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# THE POTENTIAL OF SODIUM AZIDE IN THE CREATION OF STABLE VARIATIONS AMONG $M_3$ MUTANTS OF GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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**ABSTRACT**

The study aimed to evaluate the efficacy of sodium azide as a mutagen in inducing mutations and enhancing genetic variability in groundnut varieties. Four varieties were subjected to different doses of sodium azide, with observations highlighting varying effects on different traits. Notably, Kadiri-6 exhibited the most favourable response to the mutagen at a 0.10% dose, demonstrating improved yield and several key traits compared to the control. The results indicated a dose-dependent effect of sodium azide on different characteristics, with some traits showing increased expression while others decreased with higher doses. High heritability and genetic advance for pod and kernel yield traits suggest a strong genetic control with minimal environmental influence, indicating stability in these characters. Overall, the study underscores the potential of mutagenesis using sodium azide to induce beneficial mutations and enhance genetic variability in groundnut varieties, particularly highlighting the significance of the Kadiri-6 variety at the 0.10% dose for improved yield and essential agronomic traits.

***Keywords***: Sodium azide, Kadiri-6, Gujarat Gold, Haritha Andhra, and Kadiri Amaravati.

## Introduction

Groundnut which is scientifically known as *Arachis hypogaea* L. is among the vital oil seed crops with special reference to India and is a member of the Papilionaceous subfamily of the Fabaceae family originating from South America – Brazil to be precise. This crop is allotetraploid with 2n=4x=40. An important feature of this plant is that the flowers develop on the above-ground part and after pollination, the pegs go down into the ground and develop into pods. It is grown in over a hundred countries of the six continents. World-leading groundnut producers are China, India, Nigeria, the USA, Indonesia, and Sudan. It occupies the pride of place as the biggest source of edible oils in the country while contributing to about half of the total oil seed output. It is comprised of about 50% edible oil, which is of high quality, 25% edible protein, and 20% carbohydrate, and is also a very rich source of phosphorus, calcium, and zinc – all essential minerals. Interestingly, groundnut skin is high in vitamin B and is used as the main ingredient in many processing industries: Confectionery, ice cream, coating, peanut butter, and bakery products.

Biotic stress such as pests, and diseases, low yield, and abiotic stress are major problems facing the crop. Conventional breeding strategies might not suffice when it comes to creating adequate genetic variability which is deemed indispensable for building a good foundation for the breeding strategy. Furthermore, groundnut being an autogamous crop and its genetic constitution endowed with a small gene base, which limits the range of authentic groundnut varieties that can be observed and relied on by breeders for improvement campaigns. Crop improvement by mutagenesis has been applied in several crops for yield improvement, creation of new cultivars, stress and drought tolerance, disease resistance, and horticultural or floriculture purposes. Induced mutations have been employed to increase key crops that are primarily propagated through seeds and to create new genetic variation in horticultural plants. Sodium azide is known as a "super mutagen". SA works as a well-established heavy metal enzyme that affects the metabolism and is also known as a respiration inhibitor that hinders the activity of enzymes like catalase and peroxidase. This chemical was found to be a effective mutagen for inducing mutation in field crops. It should also be noted that the outcome of mutation breeding depends on such parameters as effectiveness and efficiency, plant material, mutagen dose, and duration.

The mutagen efficiency addresses the issue of, or rate at which a given amount of mutagens brings about mutations on the other hand mutagen effectiveness speaks on the efficiency of the particular changes in as much as it brings about undesirable change such as death, harm or barrenness. Mutation rate of mutagens act as tool through which different mutagens can be measured for their efficiency as mutagens.

