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Production of n + 1 plants and tetrasomics by means of anther culture of trisomic plants in rice (*Oryza sativa* L.)

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Summary. Eleven primary trisomics of rice, variety Nipponbare, were subjected to anther culture. The 12th trisomic did not produce normal anthers. A total of 3,734 plants were obtained, which were examined morphologically at the seedling stage in the greenhouse. A number of plants appeared in the progenies of ten trisomics which had unique morphological features. The frequency of these variant types differed among different progenies. Cytological observations revealed that 43 variant plants in the progenies of nine trisomics had 13 chromosomes (n+1), and 56 were tetrasomics (2n=26). The tetrasomic plants in the progeny of a trisomic were morphologically identical. Similarly, n+1 plants in the progeny of a trisomic were also identical. Plants with 23, 25, 36, 39, and 73 chromosomes were also obtained. Results show that valuable aneuploids such as n+1 and 2n+2 can be obtained in the anther-culture-derived progenies of trisomics.

Key words: *Oryza sativa* L. – Trisomics – Anther culture – Aneuploids – Tetrasomics

Introduction

Aneuploids of rice (*Oryza sativa* L.) have been reported by several investigators (Chang 1964; Khush 1973; Nayar 1973). Primary trisomics were obtained either from the progenies of triploids (Ramanujam 1937; Yunoki and Masuyama 1945; Karibasappa 1961; Katayama 1963; Sen 1965; Hu 1968; Iwata et al. 1970; Watanabe and Koga 1975; Zhang et al. 1987) or from anther culture of diploid plants (Oono 1975; Chu et al. 1985). Primary trisomics in Japonica (Iwata and Omura 1984) and Indica (Khush et al. 1984) rice have been utilized in genetic investigations. Other aneuploids, such as monosomics, nullisomics, double trisomics, and tetrasomics, have also been reported (Sampath and Krishnaswamy 1948; Chandrasekharan 1952; Seshu and Venkataswamy 1958; Katayama 1963; Khush et al. 1984; Chu et al. 1985; Wang et al. 1988). However, their frequency was so low that detailed studies were not conducted. Haploid plants with one extra chromosome (n+1) have not reported previously.

Aneuhaploids were obtained in tobacco by means of anther culture of trisomics (Mattingly and Collins 1974; Niizeki and Kita 1975; Niizeki et al. 1984), and in wheat from anther-derived plants of disomics (Metz et al. 1988).

This study was undertaken to test the feasibility of producing aneuhaploid (n+1) and tetrasomic (2n+2) plants by means of anther culture of rice trisomics.

Materials and methods

Eleven primary trisomics in the background of Japonica variety Nipponbare were used in the study (Table 1). Trisomic M does not produce normal anthers and thus could not be included in the study.

Panicles from trisomic plants were harvested prior to emergence of the flag leaf sheath, when the base of the flag leaf was 5–10 cm above the base of the next lower leaf, and were stored in an incubator at 7 °C for a given period.

Cold-treated panicles were removed from the leaf sheath and surface-sterilized with 70% ethanol for a few seconds, and then rinsed with sterilized water three times. Anthers thought to contain uninucleate-stage pollen grains were plated on Chu's medium supplemented with 2 mg/l 2,4-dichloroacetic acid (2,4-D), and incubated in the dark at 25 °C. The induced calli were transferred onto MS medium supplemented by 2 mg/l indoleacetic acid (IAA) and 0.5 mg/l kinetin when the calli were 2–4 mm in length. Transferred calli were kept under constant biolight at 25 °C for regeneration. Regenerated plants were transplanted into pots.

Table 1. Primary trisomics of Japonica variety Nipponbare used in the study and their extra chromosomes (Kyushu University, Japan)

Trisomic	Extra chromosome	
	Somatic karyotype	Pachytene karyotype
A	K5	12
B	K6	6
C	K12	10
D	K7	8
E	K4	4
F	K11	7
G	K8	11
H	K10	9
L	K9	5
M	K3	3
N	K2	2
O	K1	1

Table 2. Frequency of callus induction and plant regeneration in different trisomics in 1988

