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AZIDE MUTAGENESIS IN BASMATI RICE (*ORYZA SATIVA L.*) CULTIVARS

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Pre-soaked seeds of three cultivars of indica rice (*Oryza sativa* L.) viz. Basmati 370, Basmati 198 and Basmati Pak were treated with various concentrations (1.5-3.5 mM) of sodium azide in 0.1M phosphate buffer at pH3. Significant intraspecific and intervarietal differences were found in their physiological sensitivity towards mutagenic treatments. On the basis of seed germination and reduction in seedling height, Basmati 370 appeared to be more sensitive towards the applied concentrations of mutagen compared to Basmati Pak and Basmati 198. Relatively high frequency of chlorophyll deficient mutations was observed in Basmati 198. Sodium azide concentration of 1.5 for Basmati 370 only and 1.5, 2.0 and 3.5 mM for Basmati 198 seemed to be more effective. In Basmati Pak, however, higher concentrations can also be applied. Early flowering and dwarf plants were frequently observed in the spectrum of viable mutants. These studies indicated high mutagenic potency of sodium azide in Basmati rice.

Key words: *Oryza sativa*, Sodium azide, Mutagenesis,Chlorophyll mutations, Differential response.

Introduction

Induction of mutations for the improvement of crop plants has now become a supplementary approach to plant breeding [1].

While radiation still remains a tour-de-force in mutation breeding, some chemicals have been found to be equally or more efficient and effective mutagen. Among many others sodium azide has a unique position since it does not induce chromosomal aberrations [2]. It has been reported to be a very effective and potent mutagen in barley [3-5], soybean [6], pea [7], wheat [8], sorghum [9,10] and rice [11-14].

Basmati Rice occupies a vital position in the economy of Pakistan. These varieties are generally low yielding, because of their tall stature and weak stem which leads to lodging. Keeping in view the importance of basmati rice in the national economy, it is imperative to improve the plant type while keeping its quality characters intact. A major rice improvement programme through the use of induced mutations in ongoing at our institute. Sodium azide mutagenesis in basmati rice is also a part of that programme. The present paper describes the results achieved in this direction.

Materials and Methods

Fully developed seeds of three cultivars of basmati rice (*Oryza sativa* L.) viz Basmati 370, Basmati Pak and Basmati 198, were used in this study. Basmati 370 is a pure line variety with tall stature (plant height = 185 cm) and late maturing habit, Basmati Pak (Basmati 370 x CM7-6) is also a late maturing variety with plant height of approximately 155cm, and Basmati 198 (Basmati 370 x Taichung Native-1) is a late maturing but semi dwarf variety (plant height approximately 130 cm).

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For each treatment, 560 seeds of each variety were hydrated in distilled water at room temperature (25°) for 24 hrs. Five different concentrations of sodium azide (1.5, 2.0, 2.5, 3.0 and 3.5 mM) were prepared in 0.1 M phosphate buffer (pH3). The hydrated seeds were treated for three hrs. with different concentrations of sodium azide along with control (distilled water) at room temperature. The treated seeds were washed in running tap water for one hr. Sixty seeds from each variety and treatment were separated. Ten seeds per treatment per variety were planted on moist filter paper in petri plates with three replications to record seed germination. The other 30 seeds were planted in wet blotter sandwiches [15]. The remaining 500 treated seeds were planted in the field in rows 6" apart in a randomized complete block design with three replications. At the time of flowering, immature panicles were fixed into alcohol: acetic acid (3:1) fixative to which few drops of ferric chloride were added [16]. Meiotic irregularities were recorded mainly at anaphase I and II and telophase. Field plant survival was recorded at maturity. Seed sterility was calculated from ten randomly selected M1 plants per variety per treatment according to the procedure used by Konzak *et.al.*[17]. Pollen fertility was checked by staining the pollen grain with 1% potassium iodide. At maturity, the first formed penicles of each M1 plant were harvested separately and planted in the field as M2 with three replications. Chlorophyll deficient mutations were recorded from 10 day-old seedlings. Mutation frequency and spectrum were worked out on M2 seedling basis. Mutagenic efficiency and effectiveness was calculated according to the formula used by Konzak *et.al.* [17]. Seeds from remaining panicles on M1 plants of each variety and treatment were sown in nursery beds. Thirty day-old seedlings were planted as M2 in the field in rows (row to row distance 6" and plant to plant 9") on single plant progeny

