhan University, and young panicles were cultured *in vitro*. Many plantlets were regenerated from several cycles of subcultures, and grown in greenhouse of the Hubei Agricultural Institute in the winter of 1984. Then, a few of them set seeds. When sown in a culture medium, some germinated and produced viable plants although others produced roots only or failed.

2. Characters of plants regenerated from cultures

Twelve plants were recovered from the subcultures of the F_1 plant. They were of two types, one showing the same characters as of the F_1 plant and being completely sterile, and the other resembling the *O. sativa* parent in some respects and producing some seeds (**Figs. 7, 8**). From the seeds, a total of 11 F_2 plants were obtained. They markedly differed from one another in seed fertility. Three of them,

Table 2. Character of F_1 and F_2 plants derived from culture.

Character		F_1	F_2				
		A4	A93	A44	A63	A79	A81
Panicle branches		Dense	Dense	Sparse	Dense	Dense	Dense
Awn		Awnless	Awnless	Short	Long	Short	Short
Apiculus		Red	Purplish red	White	Straw-yellow	Golden-yellow	Purplish red
Glume color		Straw-yellow	Straw-yellow	Straw-yellow	Golden-yellow	Golden-yellow	Straw-yellow
Stigma color		Purple	Purple	White	White	White	White
Grain shape		Medium	Slender	Medium	Slender	Medium	Medium
Plant height	(cm)	119	78	100	139	145	136
Flag leaf length	(cm)	15	18	30	40	39	32
Flag leaf width	(mm)	22	14	34	26	22	22
Panicle length	(cm)	26	21	25	33	26	23
Spikelet length	(mm)	6.7	6.6	8.3	8.0	8.7	8.1
Spikelet width	(mm)	2.7	2.0	3.3	2.9	3.2	3.0
Seed fertility	(%)	6.4	0.8	0.8	63	44	12
Spikelets/panicle		154	115	235	259	136	159

A 79, A 63 and A 81, showed a high fertility. A 93 had a low fertility and set only one seed, which died after reaching the two-leaf stage. A 44 set two seeds, one of which germinated but produced no panicle until November. Characters of these F_2 plants are given in **Table 2.**

A fertile plant, A 81, produced panicles for a period, early ones being golden in color and late ones yellow-green. The panicles had only primary branches which were dense or sparse. From their seeds, F_3 plants were raised which showed a wide range of variation in heading time and appeared to be less sensitive to photoperiod than the *O. sativa* parent. They generally showed *sativa*-like features, and some of them produced large panicles as long as 40 cm and carrying about 500 spikelets (**Fig. 6**).

The fertile F_2 plants, A 5, A 6, A 62, A 63, A 79, A 80 and A 81, had $2n=24$ chromosomes in the root-tip cells (**Fig. 11**). Their F_3 plant also showed $2n=24$. A 44 was a chimera or mixoploid having cells with 24 or 12 chromosomes. On the other hand, the sterile F_1-like plants had $2n=36$ chromosomes.

It may be asserted that fertile segregants have $2n=24$ and sterile ones $2n=36$ chromosomes. But the fertile plants could have alien chromosomes or chromosome segments derived from the *O. latifolia* parent.

The pollen grains of these plants showed remarkable variation in size (**Fig. 10**). Some pollen grains had a gemma or a bud-like body (**Fig. 9**); many had two apertures, sometimes 5 or 6. Other pollen grains were empty or having no nucleus. These irregular pollen grains were abortive.

Discussion

Different cases of anomalous mitosis and variability in number and structure of chromosomes have been reported in the tissue culture of plants. This enables us to use tissue culture as a source of genetic variation. Therefore, the tissue culture of distant hybrids has increasingly drawn the attention of scientists[4,6,14]. By this way, we have obtained fertile progenies from the AA×CCDD cross of rice. Under normal conditions, the chromosomes of these genomes are non-homologous and the ACD plants are completely sterile. Under the conditions of tissue culture, during several cycles of subcultures, there may be chances of reunion of chromosome segments between different genomes and of cross-over, resulting in reconstruction of the genome. Thus, a part of alien germplasms may be introduced into the genome of the cultivated rice.

As for the occurrence of a mixoploid, it may be inferred that the organ primordia originating from calluses are composed of meristematic cells having different chromosome numbers. Variations in chromosome number in the culture of distant hybrids have been reported by several authers[8-10]. In many of such cases, although cells of different ploidy levels or aneuploidy occur, regenerated plants are mostly diploids, suggesting that diploid cell lines are of selective advantage during organogenesis and morphogenesis[1]. This trend was also observed in the present experiment. Although diploidy remains unchanged, the transfer of genetic material from distant sources may occur during this process.

References

1) D'Amado, T., 1975. The problem of genetic stability in plant tissue and cell cultures. *In*: Crop Resources for Today and Tomorrow (ed. by Frankel, O., Hawkes, J. G.), p. 333-348, Cambridge University Press, London.
2) Katayama, T., W. Onizuka, Y. B. Shin, 1981. Jpn. J. Genet., **56**: 67-71.
3) Kurata, N., T. Omura, 1978. Jpn. J. Genet., **53**: 251-255.
4) Li, J. M., M. Zhu, T. S. Cai, 1985. Acta Genet. Sinica, **12**: 434-439.
5) Ling, D. E., W. I. Chen, M. F. Chen, Z. R. Ma, 1984. Acta Genet. Sinica, **11**: 26-32.
6) Nakamura, C., W. A. Keller, G. Fedak, 1981. Theor. Appl. Genet., **60**: 89-96.
7) Nayar, N. M., 1973. Genet. 6., **17**: 135-292.
8) Orton, T. J., 1980. Theor. Appl. Genet., **56**: 101-112.
9) Shimada, T., M. Tabata, 1967. Jpn. J. Genet., **42**: 195-201.
10) Shimada, T., 1971. Jpn. J. Genet., **46**: 235-241.
11) Shu, L. H., W. Y. Deng, G. P. Li, 1985. J. Wuhan Bot. Res., **3**: 413-417.
12) The Cooperative Team for Wild Rice Resources Survey and Exploration in China, 1984. Sci. Agric.

Sinica, **6**: 27-34.
13) Wu, M. X., Z. R. Mo, D. Y. Li, L. H. Shu, X. L. Chang, 1987. Sci. Agric. Sinica, **20**: 13-18.
14) Zhu, Z. Q., J. S. Sun, Y. X. Wang, 1985. Acta Gen. Sinica, **12**: 430-433.

≪和文要約≫

Oryza sativa×*O. latifolia* の F_1 雑種の培養から再生した植物の形態と細胞学的特性

舒 理 慧, 吴 紅 雨, 張 希 宁

武漢大学生物系遺伝研究室

O. sativa×*O. latifolia* による F_1 植物の幼穂を培養し，サブカルチャーを数回くり返す間に多数の小植物が再生した．それらは $2n=36$ の染色体をもち完全に不稔であるか，あるいは $2n=24$ をもち部分的に稔性の二倍体であった．後者は種々異なる形質を示し，それらに由来する F_2 植物も形質が異なっていた．これは *O. latifolia* の染色体の一部が *O. sativa* のゲノム中に導入されたことを暗示する．