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Somaclonal genetics of rice, *Oryza sativa* L.

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Summary. The inheritance and variations of some traits of more than 2,000 somatic cell derived plants of rice (*Oryza sativa* L.) were investigated in the second and third generations (T_2 and T_3) of regenerated plants (somaclones). The percentages of multiploids occurring in somaclones ranged from 0–13.3 in nine varieties (or hybrids) of 'Hsien' (indica) group, but no multiploid was found in nine varieties (or hybrid) of 'Keng' (japonica) group. A dwarf mutant with the height of only 20 cm was isolated in the T_2 of 'Tai-Zhong-Yu 39'. Genetic analysis indicated that it was controlled by a single recessive gene. The frequencies of chlorophyll mutations probably controlled by cytoplasmic genes decreased gradually with the advance of generations. The variations of five quantitative traits – plant height, grain weight, etc. – in 950 T_2 pedigree lines of four varieties were also studied. Only 24.4% of the lines were normal in all the traits studied. Variation frequencies of different traits were from 11.5% to 39.5%. And there was an obvious tendency for the plant height to become shorter, number of productive tillers to increase and 1,000 grain weight to be lighter, whatever the variety studied. Traits were uniform within each of more than 90% of all T_2 lines studied. What is more interesting, variations phenotyped in T_2 proved to breed true. Causes of somaclonal variations are discussed, as well as their potentials in breeding.

Key words: Somaclonal variation – Genetics – *Oryza sativa* L. – Rice improvement

Introduction

As a novel source of variability, somaclonal variations, a subject reviewed recently by Larkin and Scowcroft (1981), are attracting much attention from geneticists and breeders. Corn (Gengenbach et al. 1977), tobacco (Burk and Matzinger 1976), rice (Wang et al. 1981; Oono 1978), oats (Cummings et al. 1976) and some such vegetatively propagated crops as potato (Shepard et al. 1980) and sugar cane have been used in tissue culture and their somaclonal variations studied. D'Amato (1978) reported a more detailed cytological study of tissue culture. All the above studies indicated that there were changes in the number of chromosomes and morphology in somaclones. Progenies of somaclones were also studied by some researchers. Gene mutation occurring in tissue culture has been reported (Gengenbach et al. 1977). Oono (1978) and Kucherenko (1979) studied the inheritance and variations of traits of rice somaclones, indicating that rather high frequencies of variations could occur in such traits as chloroplast, plant height, tillering and others in progenies of somaclones. We have reported that in rice somaclones, the total number of leaves per plant decreased, date of maturity became earlier, plant height became shorter and most economic characters were inferior (Sun 1980, Sun et al. 1981). The inheritance and variation of characters in progenies of somaclones have also been preliminarily reported (Zhao et al. 1982). The present paper describes a study on somaclonal variability of rice. Causes of the variations were discussed, as well as their potentials in breeding. Abbreviations for regenerated plants (somaclones) and their following generations have been referred to as D_1, D_2 ... by Oono (1978), and as R_1, R_2 ... by Kucherenko (1979). We propose to use T_1, T_2 ..., originated from the initial of 'Tissue culture', which is a more general term used at present.

Materials and methods

Eighteen varieties (or hybrids) of rice (*Oryza sativa* L.) were used in the present study: from the 'Hsien' (indica) group

