Theor Appl Genet (1989) 77:149–151

A new approach to chromosome doubling for haploid rice plants

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Received January 4, 1988; Accepted May 5, 1988
Communicated by G. Wenzel

Summary. Rice nodal segments from three flowering haploids were excised and treated for different lengths of time with 0.3% or 0.4% colchicine (dissolved in 2% DMSO) in an attempt to induce fertile seeds. A combination of higher colchicine concentration and longer hours of treatment reduced the survival rate of treated segments, but more fertile plants were transformed. Pooled data showed that of the 842 segments used, 42.2% survived the treatment and sprouted, but only 31.9% were successfully established and grown to maturity. Among the 269 mature plants, 29,4% produced fertile seeds (panicles) with an average of 146.2 seeds per diploidized plant.

Key words: *Oryza sativa* – Haploid – Nodal segment – Colchicine – Chromosome doubling

Introduction

In recent years the production of haploids by anther culture or other means has provided a rapid method of obtaining pure homozygous lines. In rice, plants regenerated via androgenesis are predominantly diploids and haploids with occasional polyploids and aneuploids. The percentage of haploids produced varies with cultivars (Chen and Lin 1976; Oono 1981), crosses (Anonymous 1983; Chen et al. 1983), culture media or pre-treatment of anther (Lee and Chen 1982). Generally, it is within the range of 30%–60%. These haploids must be diploidized before they can be used for testing and evaluation.

The traditional method of chromosome doubling in rice entails cutting back of stems, separation and removal of newly emerged tiller and washing and trimming of roots before colchicine treatment. Another method is to carefully inject colchicine solution into the meristem region of each developing tiller (Lin 1979). These methods are very laborious and time-consuming. Therefore, an efficient method of converting a large number of haploid plants into fertile plants is surely needed.

Rice stem structurally consists of a series of nodes and internodes. It has been shown that excised upper nodes can be regenerated with high success into normal whole plants on vermiculite moistened with simple inorganic nutrient solution (Wong et al. 1987). Since each mature tillering haploid rice plant can easily furnish 10–20 regenerable nodal segment and the axillary bud borne on each excised nodal segment is well exposed, chemical treatment of nodal segments, or other means, followed by plant regeneration may provide a convenient avenue of chromosome doubling.

Materials and methods

The three haploids used were Tainung 67 (TNG 67), B_7 and Huang-Tsao-San (HTS) of *Oryza sativa* subspecies *japonica*. All were produced by anther culture in the laboratory. B_7 was an androgenetic line developed from anther culture of a seed callus-derived regenerated plant of TNG 67 having ethylmethansulfonate (EMS) treatment and NaCl in vitro selection histories. Compared with the TNG 67 haploid line, it was taller and more vigorous, with profuse tillering and somewhat larger florets, indicating genetic changes may have occurred from the EMS treatment and/or the culture procedures. It was, therefore, included in the chromosome doubling study.

The haploids were vegetatively mass-propagated via excised nodal segments (Wong et al. 1987) and planted in an experimental plot. At anthesis or a few days after anthesis, stems were detached at soil surface and washed gently in tap water. Since the topmost node (counted downward from the panicle) was not regenerable into whole plant (Wong et al. 1987), only excised

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Table 1. Responses of excised nodal segments of three haploids to colchicine treatment and their formation of seeds

Genotype	Colchicine conc. %	Hours	No. of segments treated	No. of segments survived[a]	No. of segments grown to maturity[a]	No. of mature plants producing seeds[a]	Average no. of seeds per fertile plants	Range of seeds among fertile plants
TNG 67	0.3	6	45	31 (68.9)	22 (48.9)	5 (22.7)	98.5	20–147
		8	45	31 (68.9)	19 (42.2)	5 (26.3)	178.0	46–287
		10	45	25 (55.6)	18 (40.0)	5 (23.8)	132.8	5–448
	0.4	6	44	30 (68.2)	25 (56.8)	5 (20.0)	104.4	24–250
		8	44	28 (63.6)	15 (34.1)	4 (26.7)	105.3	48–168
		10	47	25 (53.2)	10 (21.3)	3 (30.0)	57.7	15– 88
B_7	0.3	6	46	22 (47.8)	20 (43.5)	2 (10.0)	70.0	40–100
		8	45	17 (37.8)	14 (31.1)	2 (14.3)	138.5	37–240
		10	50	18 (36.0)	14 (28.0)	6 (42.9)	274.5	5–776
	0.4	6	45	19 (42.2)	17 (37.8)	3 (17.6)	47.6	19– 80
		8	44	14 (31.8)	11 (25.0)	4 (36.4)	107.2	9–380
		10	36	10 (27.8)	8 (22.2)	2 (25.0)	12.0	12– 12
HTS	0.3	6	50	17 (34.0)	14 (28.0)	3 (21.4)	108.3	70–155
		8	50	14 (28.0)	9 (18.0)	3 (33.3)	311.5	240–383
		10	58	14 (24.1)	14 (24.1)	7 (50.0)	124.4	30–283
	0.4	6	50	16 (32.0)	16 (32.0)	6 (37.5)	176.0	59–411
		8	50	13 (26.0)	11 (22.0)	7 (63.6)	372.5	142–483
		10	48	11 (22.9)	12 (25.0)	7 (58.3)	213.2	18–821
Pooled			842	355 (42.2)	269 (31.9)	79 (29.4)	146.2	

[a] ( ) = %

stem segments of node positions Nos. 2 and 3 were used for the colchicine treatment.