## Material and Methods

The investigation was carried out during the Kharif seasons of 2021, 2022, and 2023 represented by M1, M2, and M3 respectively at CRC-1 within the Department of Genetics and Plant Breeding, School of Agriculture, ITM University Gwalior, MP India. The experimental site is located at a geographical coordinate of 26 o 14' N latitude, 78 0 14' E longitude, and the altitude stands at 206 m above sea level. Four Spanish bunchy type groundnut varieties Kadiri-6, Gujarat Gold, Haritha Andhra, and Kadiri Amaravati were collected from the Agriculture Research Station, Kadiri, Andhra Pradesh. These varieties were exposed to treatment with concentrations of the mutagen Sodium azide (NaN3) with four ranges: control; 0.1, 0.15, 0.20, and 0.40 w/v. To prepare the solutions of mutagens, 0.10, 0.15, 0.20, and 0.40g of Sodium azide was dissolved in 100 ml distilled water respectively. Before treatment, the seeds were first pre-incubated in distilled water at 37℃ for two hours to make the seeds actively metabolize so that they are more sensitive to the mutagenic effects of the treatment. After this, the seeds of the four varieties were treated with Sodium azide at different concentrations of (0.10, 0.15, 0.20, and 0.40) for seventeen hours at temperature as per the instruction of Joshi (2017). After treatment, the seeds were washed in distilled water and sown in Randomized Block Design (RBD) with replication three. Planting was done using a dibbling method where seeds were placed in rows at a density of 60 cm along the row and 10 cm between rows and all recommended practices used in agriculture were practiced. These parameters incorporated germination %, survival %, seedling height, days to maturity, days to 50% flowering, no. of sterile plants, height of the plant at maturity, number of branches per plant, no. of pods per plant, no. of sound mature kernels, 100-kernel weight, biological yield per plant, harvest index, shelling percentage, pod yield per plant, pod length, pod width and kernel yield per plant. Sampling of data for these parameters was done during the growth of crops and all the results were analyzed using R Studio. Those plants that showed the desired morphological changes indicating mutation were taken for further breeding and the seeds produced were sown to get the next generation. Each potential mutant from the M1 generation was grown in three replications in the M2 and M3 generations with a plant-to-plant and row-to-row distance of 60 x10 cm by M3.