– 'Yuan-Feng-Zao', 'Guang-Lu-Ai No. 4', 'Gui-Chao No. 2', 'Zhen-Shan 97', 'Si-Hong-Ling-Yi × Gong-Qing (F_8)', 'IR26', 'Ya-Xue-Nuo', 'Ma-Lai-Hong', 'Shan-You No. 6 (Zhen-Shan 97 × IR26)'; from the Keng (japonica) group – 'Tai-Zhong-Yu No. 39', 'Nong-Hu No. 6', 'Ce21', 'Ce26', 'Dong-Xuan No. 4', 'Ai-Keng 23', 'C57', 'Bai-Ge' and 'Feng-JinA × C57'. The young panicles or dehusked seeds were used as explants. Calli were induced on N_6 medium supplemented with NAA, 2.4-D and 6-BA of differing concentrations, and plantlets were then redifferentiated on N_6 medium, omitting 2.4-D. Detailed culture procedures had been described previously (Tang and Sun 1979; Zhao et al. 1980). Conventional management measures were carried out after the somaclones were transplanted to soil. Single plants were taken in at maturity for growing the next generation (T_2). In the following year, T_2 seedlings were transplanted in individual plant progeny plots, each of which contained 70–99 plants, with single plants per hill and one plot of the parent variety was grown every 10 plots as the control. Plant height, number of productive tillers, heading date (for 50% of plants in a line) and grain weight of every T_2 line and the checks were assigned scores according to the standard evaluation system of the International Rice Research Institute (IRRI 1980). Data for all traits measured were analysed statistically. Panicles of part of T_2 lines were covered with glassine bags at the pollination stage to protect them from foreign pollens for the the pedigree growing of the following generation and for performance studies in T_3.

Table 1. Segregation of leaf sheath colour in T_2 lines of somaclones with different grain sizes of the hybrid rice Shan-You No. 6

Grain sizes	Segregation in leaf sheath colour			χ^2	P
	Observed plants		Expected ratio		
	Purple	Green			
Bigger (T_2) grain	125	6	15 : 1	0.371	0.50 – 0.75
Normal (T_2) grain	220	92	3 : 1	3.116	0.05 – 0.10
Check (T_2)	138	59	3 : 1	2.316	0.10 – 0.25

Results

Variations of ploidy and qualitative traits

1 The changes of ploidy. A few somaclones with thicker stems, larger leaves, awned and obviously bigger grains were found in some varieties and in the hybrids of Hsien (indica) rice (Sun 1980; Sun et al. 1981). Their progenies were similar to T_1 in appearance. Genetic observations were made using hybrid rise 'Shan-you No. 6' as the material by which to confirm the variations of ploidy in plants. It is known that the leaf sheath colour of 'Zhen-Shan 97A' is purple, that of 'IR26' is green, and that of F_1 ('Shan-You No. 6') is purple. Segregations occurred in leaf sheath colour in both the selfed progenies of somaclones with awned and bigger grains and with normal grains. χ^2 test showed that the segregations of somaclones with

Table 2. Frequencies of polyploid and chloroplast mutations in T_2 lines of somaclones of different genotypes of *Oryza sativa* L.[a]

Race	Genotype	Polyploid			Chloroplast mutation		
		No. of observed lines	No. of polyploid lines	Frequency (%)	No. of observed lines	No. of partly albino lines	Frequency (%)
Hsien (Indica)	Yuan-Feng-Zao	459	1	0.22	463	13	2.81
	Guang-Lu-Ai No. 4	377	6	1.59	377	7	1.86
	Gui-Chao No. 2	78	1	1.28	79	0	0
	Zhen-Shan 97	15	2	13.3	15	0	0
	Si-Hong-Ling-Yi × Gong-Qing (F_8)	97	0	0	125	3	2.40
	IR_{26}	40	0	0	29	2	6.90
	Ya-Xue-Nuo	4	0	0	4	0	0
	Ma-Lai-Hong	11	0	0	11	1	9.09
	Shan-You No. 6 (Zhen-Shan 97 × IR_{26})[b]	810	6	0.74	10	0	0
Keng (Japonica)	Tai-Zhong-Yu No. 39	44	0	0	44	22	50.0
	Nong-Hu No. 6	4	0	0	5	0	0
	Ce21	17	0	0	17	0	0
	Ce26	18	0	0	18	0	0
	Dong-Xuan No. 4	15	0	0	15	0	0
	Ai-Keng 23	4	0	0	5	0	0
	C57	3	0	0	3	0	0
	Bai-Ge	3	0	0	3	0	0
	Feng-Jin A × C57[b]	5	0	0	5	0	0

[a] Polyploid frequency refers the observation in the field; chloroplast mutation frequency refers the results of the germination test in the laboratory

[b] Hybrid rice

normal grains was the same as that of F_2 of the starting material 'Shan-You No. 6', i.e. the purple colour of leaf sheath was controlled by a single dominant gene, and their genotypes were PP or P_p. The segregation in the progenies of somaclones with awned and larger grains, however, was quite different. Only the leaf sheath of individuals of *pppp* genotype was green (Table 1), confirming that the mutant with awned and larger grains found in T_1 of 'Shan-You No. 6' was tetraploid and resulted from the spontaneous doubling of chromosomes. Its genotype was PP_{pp}.

