Mutagenic Effects of DNA Base Analogues on Agronomic Characters of Rice (*Oryza sativa* L.)

By KIICHI FUKUI

Department of Molecular Biology, National Institute of Agrobiological Resources
(Yatabe, Ibaraki, 305 Japan)

Introduction

Mutagenic effects of DNA base analogues were discovered in microorganisms in 1950s.[2] The mechanism of their mutagenesis was elucidated as transition in DNA by the detailed experiments of phage T_4.[4] Since then base analogues have become popular mutagens for induction of point mutations in microorganisms.

In higher plants, induction of point mutations has been also attempted using some base and nucleoside analogues such as 2-aminopurine (AP), 5-bromouracil (BU) or 5-bromodeoxyuridine (BUdR) etc.[5,7] because powerful mutagens such as ionizing radiations and alkylating agents have induced not only valuable mutations but also lots of deteriorative mutations like sterility or chlorophyll mutations which are thought to be mainly induced by chromosomal aberrations. Induction of growth type mutants has been reported in *Arabidopsis*.[3,9]

For agronomic characters of cereal crops, however, there have been only a few reports[6,8,11] on induction of mutations by these substances. In this paper, we report the mutagenic effects of AP and BU on agronomic characters of rice and characteristics of the induced mutants. The value of these chemicals in mutation breeding is also discussed.

Materials and methods

A rice cultivar, "Ginbozu" was used in this experiment. Air dried kernels were soaked in the AP or BU solution either saturated or half concentration of saturated solution for 7 or 14 days (Table 1). All the materials were washed thoroughly after the treatment and placed on the tray of the nursery. Fifty seedlings in each treatment were randomly selected and transplanted into the experimental paddy field. Two panicles from each M_1 plant in the AP treatments and one from the BUs were randomly selected and harvested. Heading date and seed fertility of each M_1 plant were recorded.

Table 1. Experimental design for the treatment

Treatment No.	Mutagen	Concentration (s*)	Duration (days)
AP–1	AP	0.5s	7
AP–2	AP	0.5s	14
AP–3	AP	1s	7
AP–4	AP	1s	14
BU–1	BU	0.5s	7
BU–2	BU	0.5s	14
BU–3	BU	1s	7
BU–4	BU	1s	14

* Saturated solution at 25°C.

In the next M_2 generation, 386, 200 and 45 lines were raised by one-panicle-one-line method from all the treatments of AP, BU and the original variety as the control, respectively. Fifty kernels were sown for each line in the nursery bed. Fifteen to eighteen plants from each line were selected randomly and transplanted into the experimental field. Chlorophyll mutations at the nursery stage and several agronomic characters, i.e. heading date, culm length, fertility etc. were recorded for all the plants including the control.

One panicle was harvested from all these plants which were statistically determined to possess at least one varied character. One panicle was also harvested from the plants classified as normal for every character examined. For the M_3 control, some panicles were harvested from the M_2 control. Thus 696 M_3 progeny lines derived from the treated M_2 plants (338 lines for the AP treatments, 358 lines for the BU treatments) and 19 lines for the M_3 control were raised. The same methods as used for the M_2 generation were used to raise and examined the plant materials both in the nursery and the experimental field.

Chlorophyll mutations were determined by naked eyes. While quantitative variants on heading date, culm length etc. were identified by the following criterion. That is, a plant having a character(s) whose value(s) fell out of the confidence limits ($\alpha=0.01$) set up by the following formula according to the character distribution in the control was classified as a variant of the trait.

$$X \pm s\sqrt{F(N+1)/N}$$

where X: Mean of the trait in the control
s: Standard deviation of the trait in the control
N: Number of the plants in the control
F: F value, $\alpha=0.01$, $n_1=1$, $n_2=N-1$

A critical rate of 0.01 was adopted for all the characters in both generations.

For both the M_2 and M_3 generations, the same method was used to decide variants. Only the M_2 variants that segregated the same variation also in the M_3 generation were regarded as the mutants of the trait.