**Table 1 :** Mean performance of all four varieties at four different doses for $M_3$

| Treatment | ination (%) | urvival% | Seedling height | Days to flowering | Days to maturity | Sterile plants | lant heigh at Maturity | No.of. Pods per plant | Sound mature kernel % | Shelling % | Pod yield per plant | Harvest index | Biological yield per plant | 100-Kernel weight | No. of branches | Pod length | Pod width | Kernel yield er plan |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kadiri-6 Control | 93.67 | 91.00 | 16.37 | 28.00 | 107.33 | 0.03 | 31.50 | 28.00 | 74.33 | 65.02 | 16.93 | 57.79 | 29.30 | 31.67 | 9.33 | 3.56 | 1.78 | 10.97 |
| SA-0.10 | 9.82** | 86.38*** | 7.84*** | 28.6*** | 110.46*** | .85*** | 34.38*** | 9.54*** | 74.07*** | 9.52** | 17.96*** | 0.32*** | 28.09*** | 33.96*** | 9.65*** | .39*** | .77** | 1.32*** |
| SA-0.15 | 4.15** | 86.82*** | 5.58*** | 34.11*** | 108.46*** | .66*** | 29.61*** | 7.75*** | 73.57*** | 4.01** | 17.48*** | 1.16*** | 25.62*** | 26.37*** | 10.16*** | .41*** | .83*** | 5.83*** |
| SA-0.20 | 1.94** | 82.01*** | 3.64*** | 31.33*** | 113.16*** | .68*** | 29.10*** | 2.17*** | 73.94*** | 2.63** | 14.76*** | 3.61*** | 25.18*** | 30.57*** | 10.48*** | .52*** | .87*** | 8.31*** |
| SA-0.40 | 0.77** | 75.3*** | 1.93*** | 35.38*** | 120.76*** | .54*** | 25.44*** | 5.06*** | 73.69*** | 9.59** | 13.01*** | 0.37*** | 23.65*** | 28.02*** | 8.46*** | 2.8*** | .90*** | 9.08*** |
| G.Gold Control | 93.67 | 93.33 | 16.90 | 30.00 | 109.67 | 0.00 | 34.90 | 28.67 | 81.67 | 57.58 | 16.00 | 62.01 | 25.87 | 32.67 | 8.00 | 4.02 | 1.97 | 9.20 |
| SA-0.10 | 2.43** | 88.08*** | 6.93*** | 27.72*** | 110.68*** | .67*** | 37.85*** | 9.26*** | 80.99*** | 3.12** | 16.46*** | 0.18*** | 26.47*** | 32.09*** | 9.11*** | .75*** | .90*** | 8.24*** |
| SA-0.15 | 4.15** | 86.92*** | 8.01*** | 30.11*** | 108.38*** | .70*** | 36.54*** | 7.00*** | 73.21*** | 8.30** | 14.99*** | 4.22*** | 24.85*** | 30.29*** | 7.92*** | .66*** | .85*** | 8.34*** |
| SA-0.20 | 4.74** | 80.33*** | 4.91*** | 31.63*** | 111.83*** | .92*** | 31.69*** | 4.72*** | 74.16*** | 2.44** | 15.17*** | 8.94*** | 27.37*** | 30.87*** | 7.3*** | .11*** | .15*** | 6.74*** |
| SA-0.40 | 1.9*** | 77.83*** | 1.69*** | 35.21*** | 121.3*** | .86*** | 29.99*** | 4.53*** | 73.47*** | 9.63** | 13.79*** | 5.68*** | 27.74*** | 27.92*** | 8.01*** | .35*** | .17*** | 5.38*** |
| H.Andhra Control | 92.33 | 91.67 | 16.60 | 29.00 | 111.33 | 0.00 | 32.67 | 30.67 | 77.33 | 53.78 | 18.80 | 69.83 | 26.97 | 31.67 | 10.33 | 3.97 | 1.89 | 10.10 |
| SA-0.10 | 7.54** | 91.74*** | 8.44*** | 27.98*** | 113.83*** | .90*** | 30.67*** | 9.44*** | 79.24*** | 9.27** | 14.45*** | 6.18*** | 24.49*** | 33.36*** | 9.00*** | .66*** | .78*** | 7.85*** |
| SA-0.15 | 7.29** | 83.83*** | 5.66*** | 27.46*** | 112.08*** | .31*** | 30.35*** | 8.59*** | 74.31*** | 3.14** | 15.44*** | 6.86*** | 23.55*** | 26.51*** | 10.68*** | .72*** | .84*** | 7.78*** |
| SA-0.20 | 6.56** | 78.56*** | 4.34*** | 30.45*** | 118.33*** | .74*** | 29.40*** | 8.20*** | 74.61*** | 9.55** | 14.28*** | 63.8*** | 23.67*** | 32.11*** | 10.23*** | .71*** | .81*** | 7.12*** |
| SA-0.40 | 2.42** | 75.89*** | 2.42*** | 32.38*** | 121.33*** | .85*** | 24.81*** | 1.68*** | 67.85*** | 7.39** | 10.98*** | 9.23*** | 23.58*** | 30.4*** | 8.95*** | .73*** | .77*** | 7.10*** |
| K. Amaravati Control | 92.33 | 92.33 | 16.73 | 31.33 | 106.67 | 0.00 | 33.90 | 32.33 | 77.00 | 65.72 | 16.47 | 69.67 | 23.83 | 34.00 | 9.67 | 3.35 | 1.53 | 10.73 |
| SA-0.10 | 8.96** | 86.85*** | 7.44*** | 28.24*** | 107.33*** | .96*** | 26.83*** | 1.85*** | 73.20*** | 3.50** | 14.30*** | 8.66*** | 21.16*** | 33.05*** | 8.27*** | .11*** | .70*** | 7.39*** |
| SA-0.15 | 8.32** | 82.77*** | 6.23*** | 28.37*** | 106.00*** | .42*** | 31.72*** | 5.29*** | 73.55*** | 3.23** | 11.20*** | 9.97*** | 21.91*** | 28.59*** | 8.65*** | .14*** | .71*** | 5.62*** |
| SA-0.20 | 2.89** | 79.53*** | 16.4*** | 32.51*** | 116.33*** | .20*** | 26.12*** | 3.39*** | 69.62*** | 0.69** | 11.87*** | 0.73*** | 21.21*** | 32.48*** | 7.40*** | .16*** | .73*** | 5.52*** |
| SA-0.40 | 78.3*** | 74.59*** | 1.54*** | 31.64*** | 121.67*** | .85*** | 25.55*** | 3.25*** | 74.03*** | 7.21** | 11.96*** | 1.86*** | 26.05*** | 30.71*** | 8.30*** | .25*** | .75*** | 5.69*** |
| SEM | 0.235 | 0.213 | 0.29 | 0.336 | 0.255 | 0.215 | 0.223 | 0.319 | 0.318 | 0.349 | 0.226 | 0.211 | 0.195 | 0.245 | 0.254 | 0.029 | 0.011 | 0.263 |
| CD | 0.681 | 0.619 | 0.841 | 0.974 | 0.739 | 0.623 | 0.648 | 0.927 | 0.923 | 1.012 | 0.657 | 0.612 | 0.567 | 0.71 | 0.736 | 0.084 | 0.032 | 0.765 |
| CV (%) | 0.476 | 0.449 | 3.325 | 1.887 | 0.387 | 10.197 | 1.316 | 6.248 | 2.08 | 0.815 | 1.288 | 1.482 | 0.546 | 0.777 | 3.083 | 1.916 | 2.238 | 6.124 |