The frequencies of multiploids occurring in somaclones of different eco-geographic races of *Oryza sativa* were different (Table 2). Those somaclones of the Hsien rice ranged from 0–13.3%, and no multiploid was found in those of the Keng rice.

2 Mutations of nuclear genes. From the somaclones of 'Tai-Zhong-Yu No. 39', one plant (T_1-Tai-19) achieved a height of 92 cm, quite close to that of the control (99.8 cm). In 614 T_2-Tai-19 seedlings, however, there were 132 dwarf mutants with a height of only 20cm which could reach maturity normally (Fig. 3). Single plants were harvested for segregation studies in the progenies, and a χ^2 test verified the 3 : 1 ratio (Table 3). Analyses of the T_3 pedigree lines indicated that in 101 normal plant height T_2 derived T_3 lines, there were 71 lines which continued to show segregation of plant height while the remaining 30 lines showed no segregation and were all normal. This is in agreement with the ratio of 2 : 1 ($\chi^2=0.137$, $0.50<P<0.75$). Seedlings grown from 1,000 seeds derived from 50 dwarf mutants were all dwarf. Observations on pollen mother cells showed that the number of chromosomes in the dwarf mutant was $n=12$.

The plant height of T-Tai-19, the dwarf mutant× cultivar 'Tai-Zhong-Yu 39' derived F_1, was similar to that found in the cultivar. Part of the seeds derived from F_1 were germinated in the laboratory, 693 normal height plants and 203 dwarfs were obtained. This coincided with the expected ratio of 3 : 1 ($\chi^2=2.69$, $0.10<P<0.20$). it was thus confirmed that the T-Tai-19 dwarf mutant derived from rice somaclones was controlled by a single recessive gene. The genotypes of the initial parent 'Tai-Zhong-Yu 39', T-Tai-19 and dwarf mutant segregated from T-Tai-19 were DD, Dd and dd respectively.

Table 3. Segregation of plant height in progenies of T-Tai-19

Generation	Segregation in plant height			χ^2	P
	Observed plants[a]		Expected ratio		
	Normal	Dwarf			
T_2	482	132	3 : 1	3.831	0.05 – 0.10
T_3	4,786	1,549	3 : 1	1.016	0.25 – 0.50
T_4	4,572	1,459	3 : 1	0.397	0.50 – 0.75

[a] For T_3 and T_4, only the lines within which segregation occurred in plant height were included

Table 4. Changes of albino percentage in progenies of some somaclones of Tai-Zhong-Yu 39

Line No.	Albino percentage	
	T_2	T_3
12	36.36	13.00
24	2.4	0.0
31	23.08	14.14
37	34.74	22.22
44	26.09	24.24
15	21.74	12.00
26	11.76	11.00
32	3.23	0.0
38	24.24	10.20
46	31.61	22.00
19	27.04	8.00
28	20.69	14.14
33	21.05	19.38
39	3.33	0.0
47	21.05	28.00
20	20.00	23.00
29	25.00	31.63
36	25.00	9.09
41	18.61	25.25
48	0.99	0.00

3 Chloroplast mutations. Albino occurrence in somaclones has been reported to be lower than 1% (Zhao et al. 1980; Tang and Sun 1979). However, albinos have been segregated in some T_2 lines of certain varieties (Table 2). Changes of albino percentages in T_2 and T_3 of 'Tai-Zhong-Yu 39' were investigated. A *t*-test ($t=2.86$, $t_{(0.01,\,19)}=2.86$) indicated that albino percentages dramatically decreased with advance of the generations (Table 4). It is known that the big and small subunits of some chloroplast proteins were controlled by cytogenes and nuclear genes respectively. We therefore suppose that the chloroplast variations occurred in our experiment was caused by cytogene mutations.