Trisomic	Anthers plated	Rate of callus induction (%)	Calli transferred	Rate of plant regeneration (%)
A	1,700	3.88	61	6.56
B	4,000	8.88	307	22.48
C	4,000	11.73	422	24.88
D	4,000	32.58	1,168	16.87
E	1,000	4.20	38	21.05
H	5,000	15.82	674	14.84
Total	19,700	16.26	2,670	18.09

Table 3. Frequency of callus induction and plant regeneration in different trisomics in 1989

Trisomic	Anthers plated	Rate of callus induction (%)	Calli transferred	Rate of plant regeneration (%)
A	10,900	27.41	1,753	17.86
B	5,300	15.46	415	40.24
C	10,800	15.67	1,168	43.75
D	10,000	18.97	1,050	26.00
E	11,700	14.73	1,133	33.19
F	11,700	9.99	865	14.45
G	11,100	10.05	837	41.94
H	3,700	31.41	297	35.36
L	12,200	18.29	1,047	21.78
N	10,500	21.59	1,360	41.18
O	5,500	13.29	615	39.40
Total	103,400	17.22	10,540	30.84

Plants were morphologically examined at the five- to seven-leaf stage. The root-tip chromosomes of morphologically variant plants were examined, following the technique of Kurata and Omura (1978), for chromosome preparation and staining.

Results

Anther culture of trisomics

Calli appeared about 4 weeks after plating. In the 1988 experiment, 19,700 anthers of six trisomics were plated, and 3,204 calli were induced with an average callus induction frequency of 16.26% (Table 2). The callus induction frequency among trisomics ranged from 3.88% for type A to 32.58% for type D. A total of 2,670 calli was transferred onto redifferentiation media, and 483 plants were regenerated. The average plant regeneration frequency was 18.09% (Table 2), with a range of 6.56% for type A to 24.88% for type C.

In the 1989 experiment, 103,400 anthers of 11 trisomics were plated, and 17,804 calli were obtained with an average callus induction frequency of 17.22%. From 10,540 calli transferred for redifferentiation, 3,251 plants were obtained. The average plant regeneration frequency was 30.84%. There were variations among trisomics both in callus induction frequency and plant regeneration frequency. The callus induction frequency ranged from 9.99% for type F to 31.41% for type H, and plant regeneration frequency ranged from 14.45% for type F to 43.75% for type C (Table 3).

The results show that trisomic C had the highest plant regeneration frequency in both years, and that trisomic F was poorest both in callus induction and plant regeneration responses in the 1989 experiment.

Chromosome number of anther-culture-derived plants

The chromosomes were counted in the root-tip cells of some of the regenerated plants, mainly those showing unique morphological features at the seedling stage. A total of 694 plants were cytologically examined during the 2-year period. Forty-three $n+1$ plants were obtained among regenerates of nine trisomics, and 51 tetrasomic plants were also obtained among regenerates of nine trisomics (Table 4). The extra chromosome in the root-tip cells of $n+1$ plants regenerated from trisomics G and L was a fragment chromosome (Fig. 1). Similarly, one of the $n+1$ plants derived from trisomic C was a fragment.

Other aneuploids, such as monosomics, trisomics, triploids, tetraploids, and plants with $2n=39$ and $2n=73$, were also observed in regenerants from some types of trisomics (Table 4).

Frequency of plants with $2n=13$ and $2n=26$ chromosomes

In both years, aneuploids with $2n=13$ and $2n=26$ were obtained from eight trisomics (trisomics A, B, C, D, E,

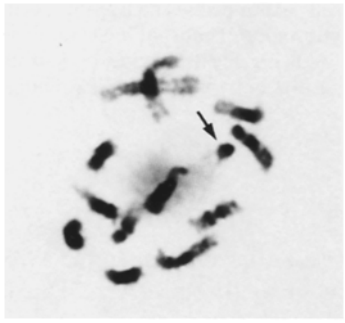

Fig. 1. Somatic chromosomes of an aneuploid plant with 2n=13 derived from trisomic type L. *Arrow* shows a fragment chromosome