basis. Selections for early flowering (1-3 weeks earlier than parent) and dwarf types (20-30% shorter in stature compared to parents) were made at the time of heading and maturity respectively. The selected plants, irrespective of the variety, were progeny tested in the M3 generation.

Results

Seed germination: Germination in non-treated seed (control) started after third day of planting. Most of the seeds germinated within five days, and complete germination was achieved within 10 days. Germination in all three varieties was inhibited significantly after treatment with mutagen. The magnitude of inhibition was maximum in Basmati 370 where mean seed germination was only 40% on the fifth day. However the seeds kept germinating and after 10 days, 100% germination was achieved even in the seeds treated with 2.5 mM of sodium azide. However, in the remaining two doses maximum seeds germination was 60% and 45% respectively. In Basmati 198, 100% seed germination was achieved only in the seeds treated with 1.5 mM concentration. Rest of the treatments significantly reduced the germination in Basmati 198 and Basmati Pak (Table 1).

Seedling height. Seedling height in all three varieties decreased progressively and significantly with increase in mutagen concentration (Table I) Sodium azide concentration of 2.5 mM caused more than 50% reduction in seedling growth of Basmati 370. In Basmati Pak, 50% reduction was observed in seeds treated with 3.0 mM of mutagen whereas in Basmati 198 even the highest concentration of mutagen (3.5 mM) was unable to cause 50% reduction in seedling growth compared to untreated control.

Frequency and spectrum of chlorophyll deficient mutations. The frequency of chlorophyll deficient mutations in M2 seedlings increased significantly in all the varieties with an increase in mutagen concentration (Table 1). The spectrum of mutations showed a composition of *albina* (white), *xantha* (yellow) *Striata* (chlorophyll deficiency in spots), *viridis* (light green), *alboviridis* (upper portion white, lower portion green) and alboxantha (upper portion white, lower portion yellow. This composition was independent of the concentration used. *Albina* and *striata* types occurred in greater proportion compared to rest of the chlorophyll mutations. The highest mutation frequency was observed in Basmati 198 and Basmati Pak showed the lowest.

Plant survival and sterility. Highly significant effects of sodium azide concentrations were observed on seed sterility and field plant survival. Seed sterility increased progressively with increase in mutagen concentration (Table 2) while plant survival decreased. Within a treatment, the differences among the varieties were non significant, based on 50% reduction in survival, Basmati 370, Basmati 198 and Basmati Pak gave an LD50 at approximately 2.9, 1.8 and 2.3 mM sodium azide

TABLE 1. MEAN VALUE OF SEED GERMINATION AND SEEDLING HEIGHT (CM) IN THREE RICE VARIETIES FOLLOWING TREATMENT WITH DIFFERENT COCENTRATION OF SODIUM AZIDE.

Azid concen tration (mM)	Seed germination after 5 days				Shoot length (cm) after 10 days				Mean frequency of mutation in 100 M2 seedlings			
	Bas. 370	Bas 198	Bas Pak	Mean	Bas 370	Bas. 198	Bas Pak	Mean	Bas. 370	Bas. 198	Bas Pak	Mean
0(control)	77 (100)	70 (100)	93 (100)	80 a	85	45	56	62 a	1.07	2.15	0.50	1.24 a
1.5	37 (100)	67 (100)	70 (91)	58 b	69	36	51	52 b	4.39	5.99	1.59	3.99 b
2.0	36 (100)	43 (90)	57 (75)	45 c	55	35	45	45 c	5.71	6.18	1.80	4.56 b
2.5	33 (80)	26 (70)	53 (60)	37 d	41	30	34	35 d	5.24	7.83	1.98	5.01 b
3.0	30 (60)	23 (50)	50 (55)	34 de	32	28	28	29 de	7.46	8.59	2.92	6.32 c
3.5	27 (45)	23 (40)	47 (55)	32 e	31	26	24	27 e	7.72	13.47	3.29	8.16 d
Mean	40 b	42 b	52 a	33 c	40 b	5.26 b	7.36 a	2.01 c				