**Table 2 :** Genetic Variability Analysis

| Genetic Variability Analysis | | | | | | |
|---|---|---|---|---|---|---|
| | | Coefficient of Variance | | | | |
| S. No. | Character | PCV | GCV | Heritability | GA as a percent of Mean | Genetic Advance |
| 1 | Germination Percentage | 4.56 | 4.53 | 98.72 | 9.27 | 7.93 |
| 2 | Survival Percentage | 6.26 | 6.25 | 99.5 | 12.84 | 10.57 |
| 3 | Seedling Height (Cm) | 15.33 | 15.15 | 97.63 | 30.84 | 4.62 |
| 4 | Days To Flowering | 8.72 | 8.51 | 95.32 | 17.12 | 5.28 |
| 5 | Days To Maturity | 4.06 | 4.04 | 99.07 | 8.29 | 9.45 |
| 6 | Sterile Plants (%) | 23.31 | 20.21 | 75.16 | 36.09 | 1.31 |
| 7 | Plant Height (cm) | 11.01 | 10.92 | 98.49 | 22.33 | 6.6 |
| 8 | Number Of Branches | 14.22 | 12.77 | 80.69 | 23.63 | 2.09 |
| 9 | Number Of Pods Per Plant | 10.74 | 10.53 | 96.07 | 21.26 | 5.66 |
| 10 | Sound Mature Kernels (%) | 4.11 | 4.03 | 96.07 | 8.14 | 6.05 |
| 11 | 100-Kernel Weight (g) | 7.9 | 7.8 | 97.36 | 15.85 | 4.83 |
| 12 | Biological Yield Per Plant (g) | 8.92 | 8.79 | 97.24 | 17.86 | 4.4 |
| 13 | Harvest Index (%) | 10.43 | 10.42 | 99.72 | 21.44 | 13.29 |
| 14 | Shelling Percentage | 8.94 | 8.91 | 99.24 | 18.28 | 9.98 |
| 15 | Pod Yield Per Plant (g) | 14.8 | 14.47 | 95.66 | 29.16 | 4.16 |
| 16 | Pod Length (cm) | 13.37 | 13.24 | 98.02 | 27 | 0.7 |
| 17 | Pod Width (cm) | 16.31 | 15.96 | 95.79 | 32.18 | 0.27 |
| 18 | Kernel Yield Per Plant (g) | 19.71 | 18.63 | 89.31 | 36.27 | 2.78 |

## Result and Discussion

A general observation about the mutagen sodium azide upon 4 varieties namely Kadiri-6, Gujarat Gold, Haritha Andhra, and Kadiri Amravati with the doses 0.10%, 0.15%, 020%, and 0.40% respectively was observed to be more or less the same. The characters displayed the effects of sodium azide in a trend where the increase in the dose of the mutagen led to the decrease in the magnitude of the characters *viz.* germination percentage, survival percentage, seedling height, number of sterile plants, plant height, number of pods per plant, sound mature kernels, shelling percentage, pod yield, biological yield per plant, 100-kernel weight, pod length, pod width, and kernel yield per plant. The characters where the magnitude displayed a rise with the increase in the dose of sodium azide are days to flowering, days to maturity, harvest index, and number of branches per plant.