The inheritance of quantitative traits

Some quantitative traits such as plant height and grain weight of T_2 populations were different from their respective check populations (Fig. 1). The probabilities of the number of T_2 lines which were out of the boundaries of 95% confidence intervals of their check populations were estimated on these quantitative traits according to the probabilities of normal distribution (Table 5). It is evident that: (1) there were very high probabilities of the induction variations of traits in T_2

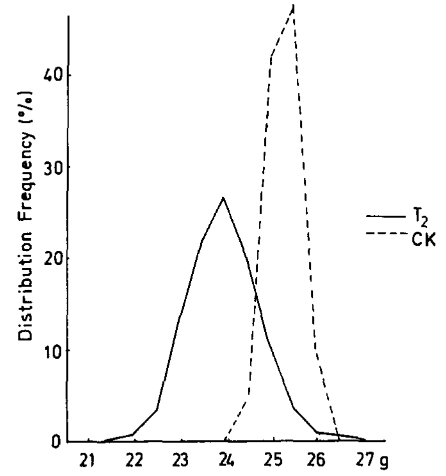

Fig. 1. Distribution of grain weight in T_2 of Guang-Lu-Ai No. 4

in the course of tissue culture, (2) except for grain number per panicle, variations of the same trait in T_2 populations of different cultivars tended to be essentially consistent, i.e. in comparison with their respective controls, T_2 populations had shorter plant height, lighter grain weight and more effective tillers. However, the means of heading date and of fertility were nearly the same although the coefficients of variations of these traits for T_2 were larger, and (3) tissue culture had different mutation effects on different traits. The number of T_2 lines in which 1 trait, and 2, 3 or more traits, showed variation simultaneously were calculated respectively for 'Tai-Zhong-Yu 39', 'Gui-Chao No. 2', 'Yuan-Feng-Zao' and 'Guang-Lu-Ai No. 4' (Table 5). Most of the 950 somaclone derived lines of 4 rice genotypes showed such variations and only about 24.2% lines were normal. Variation coefficients of

Table 5. Frequency estimates of the number of varied lines in T_2 of *Oryza sativa* L.

Trait (cm)		Genotype				Average	Total
		Guang-Lu-Ai No. 4[a]	Yuan-Feng-Zao	Gui-Zhao No. 2	Tai-Zhong-Yu No. 39		
Plant height	T_2	75.91±2.83	77.48±3.65	99.50± 2.57	97.01± 2.57		
	CK	79.92±1.63	78.47±1.67	101.55± 1.66	99.76± 1.56		
	> A[b]	7.14	12.16	2.00	1.19	5.62	36.93
	< B[b]	21.33	26.60	32.10	45.20	31.31	
No. of effective tillers	T_2	7.51±0.83	6.85±0.73	8.07± 0.78	4.96± 0.57		
	CK	7.26±0.80	6.18±0.59	7.73± 0.52	4.86± 0.35		
	> A[b]	5.73	25.11	19.16	13.86	15.97	19.78
	< B[b]	1.46	0.61	4.05	9.13	3.81	
Heading date (days)	T_2	92.55±1.56	84.01±0.91	114.58± 1.16	91.83± 2.59		
	CK	92.67±0.80	84.00±0.60	114.43± 0.78	92.50± 3.21		
	> A[b]	14.08	9.92	11.08	0.37	3.86	17.82
	< B[b]	17.80	9.18	7.37	1.50	3.96	
Grain no. per panicle	T_2	90.04±3.50	–	110.86± 8.74	116.69±23.87		
	CK	90.36±6.39	–	104.08±10.76	107.43±16.83		
	> A[b]	4.34	–	5.08	16.01	8.48	11.53
	< B[b]	5.19	–	0	3.83	3.05	
Fertility (%)	T_2	57.81±5.80	–	41.70± 7.11	68.90±11.7		
	CK	61.22±3.79	–	42.33± 4.43	64.73± 5.60		
	> A[b]	3.07	–	9.53	28.03	13.54	29.29
	< B[b]	24.44	–	12.88	9.93	15.75	
1,000 grain wt (g)	T	23.98±0.77	21.34±0.78	25.22± 0.07	23.40± 1.26		
	CK	25.21±0.40	22.26±0.55	25.45± 0.04	23.78± 0.98		
	> A[b]	0.41	0.52	6.98	2.98	2.70	39.50
	< B[b]	72.03	41.56	21.45	12.49	36.88	
No. of T_2 lines		376	455	78	41		950
No. of lines within which *n* traits varied	*n*=1	163	169	32	8		372
	n=2	75	114	17	10		216
	n=3	39	31	7	9		86
	n>3	19	15	7	5		46
Total		269	329	63	32		720
percentage		78.7	72.3	80.8	78.0		75.8