Results

1) *Plant injury observed in the M_1 generation*

The M_1 plants both in the AP and BU treatments showed slightly disturbed distribution in heading date. This tendency was clearer in the BU treatments than in the APs as shown in Fig. 1. Most of them were in the direction toward late heading, although few of them showed earlier heading than any of the plants in the control. Seed fertility of the M_1 plants is shown in Table 2. Average fertilities

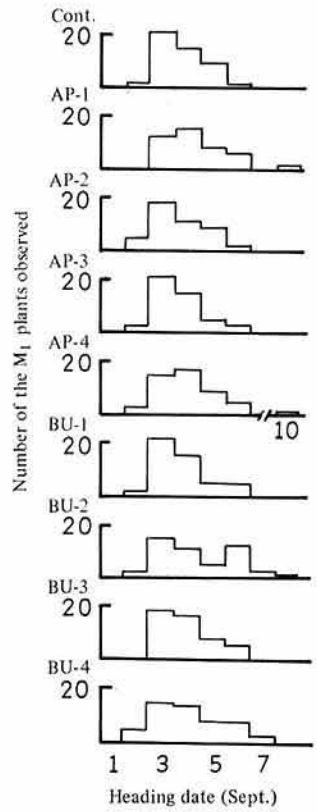

Fig. 1. Distribution of heading date in the M_1 generation for each treatment

Table 2. Seed fertility of the M_1 plants

Treatment No.	Average seed fertility (%)	Number of panicles tested
Cont.	83.8	100
AP–1	82.6	99
AP–2	81.2	92
AP–3	80.5	95
AP–4	82.9	96
BU–1	75.6	50
BU–2	78.2	50
BU–3	80.8	50
BU–4	79.1	50

of all the treatments were not better than that of the control. BU–1 treatment showed the lowest average fertility of 75.6%, which was 8.2% below that of the control. The BU treatments had a severer effect for sterility than the AP treatments.

Disturbance of heading date and decrease of the seed fertility observed in the treated population can be explained mainly by the inhibitory effect of AP and BU on DNA synthesis. Various physiological disturbances related to inhibition of the nucleic acid metabolism are also one of the main causes. Chromosomal aberration which was reported in animal cells[11] should also be taken into account. Both injurious effects on seed fertility and heading were very slight compared with the ones caused by the alkylating agents such as EI or EMS[10] and ionizing radiations in the practical doses. For example, 5 kR of gamma rays induced sterility in about 40% of M_1 rice plants.[12]

2) *Variations induced in the M_2 generation and their progeny test*

Table 3 shows the distribution of heading date of the M_2 plants in each treatment. All the frequencies of the variants induction ranging from 0.67 to 4.87% exceeded that of the control. Average variation rate of the BU treatments was 2.77% and was more than twice as much as that of the AP treatments. The AP treatments showed about the same induction frequency of variants for both directions of earliness and lateness, while the BU treatments induced early heading ones much more frequently. The earliest ones of all the variants for heading date were 7 days earlier than the average heading date of the control and the latest one was 18 days later than it. Distribution of the variants was not so widened as often shown in the M_2 population treated by ionizing radiations or alkylating agents in general.

Table 4 shows the result of the progeny test carried out in the M_3 generation. The emergence of the M_3 variants for heading date was much more frequent among the plants in the M_3 lines derived from the M_2 heading date variants than the progeny plants in the M_3 lines from the normal M_2 plants in heading date. This tendency was especially clear in the AP treatments and one of the BU treatments. Some of the M_2 plants which were classified as normal for the heading date also segregated a few number of the heading date variants in the M_3 generation. The average variant frequency of the M_3 lines derived from the M_2 normal-heading plants was 9.4%, whereas it was 28.7% among the M_3 progeny lines from the M_2 variants for heading date. The difference between the variation frequencies was highly significant (significant at 0.1% level). Segregation frequency of the variants in a M_3 line also showed a conspicuous difference between the M_3 line from M_2 variants and that from M_2 non-variants. Average segregation rate of the variants in a M_3 line

Table 3. Frequency distribution of heading date for each treatment in the M_2 generation

Treatment No.	Aug. 30	31	Sept. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	No. of M_2 plants observed
Cont.			1	1	8	26	91	144	214	102	24	4	2														647
AP-1		4	1	7	20	17	267	544	467	299	73	8	6	1	0	0	1	1									1766
AP-2			1	4	49	136	359	450	356	180	31	3	6	2	2												1566
AP-3		1	0	1	32	88	371	348	553	690	44	7	3	0	1	0	1	2	0	1	0	1					1644
AP-4	2	0	1	7	17	50	284	323	670	246	64	17	5	2	4	0	0	1	2								1692
BU-1			1	8	20	54	156	697	281	102	20	6	3	2	0	0	0	0	0	0	0	0	1	0	0	1	801
BU-2			1	3	19	47	153	180	175	127	21	6	0	0	0	3	0	1	0	0	1						729
BU-3				17	54	86	128	198	149	79	13	2	1	1	1	0	0	2									731
BU-4	1	1	7	20	54	89	104	203	160	80	10	3	1	3	2	0	0	0	1								739

Vertical lines show the confidence limits at a critical rate of 0.01.