Table 4. Chromosome number of plants derived from each trisomic in 1988 and 1989

Trisomic	Plants with chromosome no.										Total
	12	13	23	24	25	26	36	39	48	73	
A	7	11				14		1			33
B	4	8		3		11					26
C	68	7[a]	1	16	1	8	2	2	2		107
D	57	4		17	1	5		1			85
E	54	3		6		2			2		67
F	23			6	3	6		1	1		40
G	64	2[a]		10	1				1		78
H	7	6	1	4	1	3				1	23
L	44	1[a]		14	4	1	1		1		66
N	91		1	13					1		106
O	40	1		18	1	1	1		1		63
Total	459	43	3	107	12	51	4	5	9	1	694

[a] One of the n+1 plants from seven of trisomic C, and all n+1 plants of trisomics H and L, had a fragment chromosome

Table 5. Frequency of aneuploids with 2n=13 and 2n=26 chromosomes in the regenerants of trisomic

Trisomic	Plants obtained	Plants with 2n=13	Plants with 2n=26
A	317	11 (3.47)[a]	14 (4.42)
B	236	8 (3.39)	11 (4.66)
C	616	7 (1.14)	8 (1.30)
D	470	4 (0.85)	5 (1.06)
E	384	3 (0.78)	2 (0.52)
F	125	–	6 (4.80)
G	351	2 (0.57)	–
H	205	6 (2.93)	3 (1.46)
L	228	1 (0.44)	1 (0.44)
N	560	–	–
O	242	1 (0.41)	1 (0.41)
Total	3,734	43 (1.15)	51 (1.37)

[a] ( ) shows the percentage

H, L, and O). Trisomic G yielded aneuploids with 2n=13 only, and trisomic F only produced 2n=26 aneuploids. The frequencies of aneuploids with 2n=13 and 2n=26 in the regenerants of each trisomic are shown in Table 5. The frequency of aneuploids with 2n=13 ranged from 0.41% in trisomic 0 to 3.47% in trisomic A. That of aneuploids with 2n=26 ranged from 0.41% in trisomic 0 to 4.66% in trisomic B. A total of 106 regenerants from 560 of trisomic N were cytologically examined, but no plants with either 2n=13 or 2n=26 were obtained.

Cytology of aneuploid plants with 2n=13 and 2n=26

The extra chromosomes of regenerants with 2n=13 and 2n=26 derived from three trisomics (C, E, and H) were examined in root-tip cells, to verify whether or not the extra chromosomes of regenerants corresponded with the extra chromosome of the parental trisomics. The results revealed that the extra chromosomes of aneuploids derived from type H were all chromosome *K10* (Fig. 2a, b), which is the extra chromosome of trisomic H. The extra chromosomes of aneuploids derived from type E were all chromosome *K4* (Fig. 2c, d), which is the extra chromosome of trisomic E. Similarly, the extra chromosomes of aneuploids derived from type C were all chromosome *K12* (Fig. 2e, f), which is the extra chromosome of type C. The aneuploid plants derived from other types of trisomics could not be identified with certainty.

Discussion

In this study, we carried out the anther culture of rice trisomics for the first time and obtained n+1 plants which have not been reported before in rice. Similarly, many tetrasomic plants were obtained which had been observed at a very low frequency earlier. Great differences were observed in the frequency of callus formation and plant regeneration from anthers of different trisomics. It is therefore obvious that the difference in anther culture ability and plant regeneration is under genetic control.

The frequencies of aneuploids in the anther-culture-derived plants of various trisomics varied greatly. Furthermore, no aneuploids were obtained among plants derived from trisomic N. Similar observations were made by Niizeki et al. (1984) in the anther-culture-derived plants of trisomics of tobacco. Perhaps pollen grains with extra chromosomes are not totipotent or else they require different culture conditions for regeneration.

Plants with an n+1 fragment chromosome were obtained for the first time among the plants regenerated from the anther culture of rice trisomics. All the n+1 plants in the regenerants of trisomic G and L and one plant among the regenerants of trisomic C had an extra

