

Inducing Genetic Variability in Pearl Millet Using Sodium Azide and Nitrous Acid

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ABSTRACT

Background and Objective: Mutation has great possibilities which can be utilized in a breeding program. There is a growing interest in mutation breeding as reflected in the volume of work on the induction of mutations for genetic diversity. Field trial was conducted to evaluate the effect of Nitrous acid (HNO_2) and Sodium azide (NaN_3) on the yield of pearl millet varieties at different concentrations. The objective of this study was to determine the effects of the mutagens and the optimal concentration that increases the agronomic traits in pearl millet. **Materials and Methods:** Treatments consisted of two varieties of pearl millet (ICMP1970085 and ICMP1970116). The NaN_3 and HNO_2 were used for this experiment. A Randomized Complete Block Design was used for the field layout and in three replications. The seeds of the two varieties of millet were divided into 2 sets. **Results:** The mutants treated with HNO_2 in most cases show improved yield than those treated with NaN_3 in ICMP1970116 while for ICMP1970085 the reverse is the case ICMP1970116 treated with a low concentration of NaN_3 (2 Mm) performed well in yield. The high concentration of NaN_3 produces earlier mutants while, those treated with 4 mM concentration performed better in Downey mildew incidence and panicle weight after harvest. The 2 mM of HNO_2 performed better in yield traits, while 3 mM of HNO_2 performed well in days to fifty percent flowering. **Conclusion:** Sodium azide at 2 mM while, HNO_2 at 3 mM appears to be the most effective treatment for inducing variability in the two pearl millet varieties.

KEYWORDS

Sodium azide, nitrous acid, pearl millet, breeding, mutation, genetic diversity, crop improvement, induced mutation, mutant

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INTRODUCTION

Millet is a major grain crop grown around the world. It is used by humans to prepare various dishes and as feed for livestock. About 97% of millet production occurs in developing countries¹. Among millet cultivars, pearl millet is most cultivated in India and Africa². Almost 90 million people in Sub-Saharan Africa (SSA) and Southern Asia (SA) depend on the cultivation of pearl millet for their nutritional security and means of subsistence³. Future demand for pearl millet is predicted to rise due to rising populations of people and animals in SSA and SA, in addition to the desire for nutritious food and other industrial purposes⁴. Due to the depletion of water resources, it may be grown in even more regions where maize and sorghum are grown. Because of the anticipated high drought stress, increase in temperature and increased disease occurrences in SSA⁵ and SA, pearl millet.



Physical and chemical mutagenesis are advantageous because mutagens induced random changes throughout the genomes⁶. In mutation breeding experiments, consideration is given to the mutagenic effectiveness and efficiency. Mutagenic effectiveness measures the frequency of mutations induced by a unit dose of the mutagen. The mutagenic efficiency denotes the mutations due to on biological damage i.e., sterility, injury and lethality. Mutagenic effectiveness and efficiency depend on the type of genotype used and the type of mutagen⁷. Sodium azide (NaN₃) is known to induce high mutagenic effect in several organisms⁸. The deamination of DNA by Nitrous acid results in mutation which caused variability in various characters in crops⁹. The purpose of induced mutation is to enhance the mutation frequency to select appropriate variants for evaluation and possible release as varieties. The present study was designed to create genetic variation in pearl millet and select mutants with improved grain yield and other desirable agronomic traits for farmers' use.

MATERIALS AND METHODS

Study area: This research was carryout during the 2020 wet season from June to October, 2020 in the defunct department of crop production and protection now called the Department of Agronomy research farm of Federal University Dutsin-Ma, Main Campus in Katsina State. Dutsin-Ma lies between latitude 12°27'22"N and longitude 7°30'83"E. The farm is situated within latitude 12°17'40"N and longitude 7°27'19"E. (latitude 12°27'18" longitude 7°29'29"E and 605 m above sea level).

Treatment and experimental design: Treatment consisted of two pearl millet varieties namely, ICMP1970085, ICMP1970116 and four levels of Nitrous acid (HNO₂) (0.00 mM, 1.0 mM, 2.0 mM and 3.0 mM) and four levels of Sodium azide (NaN₃) (0.00 mM, 2.0 mM, 4.0 mM and 6.0 mM). Randomized Complete Block Design (RCBD) was used for the field layout and replicated three times. The ridges were 5 m long with an inter-row spacing of 75 cm and intra-row spacing of 50 cm and 4 ridges per plot. Hybrids were allocated to plots using the random-number table.

