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Seed germination response of rice (*Oryza sativa* L.) variety swarna treated with sodium azide

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Abstract
Sodium azide (NaN_3) is a chemical mutagen, and widely used in crops to improve their yield and quality traits. We studied the effect of various concentrations of NaN_3 ranged (0.01%, 0.02%, 0.03%, 0.04%, 0.05%) on germination and seedling growth of rice. Control was distilled water at pH7. Viable grains were pre-treated in sodium azide solution (pH3) for 6 hrs. Germination was recorded from second day to fourteenth day after initiation (DAI). The differences were recorded in germination percentage, plumule length, radicle length and dry weight of sprouting grain. Germination at 2DAI is fastest in the control experiment than in the NaN_3 treated rice variety. There were no germination in 0.05% NaN3 at fifth DAI. Germination% declined as NaN3 conc. increases. The 50% germination was observed in 0.03% NaN_3 at 14DAI. There was no radicle formation at 2DAI in the seed that were pre treated with 0.04% and 0.05% NaN3. Plumule length was lowest in 0.05% treated rice, starting at 5th DAI. Radicle and plumule were shorter as NaN3 treatment increases.

Keywords: rice, sodium azide, gemination, lethal dose (LD_{50})

Introduction
Rice is the most important cereal crop and it is a staple food for millions of people in the world (Chakravarti *et al*., 2012; Davla *et al.*, 2013) [1, 2]. Mutations are the primary source of all genetic variations existing in any organism, including plants. The resulting variation provides the raw material for natural selection and is also a driving force in evolution. Mutagenesis is the process whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents. (Roychowdhury *et al* 2013) [3]. Mutation breeding involves the development of new varieties by generating and utilizing genetic variability through chemical and physical mutagenesis.

Chemical mutagens are ideal for inducing dominant mutant alleles, while physical mutagens are ideal for recessive mutations. The effect of chemical mutagens on plant materials is generally considered milder. An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. The main advantage of mutational breeding is the possibility of improving one or two characters without changing the rest of the genotype. Chemical mutagen generally produce induced mutations which lead to base pair substitutions, especially GC-AT resulting in amino acid changes, which change the function of proteins but do not abolish their functions as deletions or frame shift mutations mostly do.

Over 80% of the registered new mutant plant varieties reported in the International Atomic Energy Association (IAEA) database (IAEA 2015) obtained via chemical mutagenesis were induced by alkylating agents. Of these, three compounds are significant: ethyl methane sulphonate (EMS), 1-methyl-1-nitrosourea and 1-ethyl-1- nitrosourea, which account for 64% of these varieties.

Sodium azide (NaN3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. It has been reported that sodium azide affects plant physiology and decrease cyanide resistant respiration in tobacco callus (Wen and Liang, 1995) [5]. The mutagenicity is mediated through the production of an organic metabolite of azide compound (Owais and Kleinhofs, 1988) [6]. This metabolite enters into the nucleus, interacts to DNA, and creates point mutation in the genome. In order to understand its mutagenic mechanism, many studies in barley and bacteria have been performed in recent years (Kleinhofs *et al.*, 1978; Gichner and Veleminsky, 1977) [7, 8]. Being a strong mutagen in plant, it affects the different parts of the plants and their growth developmental phenomena by disturbing the metabolic activities.

Materials and Methods

Chemical mutagen sodium azide was used as a mutagen in the experiment. The 0.01%, 0.02%, 0.03%, 0.04%, 0.05% NaN_3 solution were prepared and pH adjusted with ortho-phosphoric acid.

Grain treatments

Uniform grains, free from insect attack were selected by hand picking and transferred into labeled petri dishes. Grain were pre soaked in distilled water (pH 7) for 14 hours and then transferred to NaN_3 solution (pH 3) for 6 hours, with continuous stirring. At the end of the exposure to NaN_3 treatment, grains were rinsed in water.

Experimental procedure for lab study

The experiment was conducted in petri dish with NaN_3 treated grains. Recorded the germination% and vegetative parameters every days.

