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Effects of sodium azide on seed germination and seedling growth in Kalanamak rice

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Abstract

The aim of this study was to identify the effect of different percentages of sodium azide mutagen on the seed germination, plumule and radicle growth so that most effective sodium azide dose can be applied for successful mutagenesis research study like TILLING. For this purpose seed of Kalanamak rice cultivar were treated with 0.01%, 0.02%, 0.03%, 0.04%, 0.05% sodium azide doses. Seed germination, embryonic root length, seedling length, seedling growth rate, fresh and dry weights of root and shoot, and root to shoot ratio were evaluated in comparison to the untreated materials. Mostly, there were statistically significant differences among sodium azide treatments for all the evaluated characters. The levels of differences were more pronounced with the increased doses of sodium azide. Thus, the selected characters evaluated in this study are relevant for the assessment of effective and optimum sodium azide treatments. In the present study, results show that a higher dose of sodium azide (0.05%) significantly reduced all the recorded parameters. The maximum value of germination (96.6%). Higher radical length (26.0mm), plumule length (51.67 mm), fresh weight of plumule (39.6 g), plumule dry weight (8.6 g), radical fresh weight (14.7 g) and dry weight of radicle (4.4) were recorded in control and minimum value of these parameters at higher dose of sodium azide (0.05%).

Keywords: Rice, germination, plumule and radicle

Introduction

Rice is a nutritional staple food which provides instant energy as its most important components is carbohydrate (starch). The two major rice varieties grown worldwide today are *Oryza sativa indica* and *Oryza sativa japonica*. Rice plant is a member of Poaceae (old Gramineae) family. Origin of rice *Oryza sativa* it is believed to be associated with wet, humid climates, though it is not a tropical plant. It is probably a descendent of wild grass that was most likely cultivated in the foothills of the far eastern Himalayas. Another school of thought believes that the rice may have originated in South India, then spread to the north of the country, and then onwards to China. It then arrived in Korea, the Philippines (about 2000 B. C.) and then Japan and Indonesia (about 1000 B. C.) (<https://farmer.gov.in>).

Rice production has increasingly been exposed to the losses caused by abiotic stress like drought, flooding, salinity, heat and cold in most of the cultivated rice ecosystem. The frequency intensity and duration of these stresses is expected to rise due to the progressive effects of climate change. (Singh *et al.*, 2016) ^[12].

Mutation are the primary source of all genetic variations in any organism like plants. The resulting variation provides the raw material for natural selection and is also a driving force in evolution. Mutation is the process therefore sudden heritable changes occurs in the genetic information of an organism not caused by genetic segregation or genetic recombination, but also cause by chemical, physical or biological agents (Roy Chowdhury *et al.*, 2013).

In nature, mutations are caused by errors in the replication of deoxyribonucleic acid (DNA). This hereditary material could also be changed due to exposure to surroundings natural radiations. A resulting modified individual is then known as a spontaneous mutant. By means of inducing genetic variations, mutations have been used successfully in several crops for breeding of agronomically important traits, Conventional mutation techniques have mostly been used to improve yield, quality, and disease and pest resistance in crops. Many mutant varieties involving of more than 100 plant species have been officially released. The accurate selection techniques determines the success of induced variation and obtaining desired characteristics in breeding. One way to induce mutation is through the use of chemical mutagen. One way to induce mutation is through the use of chemical mutagens. Sodium azide (NaN_3) a chemical mutagens that act as alkylating agents and consider as the most powerful

mutagens in plants. Their applications on plant are easy, inexpensive and create mutation to improve their traits. The efficiency of mutant production depends on many conditions such as pH, soaking into water, temperature, concentration and treatment duration. They create point mutation, damage the chromosomes and thus produce tolerance in plant for numerous conditions. Thus, prior to the large scale generation of variants initial studies on induced mutations are usually conducted for finding optimum combination of these parameters together with the optimum dose to elicit the best response.

The aim of this research was to study the effect of sodium azide on the growth of Kalanamak rice variety to produce genetic variation on the vegetative growth. The objective of this study is to determine the effects of sodium azide doses on the germination of Kalanamak rice varieties seeds and the growth of seedling and roots, so that the most effective of conditions can be determined in these preliminary experiments and later can be applied for successful mutagenesis in basic research studies such as knock-out of genes for confirming particular functions.

