Effects of Sodium Azide on Seedling Injury and Chlorophyll Mutation in Rice.

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Sodium azide-induced damage in M_1 generation and mutation in M_2 were analyzed in *Japonica* rice variety. In treatments of dormant seeds, germination rate and seedling growth were reduced in proportion to both the increase of sodium azide concentration and the prolongation of treatment time. Furthermore, sodium azide delayed the seed germination. When the extent of growth reduction and of the delay of germination were plotted against the "dose" (Concentration $\times$ Soaking duration) in an entire lot of treatments, these indices each fitted into a single "dose"-response curve. The highest frequency of chlorophyll mutation was 11.1 per cent per M_1 panicle and 1.22 per cent per M_2 seedling. With regard to mutation in agronomic characters, late heading, short culm, dwarf, and sterility appeared more frequently. In presoaked seeds, chlorophyll mutation was induced more frequently in the 6, 48 and 54 hour-pres soaked seeds than 12 to 42 hour-pres soaked seeds. However, M_1 seed fertility remained almost the same level (about 90 per cent) during this presoaking period. From these results, it appears that sodium azide when applied at the DNA synthesis stage of the first cell division of germinating process, is more effective in the induction of mutation. It also appears that the mutagenic action of sodium azide can be affected by cellular metabolism during germination.

Introduction

Since NILAN *et al.* (1973) have demonstrated that sodium azide (NaN_3), known as a respiratory inhibitor, is a potent mutagen in barley, a considerable number of reports on sodium azide mutagenesis in barley have appeared (NILAN *et al.* 1973, 1976, KLEINHOFs *et al.* 1974, 1978a, CHOUDHARY and KAUL 1976, HUSSAIN *et al.* 1977, SANDER *et al.* 1978, HASEGAWA and INOUE 1980). There are some merits for the practical use in mutation breeding (NILAN *et al.* 1973). Especially, the fact that sodium azide does not induce chromosome aberration suggests that mutants induced by this agent are due to the mutations at the gene level (KLEINHOFs *et al.* 1974).

However, the lack of mutagenic capacity or the low mutagenic effect of this agent in *Saintpaulia* (WARFIELD 1974), *Streptocarpus* (CHOUDHARY 1976) and *Arabidopsis* (GICHNER and VELEMINSKY 1977) were also reported. Furthermore, the evidence that sodium azide is converted into a real mutagen in plant cells (OWAIS *et al.* 1978, VELEMINSKY *et al.* 1979) suggests that sodium azide converting potentiality is different among species or varieties. On the other hand, in rice, there are some points of difference on sodium azide mutagenesis (HARN and LEE 1975, SARMA *et al.* 1979).

In the present paper, the low mutagenic effect of sodium azide in dormant and pre-soaked seeds of *Japonica* rice is reported.

Materials and Methods

The seeds of *Japonica* rice variety, Nipponbare, were used in all the treatments. Sodium azide (Merck) was dissolved in 10^{-1} M phosphate buffer solution, adjusted at pH 3, and then diluted to each concentration just before treatment. Presoaking, sodium

azide-treatment and culture of seeds were carried out in the growth chamber controlled at $25\pm 1^\circ\text{C}$ under natural daylight. When the second leaf had fully developed, observation of chlorophyll mutation was carried out.

Treatments of Dormant Seeds

The dormant seeds were treated with 0, 10^{-4} , 10^{-3} , 5×10^{-3} , 10^{-2} , 5×10^{-2} and 10^{-1} M sodium azide for 1.5, 3, 6, 12, and 24 hours. Immediately after the treatments, they were washed with running tap-water at $20\pm 1^\circ\text{C}$ and allowed to germinate in 9 cm Petri dishes. The number of days from treatment to germination, defined by the emergence of both plumule and radicle, was measured. The seedling height of the germinating seeds was measured on the 10th day after treatment. For the analysis of seed fertility in M_1 generation and mutation frequency in M_2 generation, the seeds were treated with 0, 10^{-4} , 10^{-3} , 10^{-2} and 10^{-1} M sodium azide for 3 and 6 hours and sown in the nursery. Forty days after treatment, they were transplanted to the field and cultivated under natural conditions. At the time of maturity, three panicles of each M_1 plant were harvested as a panicle progeny and one of them was used in the measurement of M_1 seed fertility.

In the M_2 generation, chlorophyll mutations were scored using all of the harvested panicles. Thereafter, some of the seedlings were transplanted to the field. Visible and agronomic mutations appeared in M_2 generation were recorded in the field, for the materials after 0, 10^{-3} and 10^{-2} M sodium azide treatment for 3 hours.

