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Azide Induced Chlorophyll Mutants in Grain *Sorghum* Varieties

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Summary. Mutagenic action of sodium azide was investigated on two grain *Sorghum* varieties BD 569 and IS 2339. 16 h presoaking and 0.001 molar concentration were found to be most effective in inducing chlorophyll mutation spectrum at pH 3. Maximum frequency of M_1 segregating panicles and M_2 chlorophyll deficient seedlings were observed in IS 2339. Varietal differences are evident in their response to the mutagenic action of the chemical.

Key words: Sodium azide – Mutagenicity – Presoaking period – Chlorophyll mutants – Varietal difference

Introduction

Induction of useful mutations in grain *Sorghum* has gained wide interest recently due to an increasing awareness of the potential of this method for creating genetic variability. Various attempts have been made to determine the most effective and efficient mutagens and treatments for the induction of desirable traits in a crop cultivar. As a result, an extensive array of highly effective mutagenic agents are now available.

Azide, a well known respiration, catalase and peroxidase inhibitor is shown to be a potent chemical mutagen in both higher and lower organisms (Nilan et al. 1973). Sideris et al. (1969 and 1973) established that the key to the high mutagenic action of azide lies in the hydrogen ion concentration of the treatment solution. A high frequency mutations was reported in barley by Nilan et al. (1973) Konzak et al. (1975) and Choudhary and Kaul (1976). The reports by Sarma et al. (1979) and Hasegawa and Inoue (1980) clearly show the high mutational yields in rice varieties investigated. The high potency of this environmental mutagen therefore seems to offer great promise in mutation breeding programmes.

In the present study an attempt has been made to demonstrate the mutagenicity of sodium azide on grain *Sorghum* varieties with special reference to the effect of concentrations and presoaking time on the frequency of inducing chlorophyll mutations.

Materials and Methods

Dry, well filled seeds of grain *Sorghum* varieties, BD 569 and IS 2339 with 14% moisture content, presoaked for 0, 8 and 16 h at 25± 2 °C were treated with 0.0005, 0.001, 0.002 and 0.004 molar solutions of sodium azide at pH 3. The treatments were carried out for 4 h and at the end of the treatment, the seeds were washed in running water for 30 min. Afterwards, the seeds were sown in the plots along with untreated controls. Panicles of M_1 plants were harvested at random at maturity and sown in the seed beds to raise M_2 generation. The frequencies of chlorophyll mutants per M_1 panicle and 100 M_2 seedlings were recorded.

Results and Discussion

The percentage of M_1 panicles segregating for chlorophyll mutants tends to increase with increase in presoaking period in both the varieties (Table 1). The same trend is observed with respect to M_2 mutant seedlings as well. If the frequency of chlorophyll mutations induced dose-wise are considered, in BD 569, 0.001 M concentration appears to be effective in all the presoaking periods with a few exceptions. With the exception of 0.004 M solution in 0 h presoaking, 0.001 M azide in IS 2339 also induced a higher frequency of chlorophyll mutants (segregating panicles). The spectrum include albina, xantha, viridis and other categories like alboviridis, striata and zebra. The percentage of albinos was greater (70.18%) in BD 569 followed by xantha, other categories and viridis. On the other hand, in IS 2339 viridis mutants were more abundant (40.04%) followed by other categories, albina and xantha.

In the presoaked entries considerable increase in mutation frequency was observed. Hydration of seeds

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Table 1. Dose-wise frequency of chlorophyll mutants induced by sodium azide in BD 569 and IS 2339 (M_1 panicle and M_2 seedling basis)

Presoaking period (h)	Concentration of the mutagen (M)	BD 569						IS 2339					
		M_1 panicles			M_2 seedlings			M_1 panicles			M_2 seedlings		
		No. studied	No. segregating	% segregating	No. studied	No. segregating	% segregating	No. studied	No. segregating	% segregating	No. studied	No. segregating	% segregating
0	Control	5	–	–	246	–	–	5	–	–	210	–	–
	0.0005	12	–	–	1,517	–	–	28	4	14.28	6,420	20	0.31
	0.001	10	2	20.00	818	2	0.24	21	4	19.04	3,946	40	1.01
	0.002	10	–	–	1,024	–	–	27	5	18.51	5,805	35	0.60
	0.004	7	–	–	1,195	–	–	25	9	36.00	6,528	35	0.53
8	Control	4	–	–	249	–	–	5	–	–	375	–	–
	0.0005	20	2	10.00	2,466	5	0.20	22	2	9.09	3,828	4	0.10
	0.001	12	3	25.00	1,480	15	1.01	18	6	33.33	3,243	32	0.98
	0.002	11	–	–	1,392	–	–	13	3	23.07	2,206	49	2.22
	0.004	4	–	–	634	–	–	14	4	28.57	3,443	44	1.27
16	Control	5	–	–	330	–	–	6	–	–	168	–	–
	0.0005	1	1	100.00	217	27	12.44	21	6	28.57	3,945	61	1.54
	0.001	12	3	25.00	1,609	7	0.43	16	6	37.50	2178	65	2.98
	0.002	10	–	–	1,163	–	–	27	10	37.03	4,432	76	1.71
	0.004	2	1	50.00	371	1	0.26	36	13	36.11	4,923	76	1.54

through soaking in water is known to facilitate rapid uptake of the mutagen thereby increasing the sensitivity. Enhanced sensitivity due to presoaking has been attributed to various factors such as changes in the metabolic activity of the cells and initiation and progress of DNA synthesis in the growing embryo (Natarajan and Shivasankar 1965). Though the presoaking periods studied were limited, it appears that, with azide treatment, no intermittent peaks in mutation frequency were evident suggesting that the mechanism of azide mutagenesis is likely to be different from that of alkylating agents and radiations. The 0.001 M concentration seems to be more effective. The same concentration is also found to be effective in inducing maximum chlorophyll mutations in barley (Nilan et al. 1973) and rice (Sarma et al. 1979) at pH 3. The greater efficiency of azide in the acid form is probably due to better penetration of the cell membrane by the uncharged HN_3 molecule. The induction in the present study of a maximum frequency of chlorophyll mutants at 16 h presoaking in grain *Sorghum* is in conformity with the results of Nilan et al. (1975) who observed maximum frequency of mutations induced in seeds presoaked for 8 and 16 h. However, in rice 4–12 h and 48 h presoaking were proved to be effective by Sarma et al. (1979) and Hasegawa and Inoue (1980) respectively. Varietal differences are seen not only in the induction of chlorophyll mutations but also in the spectrum of chlorophyll mutants induced. In peanut, Levy and Ashri (1973) suggested that varietal difference in sodium azide sensitivity resulted from different metabolic process effecting the uptake of the mutagen. Variety IS 2339 is clearly more sensitive when compared to BD 569. In addition to further investigations on the varietal difference in sodium azide mutagenicity in grain sorghums, more efficient treatment procedure of this agent is also needed for practical utilization in mutation breeding. The effect of presoaking on azide mutagenesis appears to be complex and can be explained only if the mechanism of action of azide is known.

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