Before seeding blotting paper was put on the bottom and rinsed with distilled water. Seeds were put on blotting paper and desired% of NaN_3 solution was added and tried to maintain the NaN_3 solution in each perti dish. Petri dishes were kept at room temperature and after germination different parameters were recorded.

The observations were recorded on the parameters such as germination%, plumule and radical length, fresh weight and dry weight.

Parcentage germination

Fifty seeds were germinated in 9 cm sterile petri dishes lined with one sterile Whatman No1 sterile filter paper with 5ml of distilled water (pH 7). Number of grains from which radicles emerged were counted daily up until 14 DAI. Germination test were conducted under condition of 12h light/dark cycle at 25 °C. about 10 drops of distilled water were added into petri dish every day to maintain moist condition to support germination. Percentage germination were calculated by

$$\text{Germination\%} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds}} \times 100$$

Vegetative parameters

The length of plumules and radicles of four selected germinating grains were measured daily on up till 6 DAI with measuring tape. One hundred grains of sprouted grains were first weighted fresh then the grains were dried in hot air oven at 60 °C for 72 h and the dry weight were taken.

Experimental design and statistics

The experimental design was completely randomized design with four replicates. Mean and standard error were calculated from the data optained. Data were analyzed following two way analysis of variance using GENSTAT (8^{TH} edition) statistical software package. Where significant F value were obtained, differences between means were separated using Student Newman Keuls test (Alika 2006) [9].

Results and Discussion

Table 1: Shows the germination% of the rice variety Swarna at different conc. of Sodium Azide

Day	Control%	0.00%	0.01%	0.02%	0.03%	0.04%	0.05%
2nd	50.2	52.5	40.2	10.3	0.00	0.00	0.00
3rd	95.6	95.8	51.7	18.4	0.00	0.00	0.00
4th	98.8	99.1	60.8	24.8	1.2	0.00	0.00
5th	99.4	99.8	71.5	28.6	5.2	2.2	0.00
6th	99.4	99.8	72.1	32.4	10.4	2.4	1.4
7th	99.4	99.8	75.4	36.8	15.8	3.5	1.4
8th	99.4	99.8	75.4	42.2	21.6	10.3	1.8
9th	99.4	99.8	75.4	45.6	25.6	10.8	2.6
10th	99.4	99.8	75.4	49.5	34.7	12.4	5.3
11th	99.4	99.8	75.4	53.4	41.6	12.8	5.4
12th	99.4	99.8	75.4	57.2	47.8	18.3	8.5
13th	99.4	99.8	75.4	62.2	49.6	18.3	8.5
	Period		Conc.			PxC	
CD 5%	1.31		1.00			3.48	
SEm ±	0.47		0.36			1.25	
CV	5.31						

Germination at 2DAI is fastest in the control experiment than in the NaN_3 treated rice variety. Germination% declined as NaN_3 conc. increases. Germination was slowest in 0.05% treated rice, starting at 6th DAI. Germination% is highest in control 99.4%. After 14 DAI, germination percentage of seeds treated with 0.01, 0.02, 0.03, 0.04, and 0.05% sodium azide was 75.4%, 62.2%, 49.6%, 18.3% and 8.5% respectively. At 0.03% of NaN_3 conc. 50% seeds were germinated.

Table 2: Depicts daily change in length of radicle (mm) of the rice variety Swarna at different conc. of Sodium Azide

Radicle length							
Day	Control	0.00%	0.01%	0.02%	0.03%	0.04%	0.05%
2nd	3.75	4.45	.57	.23	.15	.00	.00
3rd	24.50	21.50	8.25	3.45	.78	.12	.00
4th	37.47	36.00	15.45	8.50	1.00	.28	.10
5th	57.12	58.75	25.50	19.25	2.95	.75	.30
6th	67.25	67.00	36.00	27.00	7.50	1.8	.30
	Period		Conc.		PxC		
CD 5%	0.58		0.69		1.54		
SEm ±	0.21		0.25		0.55		
CV	7.14						

At 2DAI the control had radicle length of 4.45mm. There was no radicle formation at 2 and 3 DAI in the seeds that were pre treated with 0.04% and 0.05% NaN3. Radicles were shorter as NaN_3 treatment increases. The radicle length in control, 0.01, 0.02, 0.03, 0.04 and 0.05% NaN3 at 6DAI were 67.00, 36.00, 27.00, 7.50, 1.8 and 30mm respectively.