Methodology: The experiment was carried out in the laboratory on the farm. Seeds were presoaked in distilled water for an hour before treatment with mutagens to allow the mutagen to diffuse more rapidly to the tissues of the seed. The seed was divided into sets. Each set of seeds was soaked for 1 hr in different concentrations of the mutagens. There are two groups of treatments in the experiment. Group A was the Sodium azide group and Group B was the Nitrous acid group. In each group, the seed was divided into four sets. Set i was soaked in distilled water (0.00 mM) which served as control and for the rest sets (set ii to iv), each set was soaked in various concentrations of Sodium azide of 2 mM, 4 mM and 6 mM for 1 hr. Group B seeds were soaked in various concentrations of Nitrous acid (HNO₂) (0.00 Mm, 1.0 mM, 2.0 mM and 3.0 mM solutions) for 1 hr. The treated seeds of the two mutagens were decanted and thoroughly washed using distilled water to remove the residual effects of mutagens.

Beakers containing the treated seeds were labeled and arranged according to their respective plots number, mutagen type and concentration having 14 plots per replication one beaker each per plot.

Planting of seeds was done in the Research farm, of the Agronomy Department, Federal University Dutsin-Ma Main Campus. The ridges were 5 m long, two ridges represented a plot with an intra-row spacing of 50 cm and inter-row spacing of 75 cm in three replications.

Data collected: Data collected for each plot include the:

- **Day to 50% flowering (DF50):** The number of days for 50% of the plants per plot to flower
- **Plant height (PH):** The distance between the ground level to the apex of the panicles measured in centimetres at maturity being averaged from 3 randomly chosen plants

- **Panicle length (PL):** Distance between the bases of the panicle to the tip in centimetres being averaged from 3 randomly chosen panicles
- **Panicle circumference (PC):** Circumference of the panicle girth (CM) being averaged from 3 randomly chosen panicles
- **Panicles per plot (PP):** Total number of panicle per plot counted at harvest
- **Stand count at harvest (SCH):** Plant stands counted at harvest per plot
- **Downey mildew incidence (DMI):** Number of infected plants over total number of plants per plot expressed in percentage
- **Panicle exersion (PanEx):** Distance (cm) between the main stalk flag leaf and ligule and the base of the panicle
- **Agronomic appreciation (AAP):** Agronomic performance of the entries based on visual observations cored before harvesting. 1-Excellent, 2-very good, 3-good, 4-medium and 5-poor
- **Panicle weight (PW):** Panicle weight in kilograms per plot before threshing
- **Grain weight per plot (GW):** Grain weight in grams per plot after threshing
- **Threshing (%):** Weight of seeds over the weight of panicle expressed in percentage

Statistical analysis: Analysis of Variance (ANOVA) was carried out using SAS statistical package (SAS, 2002) version 9.1 at $p \geq 0.05$ significant level and Duncan's Multiple Range Test (DMRT) was used to separate the means.

RESULTS AND DISCUSSION

Table 1 showed the performance of ICMP1970085 Variety mutants treated with the various concentrations of NaN_3 and HNO_2 indicating that the higher concentration of NaN_3 and HNO_2 the more the resistivity to Downy mildew disease. For days to 50% flowering, the control was earlier than all the mutants and there was no significant difference between the mutants showing that the concentrations of the two mutagens used in this study delayed earliness in the mutants. Also, for plant height, the control was shorter than all the mutants. The concentrations of 2 mM and 4 mM of NaN_3 induced an increase in height of the variety. However, the higher concentrations of NaN_3 6 Mm reduced the height. This also agreed with the findings of Sable *et al.*¹⁰. Those with 1 mM, 2 mM and 3 mM of HNO_2 were all taller than the control, but, the lower the concentrations the taller mutant indicating that a lower concentration of HNO_2 induces tallness in the mutants. A similar result was reported by Goyal *et al.*¹¹. The yield attributing traits (panicle exertion, panicle number and panicle weight and grain weight) showed no significance between all the mutants treated with the two mutagens but they all performed better than the control showing that the mutagens induced an increase in these traits. This was in line with the result obtained by Khursheed *et al.*⁶. Induction of desirable micro-mutations affecting yield is a prime goal in mutation breeding¹². Physical and chemical mutagens induce an increase in the genetic variability for certain traits. This makes for effective selection and increases the probability of getting the desired genotypes¹³. Hundreds of agro-economically important mutant crop varieties have been successfully developed globally^{14,15} reported that up to this year (2022), 3,402 mutant varieties have been developed.