[a] For this genotype, data on the traits of grain number per panicle and fertility were statistically calculated on 20 random samples in 376 lines

[b] A and B refer to the maximum and minimum of 95% confidence intervals of the respective check populations on different traits

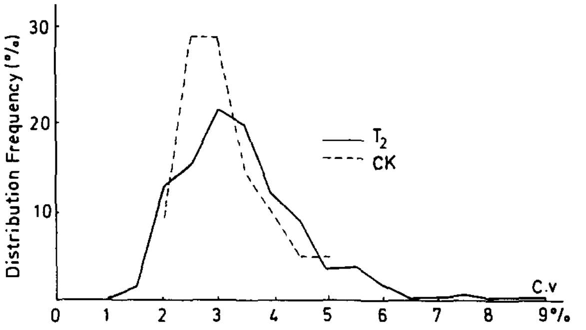

Fig. 2. Distribution of coefficients of variation of plant height in T_2 lines of Guang-Lu-Ai No. 4

measured traits within each of more than 90% T_2 populations were nearly the same as their respective checks, and lines with larger coefficients of variation numbered less than 10% (Fig. 2), although most T_2 lines obviously varied compared with their source materials. Different generations (T_3, T_4 and T_5) of lines of T-Tai-7 and T-Tai-42 were planted during the same season using the randomized block design with 3 replications. Analyses on 9 traits indicated that they were all breeding true (Table 6, Fig. 4, 5).

Discussion

1 The wide-range of somaclonal variations

From calli of 75 seeds of naturally doubled haploids of 'Norin No. 6', Oono (1978) obtained 1,121 somaclones, among which albinos comprised 7.4%. Variations of 800 somaclones and their progenies T_2 and T_3 were investigated. Wide variations in plant height, panicle shape, heading date, fertility and chloroplastids were observed. Plants normal in all traits comprised 28.1% only. In quite a number of somaclones, two or more traits varied simultaneously. Kucherenko (1979) investigated somaclones of 4 rice genotypes and their 98 progenies, and observed that in about 36% T_2 lines variations occurred as well. Our systematic study on progenies of more than 2,000 somaclones derived from 18 rice genotypes (including hybrids) indicated that variations did occur which included not only polyploids, but also mutations of nuclear genes and cytogenes, and series of continuous variations of some quantitative traits. Analyses on 950 T_2 lines of 4 rice genotypes indicated that lines normal in all traits comprised only 24.2% this coincided with Oono's results.

Oono (1978) and Wang et al. (1981) reported that dwarf mutants were obtained in rice tissue culture and anther culture respectively. Genetic analyses were not made because of sterility of the mutants obtained. In our study, fertile dwarf mutants was isolated from progenies of young panicle-derived somaclones of 'Tai-Zhong-Yu 39'. Genetic analysis indicated that dwarfness was controlled by a single recessive gene. Thus, gene mutation was for the first time confirmed in rice tissue culture.