Treatments of Germinating Seeds.

The seeds, presoaked for 0, 6, 12, 18, 24, 30, 36, 42, 48 and 54 hours, were treated with 0, 10^{-3} and 10^{-2} M sodium azide for 3 hours. Germination rate and seedling height were measured on the 7th day after treatment. M_1 seed fertility and the frequency of M_2 chlorophyll mutation were also measured in similar procedures as those with dormant seeds.

Results

Dormant Seeds Experiment

In proportion to the increase in the concentration of sodium azide and the prolongation of the period of treatment, the germination rate (Fig. 1) and seedling growth (Fig. 2) were reduced and germination were caused to be more delay (Table 1). In particular, seed germination was completely inhibited after the treatment for 12 and 24 hours at a concentration higher than 10^{-2} M. It was also seen that the degree of growth reduction and delay of seed germination in an entire lot of treatments each assigned a single "dose"-response curve (Fig. 3 and 4) as described in our previous report on barley (HASEGAWA and INOUE 1980).

Fig. 1 Germination rate on the 10th day after treatment in dormant seeds.

Table 1 Average number of days from sodium azide-treatment to germination (Days $\pm$ SD).

NaN ₃ (M)	Soaking duration (hr)				
	1.5	3	6	12	24
0	2.8 $\pm$ 0.43	2.9 $\pm$ 0.49	3.0 $\pm$ 0.40	2.9 $\pm$ 0.40	3.0 $\pm$ 0.44
10 ⁻³	2.9 $\pm$ 0.44	3.0 $\pm$ 0.47	3.0 $\pm$ 0.44	3.3 $\pm$ 0.51	3.6 $\pm$ 0.52
10 ⁻³	3.2 $\pm$ 0.53	3.6 $\pm$ 0.47	4.4 $\pm$ 0.50	6.3 $\pm$ 0.56	7.5 $\pm$ 0.99
5 $\times$ 10 ⁻³	4.2 $\pm$ 0.48	5.8 $\pm$ 0.45	7.7 $\pm$ 0.61	13.8 $\pm$ 1.92	17.3 $\pm$ 2.93*
10 ⁻²	5.5 $\pm$ 0.70	7.0 $\pm$ 0.58	10.9 $\pm$ 2.20	N. G.**	N. G.
5 $\times$ 10 ⁻²	6.2 $\pm$ 0.47	8.2 $\pm$ 0.96	14.1 $\pm$ 2.12*	N. G.	N. G.
10 ⁻²	6.7 $\pm$ 0.67	8.7 $\pm$ 1.19	15.3 $\pm$ 2.24*	N. G.	N. G.

* Germination was reduced.

** Not germinated.

M₁ seed fertility became slightly reduced with increasing concentrations of sodium azide (Table 2).

The frequency of chlorophyll mutations generally increased with an increase in the concentration and the soaking duration (Table 2), and about half of them were albina. The treatment with 10⁻¹M sodium azide for 6 hours, produced the maximum yield of mutation; 11.1 and 1.22 per cent in M₁ panicles and M₂ seedlings, respectively.

The frequency of mutations in visible and agronomic characters increased with an increase in the concentration (Table 3). Mutants, such as late heading, short culm, dwarf, short panicle and sterility, were more frequently than the other types of mutants.

Table 2 M₁ seed fertility and M₂ chlorophyll mutation frequency following sodium azide treatment in dormant seeds.

Conc. (M)	Soaking duration (hr)	No. of M ₁ panicles		No. of M ₂ seedlings		M ₁ seed fertility (%)	Mutation frequency(%)	
		tested	segregated	tested	mutated		per M ₁ panicle	per M ₂ seedling
0	3	480	0	31,989	0	95.4	0	0
10 ⁻⁴	3	471	5	33,077	23	95.4	1.0	0.06
10 ⁻³	3	315	7	23,148	35	93.6	2.2	0.15
10 ⁻²	3	219	14	13,915	84	91.2	6.3	0.60
10 ⁻¹	3	141	14	9,025	61	86.4	9.9	0.62
0	6	363	0	28,798	0	94.7	0	0
10 ⁻⁴	6	360	3	29,270	7	94.5	0.8	0.02
10 ⁻³	6	168	5	11,537	62	92.1	2.9	0.53
10 ⁻²	6	129	11	9,462	48	90.2	8.5	0.50
10 ⁻¹	6	27	3	1,719	21	88.4	11.1	1.22

Fig. 2 Seedling height on the 10th day after treatment in dormant seeds.

Fig. 3 "Dose"-response curve for seedling height on the 10th day after treatment in dormant seeds.