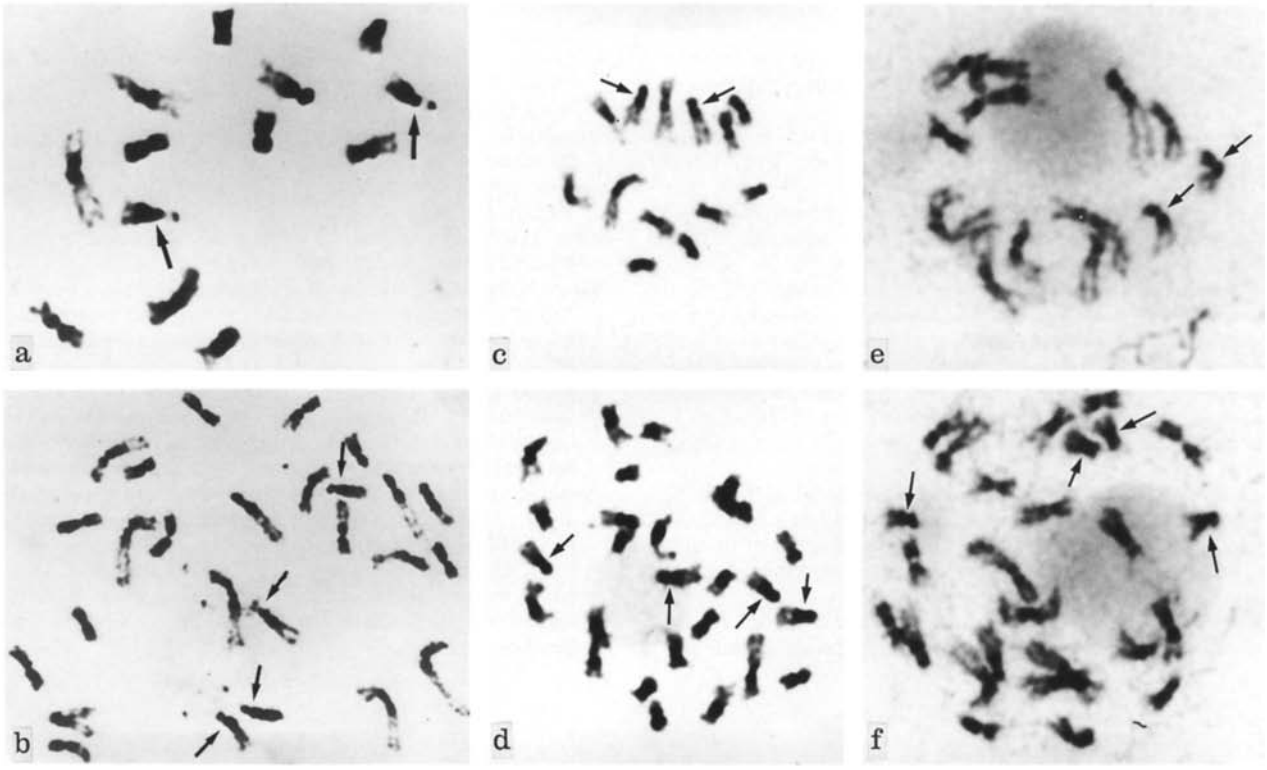

Fig. 2a–f. Somatic chromosomes of aneuploid plant with 2n=13 (**a**) and 2n=26 (**b**) derived from trisomic type H. *Arrows* show chromosome 1 (*K10*), which is the extra chromosome of trisomic type H. Somatic chromosomes of aneuploid plant with 2n=13 (**c**) and 2n=26 (**d**) derived from trisomic type E. *Arrows* show chromosome 11 (*K4*), which is the extra chromosome of trisomic type E. Somatic chromosomes of aneuploid plant with 2n=13 (**e**) and 2n=26 (**f**) derived from trisomic type C. *Arrows* show chromosome 7 (*K12*), which is the extra chromosome of the trisomic type C

fragment chromosome. These fragment chromosomes likely originated as a result of misdivision of the univalent chromosome during meiosis of these trisomics.

Aneuploids have been observed in the anther-culture-derived progenies of disomic plants (Oono 1975; Chu et al. 1985), but plants with n+1 chromosomes have not been previously reported. Plants with n+1 chromosomes in this study obviously resulted from the callus formation and regeneration of n+1 pollen grains. Chromosome doubling of such regenerants gave rise to 2n+2 or tetrasomic plants. These aneuploids will probably be useful in breeding and genetic studies. For example, trisomics were used to associate RFLP linkage groups with respective chromosomes through DNA dosage effect by McCouch et al. (1988). However, it is sometimes difficult to determine the dosage effect with certainty, since the difference in three versus two dosages is not that great. However, if we use tetrasomics, the dosage difference would be four versus two, and it would be easier to determine the linkage group chromosome associations.