Figures followed by the same letters in one row and column are not significantly different at 5% level of significance according to Duncan Multipal Range Test (DMRT)

TABLE 2. SEED STERILITY AND PLANT SURVIVAL OF THREE RICE VARIETIES AS AFFECTED BY VARIOUS CONCENTRATIONS OF SODIUM AZIDE

Aazd concen- tration	Seed sterility (%)				Plant survival (%)			
	Bas 370	Bas 198	Bas Pak	Mean	Bas 370	Bas 198	Bas Pak	Man
O(control)	11	10	10	10 a	100	100	100	100 d
1.5	14	10	19	14 ab	72	65	59	65 c
2.0	23	14	27	21 b	62	46	53	54 bc
2.5	26	27	31	28 bc	54	46	48	49 b
3.0	29	34	32	32 c	49	44	42	47 ab
3.5	31	40	41	37 b	45	39	41	42 a
Mean	22 a	22 a	27 a		64a	57a	57a	

Figures followed by the same letters in one row and column are not significantly different at 5% according to DMRT.

respectively.

Mutagenic efficiency and effectiveness. Based on low seed sterility and high rate of mutations per concentration, 1.5 mM of sodium azide in Basmati 370 and 1.5, 2.0 and 3.5 mM in Basmati 198 were found to be effective and efficient Table3) whereas all the applied doses of mutagen were proved less effective and efficient in Basmati Pak as evidenced from low rate of mutation frequency and higher percentage of induced sterility.

TABLE 3. MUTAGENIC EFFICIENCY AND EFFECTIVENESS OF SODIUM AZIDE IN THREE VARIETIES OF RICE.

Variety	Concentration(mM)	Seed sterility %	Mutated seed (Msd) %	Mutagenic effectiveness(Msd/tc)	Mutagenic efficiency (Msd/S)
Basmati 370	1.5	14	5.0	1.11	0.36
	2.0	23	5.0	0.83	0.21
	2.5	26	5.0	0.66	0.19
	3.0	29	6.27	0.69	0.02
	3.5	31	7.6	0.72	0.02
Basmati 198	1.5	10	6.27	1.39	0.63
	2.0	14	6.83	1.14	0.49
	2.5	27	7.43	0.99	0.27
	3.0	34	8.27	0.91	0.24
	3.5	40	13.83	1.32	0.34
Basmati Pak	1.5	19	1.14	0.25	0.06
	2.0	27	1.14	0.19	0.04
	2.5	31	1.70	0.22	0.05
	3.0	32	2.19	0.24	0.07
	3.5	41	2.92	0.28	0.07

Effect of sodium azide on meiosis and pollen fertility. The analysis of root tip mitosis of the treated plants revealed normal mitosis except in a few cells with sticky chromosomes. However the meiosis of the treated plants did show certain abnormal chromosomal configurations at anaphase I and II up to the stage of pollen formation (Table 4). Bridges were the predominant anomaly. Unequal separation of chromosomes and micronuclei were also observed frequently. The increase in mutagen concentration resulted in the reduction of pollen fertility.

A highly positive correlation was observed between azide concentration and pollen fertility, azide concentration and seed sterility and between abnormal pollens and seed sterility (Table 4).