Table 3: Shows the daily change in length of plumule (mm) of the rice variety Swarna at different conc. of Sodium Azide

Plumule length							
Day	**Control**	**0.00%**	**0.01%**	**0.02%**	**0.03%**	**0.04%**	**0.05%**
2nd	2.75	3.00	.85	.00	.00	.00	.00
3rd	5.25	5.50	2.50	.00	.00	.00	.00
4th	20.00	15.00	7.25	2.00	.00	.00	.00
5th	28.25	27.00	7.00	4.25	2.25	1.00	.00
6th	35.25	40.00	28.00	18.00	8.50	2.00	.20
	Period		Conc.		PxC		
CD5%	0.30		0.35		0.79		
SEm ±	0.11		0.13		0.28		
CV	7.41						

At 2DAI the control had plumule length of 3.00mm. There was no plumule formation at 2DAI in the seed that were pre treated with 0.02, 0.03, 0.04 and 0.05% NaN3. Generally, high conc. of NaN_3 in solution were reduce plumule length. Plumule length were shortest in 0.05% treated rice, starting at 5th DAI. The plumule length in control, 0.01, 0.02, 0.03, 0.04 and 0.05% NaN3 at 6DAI were 67.00, 36.00, 27.00, 7.50, 1.8 and 30mm respectively.

Table 4: Shows the fresh weight (g) and dry weight (g) of rice variety swarna at different conc. of sodium azide:

NaN3 treatment level	Fresh weight (g)	Dry weight (g)
Control	5.25	2.50
0.00%	5.65	2.30
0.01%	4.50	2.50
0.02%	4.18	2.45
0.03%	4.35	2.50
0.04%	4.25	2.46
0.05%	4.18	2.35
CD 5%	0.265	0.135
SEm ±	0.090	0.046
CV	3.902	3.753

Fresh weight of sprouting grain ranged from 4.18g in 0.05% NaN_3 to 5.25g in untreated. The fresh weight and dry weight in 0.03% NaN_3 were 4.35 and 2.50 respectively. The dry weight ranged from 2.50 to 2.35.

Discussion

Percentage germination was delayed significantly as sodium azide (NaN_3) concentration increased (Table-1). Cheng and Gao (1983) treated barley seed and found significant decrease in percentage germination. Ujomonigho, E. *et al* (2012) [14] found significant decrease in germination response of five rice varieties treated with NaN_3. Khan et al (2004, 2005) [11, 12] also reported decreases in germination in chick pea and Mung bean.

The length of plumule and radicle were decrease as sodium azide concentration increased (Table-2 and 3). Lal et al (2009) previously reported marked decrease in seedling height at high concentration of mutagen. Singh and Yadav (1987) [15] also established that reduction in seedling height correlated with increased concentration of mutagen.

Dry weight of sprouting grain were significantly higher in sodium azide treated plants than their control (Table-4). Seed germination and seedling emergence have been described as the beginning of the life cycle of plants and is critical for the establishment of plant population (Khan and Gulzar 2003) [16]. After overcoming the initial sodium azide inhibition to germination, sodium azide treated plants were observed to have accumulated to more biomass than untreated plants, thereby improving their chances of survival and establishment.

Conclusion

This study reveals that sodium azide is a potent chemical mutagen in the rice varieties used for the experiment which correlates its effect on other plants also studied by researchers. It has also provided baseline information on sodium azide lethal dose for the varieties studied. The research can therefore proceed to study its effects on yield parameters and also identify and select positive mutations for further breeding experiments.

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