Fig. 4 "Dose"-response curve for the delay of seed germination.

Presoaked Seeds Experiment

Fig. 5 shows the changes in germination rate and seedling height on the 7th day after treatment of the seeds which had been pre-soaked for 0 to 54 hours. The growth-inhibiting effect of sodium azide remained almost the same during the presoaking periods for 0 to 36 hours. On the other hand, seed germination was markedly reduced in presoaked seeds. However, in seeds pre-soaked for 42 to 48 hours, no growth-inhibiting effect was observed (in seeds with 10^{-3} M sodium azide), and a higher germination rate was obtained (in seeds with 10^{-2} M sodium azide) when compared to that of seeds treated at other stages.

As seen in Fig. 6, M_1 seed fertility remained almost constant (about 90 per cent).

Table 3 Number of visible and agronomic mutants appeared in the M_2 generation.*

NaN ₃ (M)		0	10^{-3}	10^{-2}
No. of M_2 seedling tested		97	1,310	706
Mutation type	Late heading	2	104	57
	Long culm	0	3	2
	Short culm	0	25	50
	Dwarf	0	10	25
	Short panicle	1	17	35
	Sterility	0	9	61
	Lethal	0	0	1
	Uniculm	0	0	4
	Large grain	0	0	1
	Curly leaf	0	0	4
	Others	0	0	6

* The mutants for heading date, culm length and panicle length were defined as plants falling into the critical region ($\alpha=0.01$) of the control.

Fig. 5 Effects of sodium azide on germination rate and seedling height on the 7th day after treatments of germinating seeds.

Fig. 6 Changes of M₁ seed fertility and chlorophyll mutation frequency, as influenced by the stage of sodium azide treatment.

However, the treatment of the seeds presoaked over 48 hours, slightly reduced M₁ seed fertility.

The yield of chlorophyll mutation was apparently higher in the seeds pre-soaked for 6, 48 and 54 hours than that of dormant seeds or the seeds pre-soaked for 12 to 42 hours (Fig. 6). In particular the highest frequency (11.4 per cent per M₁ panicle) was obtained in the seeds presoaked for 48 hours.

Discussion

In the present experiments, sodium azide-induced damage showed two interesting characteristics: delay of seed germination and slight reduction of M₁ seed fertility. Furthermore, the degree of these indices in an entire lot of treatments each corresponded to one "dose"-response curve at least in the range of low "dose". PEARSON *et al.* (1975) have shown that sodium azide inhibits ATP production, DNA synthesis, mitotic division and seed germination, and also that growth rate of germinating seeds remains equal under any treatment. KLEINHOFs *et al.* (1978 b) have indicated that the primary and physiological effect of sodium azide was the inhibition of catalase, peroxidase and cytochrome oxidase. From these references, one explanation for the delay of germination might be considered: sodium azide induces the delay and/or the inhibition of metabolic activation necessary for seed germination. Furthermore, the fact that sodium azide has a very limited effect on the reduction in M₁ seed fertility, tends to support the view that sodium azide-induced damage and mutation are not accompanied by chromosome aberrations (SIDERIS *et al.* 1973, CHOUDHARY and KAUL 1976, NILAN *et al.* 1976, SANDER *et al.* 1978).

In respect to the mutagenic ability of sodium azide, SARMA *et al.* (1979) reported that in *Indica* rice, the maximum frequency of chlorophyll mutation was 42 and 7.89 per cent per M₁ panicle and M₂ seedling, respectively. ANDO (personal communication) demonstrated that an extremely high frequency of chlorophyll mutation (A maximum of one mutation per M₁ panicle) was obtained with a sodium azide treatment of 10⁻² M for 8 hours in 5 hour-presoaked seeds in Brazilian rice. In Korean rice, HARN and LEE (1975) showed that the highest frequency of chlorophyll mutation was only 2.2 per cent per M₁ panicle (10⁻³ M for 4 hours in dormant seeds). The present experiments exhibit that in

Japonica rice the maximum frequency of chlorophyll mutations is 9.9 and 11.4 per cent per M_1 panicle in the treatment of dormant and pre-soaked seeds, respectively. These results pose the question of whether the difference in chlorophyll mutation frequency is dependent on the treatment procedure or material used. In peanut, LEVY and ASHRI (1973) suggested that varietal difference in sodium azide-sensitivity resulted from the different metabolic process affecting the uptake of the agent. Considering both the fact that sodium azide-sensitivity is different among *Japonica* rice varieties (HASEGAWA *et al.* 1975), and that sodium azide is converted into a real mutagen in plant cells (OWAIS *et al.* 1978, VELEMINSKY *et al.* 1979), it may be conceivable that the mutagenic effect of sodium azide is different among varieties. GICHNER and VELEMINSKY (1977) showed that the lack of cellular activating components in seeds could be the reason for the low mutagenic activity of sodium azide in *Arabidopsis thaliana*. However, in addition to further investigations on the varietal difference in sodium azide mutagenicity in rice, more efficient treatment-procedure of this agent is also needed for the practical utilization in mutation breeding.