Low concentrations of NaN_3 induced an increase in yield attributing traits while a higher concentration of HNO_2 favored better performance in these traits.

The result of the ICMP1970116 variety treated with the various concentrations of NaN_3 and HNO_2 were presented in Table 2. There were more variabilities with the different concentrations of NaN_3 in ICMP1970116 variety. The downy mildew incidence no significant differences in the resistance level of the mutants exposed to the two mutagens. They were all highly resistant to the disease. Though, there was a significant difference between the mutants and the control. In the days to 50% flowering, there were no significant differences between the mutants, but the control was earlier than all the mutants treated with

Table 1: Effect of Sodium azide (SA) and Nitrous acid (NA) on downy mildew incidence, agronomic characters and yield of ICMP1970085 variety

Designation	DMI (%)	D50%F	PHT (cm)	PANLT (cm)	PANCIR. (cm)	PANEX. (cm)	AAP	SCH	PAN No.	PAN WT (kg)	GWT (kg)	Thresh (%)
Control	1.67 ^{bc}	43.33 ^b	127.44 ^b	15.11 ^b	8.94 ^b	10.11 ^b	2.33 ^b	11.33 ^b	59.67 ^b	1299.70 ^c	621.00 ^d	42.53 ^c
NaN ₃ 2 mM	8.33 ^a	63.33 ^a	186.78 ^a	24.00 ^a	10.29 ^a	12.56 ^a	1.33 ^a	19.67 ^a	67.00 ^a	1602.7 ^{ab}	1020.67 ^{ab}	63.61 ^{ab}
4 mM	1.67 ^{bc}	61.67 ^a	183.78 ^a	24.11 ^a	9.66 ^a	8.42 ^a	1.00 ^a	19.00 ^a	60.67 ^a	1397.30 ^{bc}	925.00 ^{bc}	65.73 ^a
6 mM	5.17 ^{ab}	53.33 ^a	177.56 ^{ab}	23.89 ^a	9.78 ^a	8.81 ^a	1.33 ^a	19.33 ^a	67.00 ^a	1325.30 ^{bc}	817.67 ^{cd}	62.24 ^{ab}
HNO ₂ 1 mM	1.67 ^{bc}	53.33 ^a	181.56 ^{ab}	23.60 ^a	9.79 ^a	9.33 ^a	1.67 ^a	19.67 ^a	62.33 ^a	1322.30 ^{bc}	881.67 ^{bcd}	66.04 ^a
2 mM	0.00 ^c	63.33 ^a	176.78 ^{ab}	24.36 ^a	9.56 ^a	10.41 ^a	1.67 ^a	19.33 ^a	59.33 ^a	1265.30 ^c	757.33 ^d	59.31 ^b
3 mM	0.00 ^c	66.67 ^a	172.78 ^{ab}	21.61 ^a	9.76 ^a	8.95 ^a	1.67 ^a	19.00 ^a	64.33 ^a	1591.70 ^{ab}	1000.33 ^{ab}	63.79 ^{ab}

Means with the same letter within a column are not significantly different at $p \geq 0.05$, DMI: Downy mildew incidence, D50%F: Days to 50% flowering, PHT: Plant height at maturity, PANLT: Panicle length at maturity, PANCIR.: Panicle circumference, PANEX.: Panicle exertion, AAP: Agronomic appreciation, SCH: Stand count at harvest, PAN No.: Panicle number at harvest, PAN WT: Panicle weight after harvest, GWT: Grain weight after threshing and Thresh (%): Thresh percentage

Table 2: Effect of Sodium azide (SA) and Nitrous acid (NA) on agronomic characters and yield of ICMP1970116 variety