Table 4. Variation frequencies of heading date in the M_2 generation and their progeny test

Treatment No.	M_2			M_3				
	Number of plants tested	Number of variants	Variation frequency (%)	Number of lines tested*			Number of variant lines	Variation frequency (%)
Cont.	647	4	0.62	57	N	57	3	5.3
AP-1	1766	21	1.19	106	N	92	5	5.4
					A	14	6	42.9
AP-2	1566	15	0.96	1	N	1	0	0.0
					A	0	–	–
AP-3	1644	11	0.67	101	N	99	5	5.1
					A	2	2	100.0
AP-4	1692	24	1.42	103	N	89	18	20.2
					A	14	6	42.6
BU-1	801	15	1.87	93	N	83	12	14.5
					A	10	5	50.0
BU-2	729	9	1.24	45	N	43	0	0.0
					A	2	0	0.0
BU-3	731	22	3.01	92	N	77	7	9.1
					A	15	3	20.0
BU-4	739	36	4.87	110	N	81	6	7.4
					A	29	3	10.3

* N: Lines derived from the plants of normal heading in the M_2 generation.
A: Lines derived from the variants for heading date in the M_2 generation.

Table 5. Variation frequencies of culm length in the M_2 generation and their progeny test

Treatment No.	M_2			M_3				
	Number of plants tested	Nember of variants	Variation frequency (%)	Number of lines tested*			Number of variant lines	Variation frequency (%)
Cont.	642	13	2.03	57	N	57	3	5.3
AP-1	1428	52	3.64	106	N	89	28	31.5
					A	17	9	53.0
AP-2	1342	57	4.25	1	N	1	0	0.0
					A	0	–	–
AP-3	1401	65	4.64	101	N	84	19	22.6
					A	17	4	23.5
AP-4	1424	62	4.35	103	N	85	15	17.6
					A	18	5	27.8
BU-1	699	34	4.86	93	N	81	22	27.2
					A	12	8	66.7
BU-2	680	23	3.38	45	N	44	10	22.7
					A	1	0	0.0
BU-3	703	26	3.70	92	N	89	6	6.7
					A	3	3	100.0
BU-4	704	50	4.26	110	N	106	12	11.3
					A	4	1	25.0

* N: Lines derived from the plants of normal culm length in the M_2 generation.
A: Lines derived from the variants for culm length in the M_2 generation.

from the M_2 variant was 52%, whereas that in a M_3 line from the M_2 non-variant for heading date was 8%.

Table 5 shows the variation frequencies for culm length in the M_2 generation and the result of the progeny test in the M_3 generation. In the M_2 generation, emergence of the variants ranged from 3.4 to 4.9% and all of them exceeded that of the control. In the case of culm length, contrary to the heading date, much difference was not found in variation frequencies nor the spectrum of the variation between the AP and the BU treatments. Both treatments induced the short culm variants much more frequently than the long culm ones. Average culm length of the control plants was 74 cm and the lower and upper confidence limits were 62 and 85 cm, respectively. Culm length variation of the M_2 plants ranged from 20 cm in the shortest to 90 cm in the longest.

In the progeny test, the same tendency as in heading date was also recognized for culm length, that is far more variants emerged in the M_3 lines derived from the M_2 variants than in the M_3 lines from the M_2 non-variants for culm length. The average frequency of the variation induction for culm length within the M_3 lines derived from the M_2 culm length variants was 41.7%, whereas it was 19.3% for the M_3 lines derived from the M_2 normal plants in culm length (significant at 0.1% level). Some of the M_3 lines which were derived from the M_2 culm length variants showed biassed distribution in culm length toward the longer or shorter side of the confidence limit, although all the plants in the line stayed within the confidence limits. In most of these lines, environmental effects such as boarder effects of the experimental field etc. were avoided by the detailed examination of the adjacent lines and the control lines nearby.

Chlorophyll mutants including *albina*, *viridis* and *striata* and sterile mutants were induced only in low frequencies in every treatment.