Furthermore, the fact that sodium azide treatment produced the most chlorophyll mutation in the seeds presoaked for 48 hours was also recognized. In view of the evidence by NISHIMURA and FUTSUHARA (1976), which demonstrated that in *Japonica* rice, the most active synthesis of DNA in apical cells of germinating seeds was found at 48 to 54 hours after soaking, it appears that the time of sodium azide treatment to the 48 hour-pres soaked seeds seems to correspond to the first S-phase during the germinating period. Accordingly, the most chlorophyll mutation in the seeds pre-soaked for 48 hours, is support of the view that sodium azide is the most effective when it is applied in the S-phase (NILAN *et al.* 1976, SILHANKOVA *et al.* 1979, HASEGAWA and INOUE 1980). Such mutagenic action of sodium azide is almost in agreement with the reports that the treatment with ethylene imine (NISHIMURA and FUTSUHARA 1976) and ethyl methanesulfonate (YAMAGUCHI and MATSUBAYASHI 1973). On the other hand, in *Indica* rice seeds, SARMA *et al.* (1979) indicated that the yield of chlorophyll mutation induced by sodium azide is reduced by presoaking over 12 hours. In the present experiment, there was the second highest frequency of chlorophyll mutation in 6 hour-pres soaked seeds. This result seems to be related to the increase in the rate of the absorbed mutagen into the embryo cells (KONZAK *et al.* 1965). However, further analysis of the mutagenic action of sodium azide in relation to cellular metabolism during the germination period is necessary.

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イネにおけるアジ化ナトリウムの処理障害ならびに突然変異誘起効果

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アジ化ナトリウム (NaN_3) はオオムギにおいて強力な突然変異誘起効果が確かめられているが、一方その突

突然変異誘起効果が認められない植物も知られている。本報告は水稻品種「日本晴」の休眠種子および浸漬種子(0~54 時間)に、pH 3 のリン酸緩衝液中において、アジ化ナトリウムを処理(0~ 10^{-1} M, 1.5~24 時間)し、その処理障害および突然変異誘起効果を明らかにしたものである。

休眠種子処理における幼苗期の処理障害の指標として、処理後 10 日目における発芽率、幼苗草丈を調べ、さらに発芽遅延日数についても調査した。処理障害は濃度および処理時間の増加とともに増大した。ことに、幼苗草丈および発芽遅延日数については著者らが前報(HASEGAWA and INOUE 1980)において示したアジ化ナトリウムの“dose”(濃度×処理時間)反応曲線が得られた。 M_1 種子稔性は各処理区において著しい低下は認められなかった。この結果はアジ化ナトリウムは染色体異常を生じないという報告を支持するものである。最高葉緑突然変異率は M_1 穂あたり 11.1%, M_2 個体あたり 1.22% であり、イネにおけるアジ化ナトリウムの突然変異誘起効果はオオムギにおける効果よりも低いことが明らかになった。 M_2 代における農業形質の変異もあわせて調査した結果、晩生、短稈、矮性、不稔等の変異が多く見いだされた。

浸漬種子におけるアジ化ナトリウムの効果について調査したところ、処理種子のステージの差により処理障害および突然変異誘起効果に著しい差が認められた。浸漬種子におけるアジ化ナトリウム感受性は休眠種子における感受性よりも高かったが、前浸漬 42~48 時間の種子はやや抵抗性を示すことが認められた。突然変異誘起効果については、前浸漬 6 時間および 48~54 時間の種子は他のステージの種子よりも明らかに効果が大きいことが認められた。得られた最高突然変異率は、前浸漬 48 時間・ 10^{-2} M・3 時間処理区における、 M_1 穂あたり 11.4% であった。前浸漬 48~54 時間の種子は頂端分裂組織の発芽第 1 回目の DNA 合成期に相当すると考えられるので、本実験の結果はアジ化ナトリウムが S 期にもっとも有効な突然変異原であることを示している。

なお、本実験の結果は、突然変異率および浸漬種子における突然変異率の変動の 2 点に関して、SARMA *et al.* (1979) の報告と異なっている。これらの差異のなかで、ことに突然変異率の差異はアジ化ナトリウムの突然変異誘起効果の品種間差異の存在を示唆している。