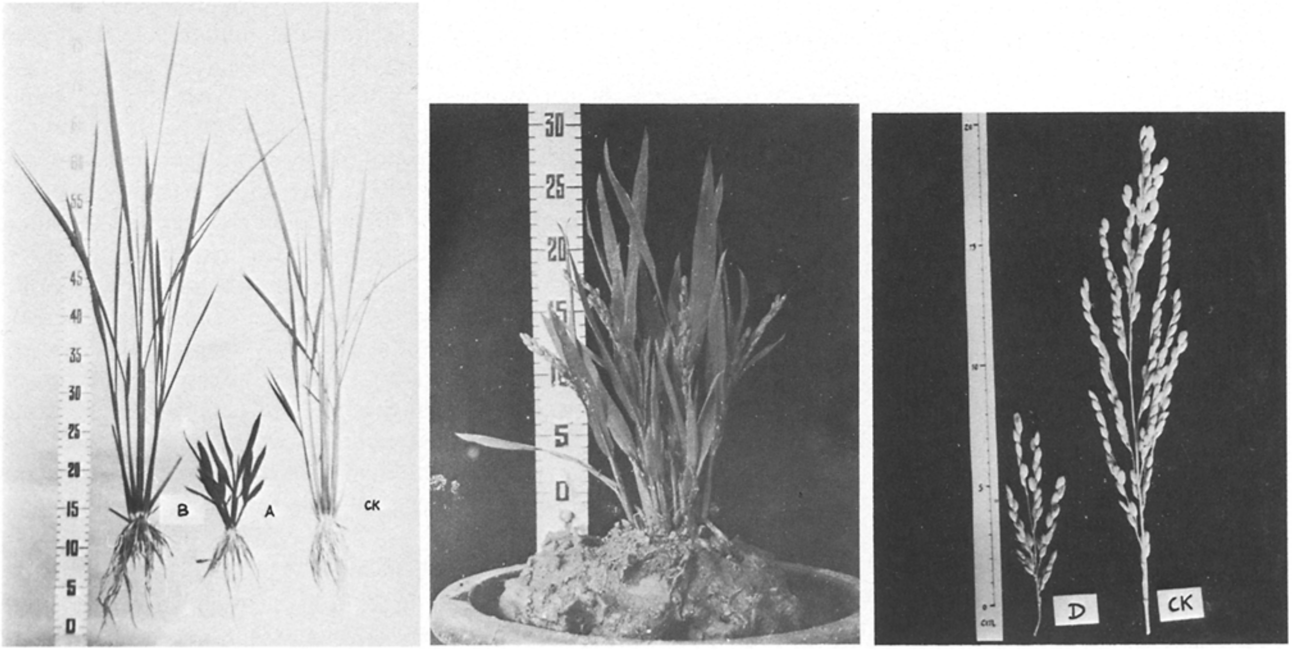

Fig. 3 a–c. Comparison of plants of Tai-Zong-Yu No. 39 (*ck*) and progenies of T-Tai-19. **a** dwarf (*A*) and normal (*B*); **b** normal seeds set on dwarf mutant; **c** morphology of the panicle of dwarf mutant

Table 6. The stability of the traits in different generations of rice somaclones. Values followed by the same letter show no significant difference at 5% level by Duncan's multiple range test

Line generation		Plant height (cm)	No. of effective tillers	Days from sowing to heading	1000 grain wt (g)	Length of flag leaf (cm)	Panicle length (cm)	Total grain no. per plant	Grain no. per panicle	Fertility (%)
T-Tai-42	3	84.35a	6.67a	99.67a	26.32a	22.3a	15.77a	539.4a	87.57ab	60.69a
	4	83.52a	6.47a	100.00a	26.19a	22.9ab	15.76a	526.3a	86.27b	62.09a
T-Tai-7	3	91.77b	5.50bc	100.00a	28.76b	25.3bc	17.50b	556.8a	98.87acd	47.55b
	4	91.11b	5.47bc	100.00a	28.79b	24.6c	17.30b	490.8a	97.10ac	43.17b
	5	91.11b	5.57b	100.00a	28.18b	25.7c	17.67b	524.4a	102.80cd	44.56b
CK		92.68b	4.90c	94.00b	24.19c	37.7d	19.00c	511.4a	108.37d	63.27a