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References

Chandrasekharan P (1952) The occurrence of barren tillers in a heterozygous rice. Madras J Agric 39:605–610

Chang TT (1964) Present knowledge of rice genetics and cytogenetics. Int Rice Inst Tech Bull 1, Los Banos, The Philippines

Chu QR, Zhang ZH, Cao YH (1985) Cytogenetical analysis on aneuploids obtained from pollenclones of rice (*Oryza sativa* L.). Theor Appl Genet 71:506–512

Hu CH (1968) Studies on the development of twelve types of trisomics in rice with reference to genetic study and breeding program. J Agric Assoc China 63:53–71 (in Chinese with English summary)

Iwata N, Omura T (1984) Studies on the trisomics in rice plants (*Oryza sativa* L.). VI. An accomplishment of trisomic series in *japonica* rice plants. Jpn J Genet 59:199–204

Iwata N, Omura T, Nakagahra M (1970) Studies on the trisomics in rice plants (*Oryza sativa* L.). I. Morphological classification of trisomics. Jpn J Breed 20:230–236

Karibasappa BK (1961) An auto-triploid of rice and its progeny. Curr Sci 30:432–433

Katayama T (1963) Study on the progenies of autotriploid and asynaptic rice plants. Jpn J Breed 13:83–87

Khush GS (1973) Cytogenetics of aneuploids. Academic Press, New York

Khush GS, Singh RJ, Sur SC, Librojo AL (1984) Primary trisomic of rice: Origin, morphology, cytology, and use in linkage mapping. Genetics 107:141–163

Kurata N, Omura T (1978) Karyotype analysis in rice. I. A new method for identifying all chromosome pairs. Jpn J Genet 53:251–255

Mattingly CF, Collins GB (1974) The use of anther-derived haploids in *Nicotiana*. III. Isolation of nullisomics from monosomic lines. Chromosoma 46:29–36

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Metz SG, Sharma HC, Armstrong TA, Mascia PN (1988) Chromosome doubling and aneuploidy in anther-derived plants from two winter wheat lines. Genome 30:177–181

Nayar NM (1973) Origin and cytogenetics of rice. Adv Genet 17:153–292

Niizeki M, Kita F (1975) Production of aneuploid plants by anther culture in *Nicotiana tabacum* L. Jpn J Breed 25:52–58

Niizeki M, Hayashi H, Saito K (1984) Production of disomic haploid by anther culture of a series of trisomic plants in *Nicotiana sylvestris*. Jpn J Breed 34:1–8

Oono K (1975) Production of haploid plants of rice (*Oryza sativa*) by anther culture and their use for breeding. Bull Natl Inst Agric Sci Ser D 26:139–222 (in Japanese with English summary)

Ramanujam S (1937) Cytogenetical studies in the Oryzae. II. Cytological behaviour of an autotriploid in rice (*Oryza sativa*). J Genet 35:183–221

Sampath S, Krishnaswamy V (1948) A chromosome deficient paddy type. Curr Sci 17:271–272

Sen SK (1965) Cytogenetics of trisomics in rice. Cytologia 30:229–238

Seshu DV, Venkataswamy T (1958) A monosome in rice. Madras J Agric 45:311–314

Wang ZX, Iwata N, Sukekiyo Y, Yoshimura A, Omura T (1988) A trial to induce chromosome deficiencies and monosomics in rice by using irradiated pollen. Rice Genet Newslett 5:64–65

Watanabe Y, Koga Y (1975) Cytogenetic studies on rice and its wild relatives. II. Genetic and cytogenetic studies on the trisomic plants of rice, *Oryza sativa* L. Bull Natl Inst Agric Sci Ser D 26:91–138 (in Japanese with English summary)

Yunoki T, Masuyama Y (1945) Investigations on the later generations of autotriploid rice plants. Sci Bull Fac Agric Kyushu Univ 11:182–216 (in Japanese with English summary)

Zhang T, Zhu H, Shu L, Wang S, Li X (1987) Studies on aneuploids from a Chinese indica rice variety. I. Plant morphological part. Acta Genet Sin 14:255–261 (in Chinese with English summary)