Discussion

The AP and BU treatments induced variants in agronomic characters of rice at fairly high frequencies. The M_2 variation frequencies of these chemicals calculated on the basis of the standard used in this experiment are reduced to less than a half of them when the results of the M_3 generation is taken into account. However, it can be said that the mutation rate confirmed by the M_3 progeny test is not very low in comparison with results obtained by gamma ray irradiation for these characters using the same material and method.[12] From the viewpoint of plant breeders, this point can be strengthened by the fact that there emerged a very few sterile and chlorophyll mutants in the M_2 population. Although some differences were observed, it can be said that the characteristics of mutation induction for agronomic traits were very similar in the AP and BU treatments. These results suggest that both the AP and BU treatments are likely to induce point mutations in rice by DNA transition. It seems to be a unique characteristic against ionizing radiations or alkylating agents.

It is worth noticing that the number of sterile plants among the M_2 population was much less than that in the progenies treated by the alkylating agents or ionizing radiation.[10,12] For example, 76.7% of the variants obtained in the M_2 generation after γ-ray irradiation (1 kR–30 kR) was reported to be sterile ones. This characteristic will be able to enhance the efficiency of mutation breeding. The AP and BU treatments seem to be quite promising for obtaining desired mutants in agronomic characters without accompanying any deteriorative mutation. It implies that the treatment by DNA base analogues shows a possibility of opening the door toward the direct use of mutants, which has been prevented mainly so far by the co-occurrence of both valuable and deteriorative mutations within a single plant. The limited extent of changes induced by mutagenic treatment is also favorable for the direct use of mutants in a practical breeding program. Such slight

modification of a character without any change in other characters would be especially useful in the case of the breeding program for multilines of a variety.

Taking all the results obtained in the experiments into account, it can be concluded that the DNA base analogues have unique characteristics in inducing mutations and they look highly promising in mutation breeding programs aiming at the direct use of mutants.

References

1) Bender, M. A., Bedford, J. S. & Mitchell, J. B.: Mechanisms of chromosomal aberration production. II Aberrations induced by 5-bromodeoxyuridine and visible light. *Mutation Res.*, **20**, 403–416 (1973).
2) Benzer, S. & Freese, E.: Induction of specific mutation with 5-bromouracil. *Proc. Natl. Acad. Sci. USA*, 44, 112–119 (1958).
3) Brown, J. A. M. & Smith, H. H.: Incorporation and effects of thymidine analogues in gametophytic tissue of *Arabidopsis thaliana*. *Mutation Res.*, 1, 45–53 (1964).
4) Freese, E.: The specific mutagenic effect of base analogues on phage T_4. *J. Molec. Biol.*, 1, 87–105 (1959).
5) Fukui, K.: Studies on the mutagenic effects of DNA base and amino acid analogues in higher plants. Thesis of Dr. Agr., Kyoto Univ. (1985) [In Japanese].
6) Fukui, K. et al.: Studies on the utility of artificial mutations in plant breeding XXXIII. Mutagenic effects of base analogues in rice plant. *Jpn. J. Breed.*, **23** (Suppl. 2), 52–53 (1973) [In Japanese].
7) Hirono, Y. & Smith, H.H.: Mutations induced in *Arabidopsis* by DNA nucleoside analogues. *Genetics*, **61**, 191–199 (1969).
8) Inoue, M., Mori, S. & Hori, S.: Mutagenic effect of base analogues incorporated into rice. *Jpn. J. Breed.*, **26**, 110–120 (1976).
9) Jacobs, M.: Studies on the genetic activity of thymidine-base analogue in *Arabidopsis thaliana*. *Mutation Res.*, 7, 51–62 (1969).
10) Ko, T. B.: Studies on the mutations induced for agronomic characters in rice. Thesis of Dr. Agr., Kyoto Univ. (1971) [In Japanese with English summary].
11) Yamagata, H. & Fukui, K.: Studies on the utility of artificial mutations in plant breeding XI. Mutagenic effects of BUdR in rice. *Mem. Coll. Agr. Kyoto Univ.*, No. 113, 25–37 (1979).
12) Yamagata, H. & Syakudo, K.: Studies on the utility of artificial mutations in plant breeding I. γ-ray sensitivity of rice and induced aberrant plants in the X_2 generation. *Jpn. J. Breed.*, **10**, 153–162 (1960) [In Japanese with English summary].

(Received for publication, December 19, 1984)