Fig. 4. Comparison of plants of Tai-Zhong-Yu No. 39 (*left*) and T-Tai-42 progeny (*right*). Differences in plant type are evident

Fig. 5. Different generations (T3, T4, T5 = D3, D4, D5 in the photo respectively) of the progenies of T-Tai-7 in the field. Randomized design with three replicatons

2 *Causes of variability of somaclones*

In conventional breeding, stable lines can be obtained only after 6–8 generations due to gene segregations and recombinations in hybrid progenies. Stable lines can be obtained through one generation by the spontaneous or artificial doubling of anther or pollen culture derived haploids. Many traits show diversified variations amongst these H_2 lines, while traits within each of lines are uniform. Hu et al. (1979) attributed this to the recessive traits and recombinants being able to express under homozygous conditions due to the segregation of n pairs of independent genes. It is of great interest that variations present in the progenies of somaclones were wide-ranging, their frequencies high, and they were inherited from the T_2 generation onwards.

It was generally believed that there were two possible sources for variations induced by tissue culture: the expression of mutant cells existing in explants and the induced mutational effect of plant hormones (esp. 2.4-D) contained in the media. Opinions varied as to the mechanism for causing such variations. Keyes et al. (1980) held that the variations appearing in the tissue culture of red clover were mainly due to the additive effect of mutated genes. Shepard et al. (1980) considered that changes of traits were the results of expression of the genes, rather than gene mutation. Larkin and Scowcroft (1981) reviewed 7 possible origins of somaclonal variations, including karyotypes changes, transposable elements and so on. Locus specific mutations found in the course of physical and chemical introduction (Jain et al. 1968) could also be the possible origin of somaclonal variation. Ploidy changes and mutation did occur in our experiment. The frequencies, however, were very low so that all results obtained could not be explained simply by karyotype change only. In addition, Sacristân (1982) recently reported the segregation of resistance to blackleg disease in the inbred progeny of *Brassica napus* somaclone with increased resistance derived from susceptible starting material, which is quite different from our results. Further investigations seem necessary to reveal the mystery.

3 *Tendencies of somaclonal variations*

Some researchers on the basis of the cytological evidence, have reported that somaclones with normal karyotypes usually prevail, though polyploid, aneuploid and haploid cells exist in calli (D'Amato 1978). This supposedly results from the full vigour and better regeneratability of diploid cells. The tendency of a shortening in plant height in anther culture derivatives has been observed in wheat (Hu et al. 1979) and rice (Wang et al. 1981). In our experiment, average probability of the decreasing of plant height was 31.3% in progenies of somaclones of 4 rice genotypes, while that of increasing of plant height was 5.6% only. Similarily, average probabilities of decreasing and increasing of grain weight were 36.9%, 2.7% respectively. Such tendencies of unidirectional variations of certain traits in T_2 might be related to regenerability of the callus cells in which these variations had occurred. Hoffmann et al. (1982) indicated that in the process of regeneration, genetic selection can occur and that the regeneration capacity gene might be closely linked with certain characters which could be observed in the field. Our results agree with this viewpoint.

4 Somaclonal variations in relation to eco-geographic races of Oryza sativa L.

In rice anther culture, the redifferentiation rate of green plantlets was closely related to ecogeographic races. The redifferentiation rate of green plantlets from the Hsien group was much higher than that from the Keng group in somatic tissue culture (Zhao et al. 1981). In the present study the frequencies of polyploidy ranged from 0–13.3% in the Hsien T_2 lines, while no polyploidy was observed in Keng. Albinos occurred in T_2 lines of only one Keng genotype, but in the T_2 lines of 5 Hsien genotypes. Thus it seems that somaclonal variations are related to races of *Oryza sativa.*

Somaclonal variation is a noval source of variability for plant improvement. Due to its inheritance, breeding of crops could be accelerated. It is promising, therefore, that the combination of somaclonal variations and mutant selection at the cell level could be a helpful and effective aid in crop improvement.

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