Frequency and spectrum of viable mutations. Early flowering and dwarf type mutants were frequently observed in all the treatments of Basmati 370 and Basmati 198. In Basmati Pak, viable mutants were only observed in the seed treated with 1.5mM of sodium azide (Table 5). Of the 400 mutant plants selected, approximately 57% were from Basmati 198, 27% from Basmati 370 and 16% from Basmati Pak. Most of the plants selected in M2 segregated for heading date and plant height in M3 generation. However 28 homozygous lines were selected on the basis of earliness and short stature. The selected plants were 30-50% shorter as compared to parent whereas early flowering selections were 10-20 days earlier. Overall field performance of the selected plants in M3 was also superior compared to parents (Table 6).

TABLE 4. CHROMOSOMAL ABNORMALITIES AT VARIOUS STAGES OF MEIOSIS AND THEIR CORRELATION WITH SEED STERILITY IN BASMATI 370 AFTER TREATMENT WITH DIFFERENT CONCENTRATIONS OF SODUM AZIDE.

Azide concentration (mM) (A)	Total PMC's Observed	Normal PMC's (%)	%PMC's with abnormal configuration			Total abnormal PMC's (%) (B)	pollen fertility (%) (C)	Seed sterility (%) (D)
			Bridges	Un-equal distribution	Micro-nuclei			
0(control)	100	99.01	0.37	–	0.62	0.99a	96.00f	11.00a
1.5	80	90.95	2.02	4.03	3.00	9.05b	80.00e	14.00a
2.0	90	85.8	4.80	6.97	2.43	14.2c	69.00d	23.00b
2.5	120	83.21	5.26	8.81	2.72	16.79c	57.00c	26.00b
3.0	78	79.26	7.23	10.51	3.00	20.74cd	50.00b	29.00bc
3.5	85	75.25	9.00	11.00	4.75	24.75d	43.00a	31.00c

	B	C	D
	**	**	**
A	0.996	-0.989	0.998
		**	**
B		-0.997	0.999
			**
C			-0.996

**Highly significant at 5% level of significance.

Figures followed by the same letters in one column are not significantly different at 5% level of significance according to DMRT.

TABLE 5. FREQUENCY AND SPECTRUM OF VIABLE MUTANTS IN THREE RICE VARIETIES FOLLOWING SODIUM AZIDE TREATMENT

Variety	Azid concentration	Total M2 analysed	Total plants selected	% of total plants	
				Early lowering	dwarf
Basmati 370	1.5	336	31 (9.22)	(84)	(16)
	2.0	336	29 (8.63)	(79)	(21)
	2.5	252	26 (10.31)	(81)	(19)
	3.0	252	14 (5.50)	(79)	(21)
	3.5	336	13 (3.86)	(100)	(0)
Basmati 198	1.5	336	72 (21.42)	(90)	(10)
	2.0	280	62 (22.14)	(81)	(11)
	2.5	280	25 (8.92)	(68)	(32)
	3.0	336	19 (6.78)	(68)	(32)
	3.5	336	53 (15.77)	(70)	(30)
Basmati Pak	1.5	336	64 (19.0)	(69)	(31)
	2.0	336	-	-	-
	2.5	336	-	-	-
	3.0	336	-	-	-
	3.5	252	-	-	-

Figures in parentheses are % of total plants analysed.