Materials and Methods

Plant material

The experiment were conducted in greenhouse and laboratory Department of Plant Molecular Biology and Genetic Engineering of the Acharya Narendra Deva University of Agriculture and Technology Kumarganj Ayodhya. The rice variety Kalanamak has been used as the plant material in this study. The seeds were induced in sodium azide mutagenesis and different percentages of sodium azide doses (0.10%, 0.20%, 0.30%, 0.04%, 0.05%) and a control dose (0% sodium azide) were applied. First of all, 25 seeds for each technical replicates were presoaked in 15 mL (0.6 mL/seed) for 8 h in 0.05 M phosphate buffer, pH 8.0 for 16 h, at 25 °C by 100 rpm constant shaking. Treated seeds were rinsed under running tap water for 1 min to remove excess sodium azide solution from seed surfaces and transferred onto the Petri dishes containing water soaked filter paper and let grown in growth chamber at 25°C as triplicates of 25 seeds of each dose treatment. Following the next day of the treatments, the seeds were continuously assessed for the germination and developmental stages daily.

Result and Discussion

Results presented in Table 1. Showed that germination

significantly effected with increasing level of sodium azide and recorded highest germination % in control 96.6% while after 7 DAI, it was show lowest germination percentage. The germination of seeds treated with 0.01, 0.02, 0.03, 0.04, 0.05% sodium azide was 96.60%, 93.30%, 90.00%, 66.00%, 43.30% respectively. The reduction in seed germination in mutagenic treatments has been explained due to the delay or inhibition of physiological and biological processes necessary for seed germination including enzyme activity (Kurobane *et al.*, 1979). Cheng and Gao (1983) ^[1, 8] treated barley seed and found significant decrease in percentage germination. Ujomonigho, E. *et al* (2012) ^[13] found significant decrease in germination response of five rice varieties treated with NaN₃. Khan *et al.*, (2004, 2005) ^[4, 5] also reported decrease in germination in chick pea and mung bean.

The length of plumule and radicle were decrease as sodium azide concentration increased. The maximum radicle length was observed under control (28.33 mm) whereas shortest length was found at higher dose of sodium azide. The value of radicle length in control, 0.01, 0.02, 0.03, 0.04, 0.05% NaN₃ at 7DAI were 26.00, 28.33, 24.00, 8.67, 1.33, 1.00mm respectively. Similarly Plumule length 0.05% NaN₃ at 7DAI were 51.67, 49.33, 35.67, 22.00, 8.00, 1.00mm under in control, 0.01, 0.02, 0.03, 0.04, respectively. Lal *et al.*, (2009) ^[9] previously reported marked decrease in seedling height at high concentration of mutagen. Singh and Yadav (1987) ^[11] also established that reduction in seedling height correlated with increased concentration of mutagen.

Fresh and dry weight of sprouted grain were significantly higher in sodium azide treated plants than their control. Fresh weight of sprouting grain ranged from 5.8g in 0.05% NaN₃ to 39.65.9g in untreated. Fresh weight and dry weight in 0.03% NaN₃ were 17.4 and 2.0 respectively. The dry weight ranged from 6.9 to 1.1.

Fresh weight of sprouting radicle ranged from 2.0 in 0.05% NaN₃ to 14.7g in untreated. Fresh weight and dry weight in 0.03% were 2.3 and 0.6 respectively. The dry weight ranged from 4.4 to 0.23. 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) ^[3]. After overcoming the initial sodium azide inhibition to germination, sodium azide treated plants were observed to have more biomass than untreated plants, thereby improving their chances of survival and establishment.

Table 1: Effect of sodium azide concentration on different growth parameters of Kalanamak rice

NaN ₃ treatment	Germination %	Length of radicle (mm)	Length of plumule (mm)	Plumule fresh weight (g)	Plumule Dry weight (g)	Radicle fresh weight (g)	Radicle dry weight (g)
Control	96.60	28.33	51.67	39.6	8.6	14.7	4.4
0.01%	96.60	26.00	49.33	33.9	6.9	6.0	3.2
0.02%	93.30	24.00	35.67	32.3	5.0	5.1	1.6
0.03%	90.00	8.67	22.00	17.4	2.0	2.3	0.6
0.04%	66.00	1.33	8.00	12.5	2.0	2.8	0.5
0.05%	43.30	1.00	1.00	5.8	1.1	2.0	0.4
CD 5%	3.9	2.9	1.25	0.493	0.513	2.3	0.23
SEm±	1.85	1.04	3.71	0.809	0.167	0.778	0.075
CV	7.05	7.5	11.5	5.9	6.7	10.2	7.2

Conclusion

It was concluded that different doses of sodium azide

influenced the performances of Kalanamak rice. Very low doses of sodium azide 0.01-0.05% might be used to study the

improvement of Kalanamak rice diversity. NaN_3 significantly decreasing capacity of seed germination $>0.05\%$. The surviving seedlings are possible for further investigation.

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