Designation	DMI (%)	D50%F	PHT (cm)	PANLT (cm)	PANCIR. (cm)	PANEX. (cm)	AAP	SCH	PAN No.	PAN WT (kg)	GWT (kg)	Thresh (%)
Control	1.33 ^c	36.67 ^c	153.78 ^b	19.66 ^b	08.05 ^{bc}	7.84 ^c	1.33 ^b	09.00 ^b	15.33 ^d	198.70 ^c	352.30 ^b	67.72 ^b
NaN ₃ 2 mM	3.90 ^a	56.67 ^a	160.45 ^a	24.06 ^a	10.39 ^{ab}	10.22 ^a	1.67 ^a	11.33 ^{ab}	34.33 ^{bc}	778.70 ^b	555.30 ^a	70.96 ^a
4 mM	3.00 ^a	61.67 ^a	153.11 ^a	23.39 ^a	10.32 ^{ab}	8.17 ^a	1.33 ^a	9.33 ^b	29.67 ^c	796.00 ^b	553.00 ^{ab}	70.17 ^a
6 mM	3.3 ^a	53.33 ^a	156.55 ^a	22.33 ^a	9.69 ^c	7.67 ^a	1.33 ^a	9.00 ^b	27.67 ^c	697.70 ^b	440.70 ^b	63.02 ^{ab}
HNO ₂ 1 mM	0.00 ^a	63.33 ^a	162.22 ^a	24.44 ^a	10.75 ^{ab}	7.95 ^a	1.00 ^a	10.33 ^{ab}	31.00 ^{bc}	763.30 ^b	553.30 ^{ab}	72.41 ^a
2 mM	0.00 ^a	61.67 ^a	158.55 ^a	22.72 ^a	10.32 ^{ab}	7.17 ^a	1.67 ^a	12.67 ^{ab}	44.00 ^a	998.00 ^a	682.70 ^a	68.31 ^a
3 mM	1.67 ^a	58.33 ^a	162.44 ^a	23.44 ^a	10.50 ^a	7.14 ^a	1.00 ^a	9.67 ^{ab}	33.00 ^{bc}	891.30 ^{ab}	636.70 ^{ab}	72.69 ^a

Means with the same letter within a column are not significantly different at $p \geq 0.05$, DMI: Downy mildew incidence, D50%F: Days to 50% flowering, PHT: Plant height at maturity, PANLT: Panicle length at maturity, PANCIR.: Panicle circumference, PANEX.: Panicle exertion, AAP: Agronomic appreciation, SCH: Stand count at harvest, PAN No.: Panicle number at harvest, PAN WT: Panicle weight after harvest, GWT: Grain weight after threshing and Thresh (%): Thresh percentage

the two mutagens. The mutants were longer in height and panicle length than the control though there were no significant differences in plant height and panicle length between the mutants^{15,16} reported contrary results. The mutants treated with higher doses of NaN₃ and HNO₂ have fatter panicles than those treated with lower doses. Moreover, all the mutants performed better with lower concentrations of NaN₃ while, a higher concentration of HNO₂ increase yield attributes and yield. Similar dose-dependent variations in quantitative traits were reported by Oladosu *et al.*¹⁷ foxtail millets. The mutants treated with HNO₂ in most cases showed improved yield than those treated with NaN₃ in ICMP1970116 variety while for ICMP1970085 variety the mutants treated with NaN₃ in most cases showed better yield than showed those treated with HNO₂. Pearl millet treated with Nitrous acid and Sodium azide induces increased genetic variability and heritability for agronomic traits this will provide wide scope for further selection in breeding programs for pearl millet.

LIMITATIONS

Mutations are primarily responsible for variety in nature and plant breeding would not be possible without mutations. In this situation, the main goal of mutation-based breeding is to create and enhance well-adapted plant types by altering one or two key features to boost their quality or output. Improved varieties can use for commercial cultivation. Moreso, compared to conventional breeding, induced mutagenesis, have the potential to accelerate the improvement of pearl millets' quantitative and qualitative qualities. Mutagenic treatment of seeds and other plant components is still an effective method for generating resistance to biotic and abiotic stressors in different crops because of its relative ease of usage and cheap cost. Since mutant pearl millet cultivars are still being developed and are not yet being produced commercially, the economic benefits are difficult to quantify. Several fresh crop mutant varieties exhibit better quality attributes that haven't yet translated into financial gains. Also, it might be challenging to quantify some effects, such as decreased environmental contamination.

CONCLUSION

The two varieties responded differently to the two mutagens. Consequently, it is concluded that Sodium azide at (2 mM) low concentration and high concentrations of HNO₂ (3 mM) appear to be the better effective treatment for inducing variability in pearl millet varieties such as ICMP1970085 variety and ICMP1970116 variety.

SIGNIFICANCE STATEMENT

The outcomes of this research showed that Sodium azide and Nitrous acid can be used for inducing mutation that will produce genetic variability in pearl millet. Mutants with low biological damage and high mutation frequency can be developed at low concentrations of Sodium azide and Nitrous acid.

This study offers useful material for discovering desirable quantitative and agronomic traits in pearl millet. It offers the possibilities of improving one or two traits without changing the rest of the genotype and produces raw material for genetic enhancement of economic crops which can possibly be utilized in the future breeding programs.

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