The stem segments, each containing a node (and about 3 cm long on either side of the node) were placed in a plastic bag and incubated at 30 °C overnight (12–18 h) to promote cell divisions. Fifty to sixty segments were then removed and allowed to float freely in 500 ml Erlenmeyer flasks each containing 180 ml of either 0.3% or 0.4% (w/v) colchicine solution. The colchicine solution was freshly prepared and contained 2% (v/v) dimethylsulfoxide (DMSO) as a tissue penetrant. Treatment was conducted at 30 °C under subduced eight on a shaker with continuous agitation of 100–120 rpm. Treatment durations were 6, 8 and 10 h, respectively. Upon completion of treatment, the segments were washed vigorously with tap water and left in the water overnight (12–18 h). Cut ends of the treated segments were then dipped into fungicide 50% Benlate powder (active ingredient Benomyl 50%, Du Pont/DE, USA) and planted vertically on vermiculite moistened with nutrient solution (Yoshida et al. 1976). Planted segments were covered with a plastic tent and placed in the shade in an unheated glasshouse (28°–34 °C). Water or nutrient solution was added every 3–4 days or as needed. The plastic tent was removed 2 weeks later. The number of sprouting segments was recorded a month later. Established cuttings were then removed and individually transplanted in a plot for obtaining and formation of diploid sectors (seeds) or panicles.

Results

Compared with controls, treated segments were stunted in growth with sprouting delayed by about 10–20 days. A total of 842 segments were used and 42.2% survived the treatment (Table 1). There was a tendency toward longer hours of treatment resulting in more deaths at either 0.3% or 0.4% colchicine. Efforts were made to transplant all sprouting segments in the field, but growing conditions at that time were hot and dry. Therefore, some of the segments succumbed after transplanting and only 269 segments (31.9%) were successfully grown. At maturity, 29.4% of the stem-derived plants bore fertile seeds and/or panicles with an average number of 146.2 seeds per diploidized plant. Interestingly, a small number of the fertile plants appeared to be diploid or diploid-like in stature and plant height, with the result that hundreds of seeds could be obtained (Table 1). Direct transformation into fertile diploids from excised haploid nodal segments in one single generation has great value in a haploidy breeding program and will be discussed later.

When the data were further classified on the basis of colchicine concentration and hours of treatment, it was shown that with higher concentration and longer duration of treatment, survival rate among treated segments was reduced, but more segments could be induced to form seeds (Table 2).

Discussion

Even though only 42% of the treated segments survived and about 29% of the stem-propagated plants produced fertile seeds, with further refinement of the technique such as the addition of phytohormones and proper post-

Table 2. Survival rate and formation of seeds of treated haploid segments, based on colchicine concentration and hours of treatment

Colchicine conc. %	Hours	No. of segments treated	No. of segments survived[a]	No. of segments grown to maturity[a]	No. of mature plants producing seeds[a]
0.3	6	141	70 (49.6)	56 (39.7)	10 (17.9)
	8	140	62 (44.3)	42 (30.0)	10 (23.8)
	10	153	57 (37.2)	46 (30.1)	18 (39.1)
		434	189 (43.7)	144 (33.2)	38 (26.9)
0.4	6	139	65 (46.8)	58 (41.7)	14 (24.1)
	8	138	55 (39.9)	37 (26.8)	15 (40.5)
	10	131	46 (35.35)	30 (22.9)	12 (40.0)
		408	166 (40.60)	125 (30.5)	41 (34.9)

[a] ( ) = %

treatment care, treatment efficiency would certainly be increased (Thiebaut et al. 1978). In a later experiment with haploid HTS the survival rate was increased from the present 27.8% to 85% by growing the treated segments at a lower temperature of 26 °C. Indole-butyric-acid (IBA) at 50–100 ppm could also increase rootings in some of the segments (unpublished results). Perhaps the most interesting facet of this study was that 12% of the plants that produced seed were diploid or diploid-like in stature and plant height, with the result that hundreds of seeds could be obtained in one single plant. Large amounts of seeds could be used directly for field testing and thus reduce the need for an additional growing cycle for seed increase. Further studies should, therefore, be directed at optimizing the conditions for the formation of these 'diploid' plants. The size of the axillary bud on the nodal segment seems to play a crucial role.

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