Discussion

The knowledge of mutagenic sensitivity of crop plants is of great importance in mutation breeding studies. Different species and even varieties of the same species may have different mutagenic requirements as has been reported for pea [18], peanut [19], rice [20-22], wheat [8] and barley [3-5]. Sodium azide mutagenecity has been tested previously in Indica [11] and Japonica rice cultivars [12]. Azide concentrations used in both these studies were lower compared to the concentrations used in the present study. The highest concentration (1.75 mM) used to treat Japonica rice cultivar "M5" resulted in approximately 35% reduction of seedling height, 62% induction of sterility and 14% of chlorophyll deficient mutations [12]. However the highest concentration (2.0 mM) used for Indica rice cultivar "China 45" resulted in approximately 30% reduction of seedling height, 35% induction of sterility and approximately 0.3% of chlorophyll deficient mutations [11]. In the present study, 2.0 mM concentration caused 35% reduction of seedling height (Basmati 370), 27% induction of sterility (Basmati Pak), and approximately 6% induction of chlorophyll deficient mutations (Basmati 198). Despite the fact that the difference between the two concentrations (1.75 and 2.0 mM) was negligible, the induced sterility and the number of chlorophyll mutations were significantly lower in Indica compared to Japonica rice. Since the induction of chlorophyll mutations is directly related to the efficiency of a mutagen [17], it was logical to infer from these results that for the induction of mutations in Indica rice, comparatively higher concentrations are required. Contrary to this, the results reported recently by Reddi and Rao [14] indicated that Indica rice cultivars are more sensitive towards azide mutagenesis compared to Japonica rice cultivars. These studies were conducted on two indica varieties 'Jaya' and IET 5656 and one Japonica cultivar 'Fujiminori' treated with four different concentrations (1,2,4 and 5 mM) of sodium azide for 4 hrs. at pH3. Although the authors did not mention plant survival and extent of sterility caused by these concentrations, it was interesting to note that all three cultivars yielded different number of chlorophyll mutations. In the present study the maximum chlorophyll mutation frequency (app. 14%) was observed in Basmati 198 after treatment with 3.5 mM sodium azide. The same concentration yielded approximately 8% and 3% chlorophyll mutations in Basmati 370 and Basmati Pak respectively. The response of the three cultivars was also different for other parameters.

Sodium azide perse is not mutagenic [23] however, it converts into an active mutagenic metabolite [24-25]. The differential behaviour of the three varieties could be due to the cultivar dependent differential metabolic rate which affects the production of active mutagen in the cell. Basmati 370 seems to have high metabolic rate as indicated through the efficiency of 1.5 mM concentration and the frequency of viable mutants. Conversely, Basmati Pak may have either low metabolic rate or very efficient. DNA repair or azide metabolite degrading system as evidenced from the inefficiency of even

TABLE 6. FIELD PERFORMANCE OF NON SEGREGATING MUTANTS OF BASMATI 370 IN M3 GENERATION (MEAN OF 10 PLANTS).

Azide Concentration	No.of selections	Performance in M2	Plant height (cm)		Tillers/Plant		Plant weight (g)		100 graom weight(g)	
			Meam	Range	Meam	Range	Meam	Range	Meam	Range
Control	-	-	184	140-190	12	8-15	14	10-17	2.00	1-2.5-0.00
1.5nM	5	8 daya earlier	125	115-125	13	10-18	15	10-19	2.18	2.01-2.1
2.0mM	5	8 daya earlier	110	100-119	9	8-13	6	2-10	1.58	1.5-1.8
2.5mM	7	13 8 daya earlier	116	110-123	12	7-23	13	7-36	1.97	1.6-2.1
3.0mM	3	25 8 daya earlier	113	98-109	10	6-22	9	3-18	2.21	2.0-2.5
1.5mM	3	Dwarf	108	100-120	14	7-24	21	9-46	1.90	1.8-2.0
2.0mM	1	Dwarf	79	69-88	22	8-37	8	3-10	1.90	1.8-2.1
2.5mM	4	Dwarf	106	103-109	16	9-24	17	8-26	1.90	1.9-2.0

Standard error range is 0.5-5%.

the highest applied dose. The observed chromosomal abnormalities and sterility may also be the outcome of altered physiology of the cell as a consequence of differential production and activity of O- acetyleserine-sulphydrylase which produces the active mutagen.

Sodium azide is a relatively new mutagen. It is easier to use and safer to handle, yet it has been tested only in a few crops and the present study is a first detailed study conducted on Indica rice particularly on basmati varieties. The results indicated significant intraspecific and intervarietal differences for the concentration used. It is, therefore, imperative to standardize the treatment conditions for each and every cultivar prior to mutation induction.

The selected mutants are now at fairly advanced stages. The stability and performance of these mutants is better compared to those selected through irradiation. Hence sodium azide can be effectively utilized in mutation breeding programmes of rice particularly at the places where radiation facilities are not available.

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