The most responsive dose for the variety Kadiri-6 was observed to be 0.10% of sodium azide. The kernel yield per plant (11.32 gm) was significantly higher than the control i.e., 10.97gm. The contributing characters were observed to be the number of pods per plant (29.54), pod yield per plant (17.96gm), 100-kernel weight (33.96gm), seedling height (17.84cm), and plant height (34.8cm).

The variety Gujarat gold displayed considerable effect of different doses of sodium azide leading to the reduction in the magnitude of most of the characters where the kernel yield per plant raised from 8.24gm to 5.38gm in contrast to the yield of the control i.e., 19.20gm. The number of pods per plant and number of branches per plant were recorded to be 16.46 and 9.11 respectively, which is higher than the control, still, the yield is less due to the lower magnitudes for the contributing characters such as sound mature kernels percentage (80.99%), shelling percentage (53.12%), and pod length (2.75cm).

The effect of different doses of sodium azide on the character yield varied from 7.85 gm to 7.10 gm. There is a slight rather non-significant variation among the doses concerning the control (10.10gm). The contributing characters were seedling height(18.44cm), sound mature kernel percentage (19.24 %), shelling percentage (59.27%), and 100-kernel weight (33.46 gm) for the 0.10% of sodium azide.

None of the doses seem effective on the variety Kadiri Amravati. The effect of all 4 doses of sodium azide for the kernel yield per plant showed variation in between the range of 7.39 gm to 5.62 gm. The yield recorded was less than the control (10.73gm) because of the lower values of the contributing characters such as plant height at maturity (26.83 cm), number of pods per plant (31.85), sound mature kernel (53.50) shelling percentage (14.30%), pod yield per plant (68.66 gm), and 100-kernel weight (33.05 gm).

A moderate range of variation was observed for all the characters under the study in the M3 generation

which is supposed be a stable variation as the M3 generation is an advancement to the generation (M2) where the mutants were identified. Rajshekhar (2013); Jain (2016); Kumar (2018) and Shiva *et al*. (2024) have reported medium to wide variation in the mean performance of genotypes under the study.

In the present study of M3 generation of mutation (populations), the PCV was greater that the GCV for all the traits under the study. Similar results were also reported by Terkimbi and Terkula (2014), Gunasekaran and Pavadai (2015); Kavera and Nadaf (2017); Hampannavar *et al*. (2018); and Shiva *et al*. (2024). This signifies that the variation is majorly due to genotypes and very small to negligible magnitude of environment influences the character. This indicates that the characters have quantitative inheritance and considerably less influenced by environmental factors.

In the present study of M3 generation, high heritability (above 60%) was observed for all the 18 characters whcich is in accordance with the reports made by Azad and Hamid (2000); Venkataramana (2001); Abhay and Nagda (2002); Wadikar *et al*. (2018); Lunagariya (2018), Ramani (2019); and Shiva *et al*. (2024).

In the present study, high genetic advance as percent of mena was observed for seedling height, sterile plants, plant height, number of branches per plant, number of pods per plant, harvest index, pod yield per plant, pod length, pod width, and kernel yield per plant

indicating nominal role of environment in the character expression. Similar observations were made by Prakash *et al*. (2000); Venkataramana *et al*. (2001); Rangrao (2013); Kavera and Nadaf (2017); Kumar (2018); Meena (2021); and Shiva *et al*. (2024).

Moderate genetic as percent of mean (10-20%) was observed for the characters survival percentage, days to flowering, 100-kernel weight, biological yield per plant and shelling percentage. These findings were in consonance with the reports made by Karikari (2002); Nagaraj (2013); Kar (2015); Chavadhari *et al*. (2017); Bheemareddy (2018); Ramani (2019); and Shiva *et al*. (2024).

Characters *viz.* germination percentage, days to maturity, and sound mature kernels displayed low genetic advance as percent of mena (below 10%) indicating fluctuative expression of the character signifying role of environment in the expression. The scientists Venkataramana (2001); Jonnada (2006); Kar (2015); Bhargavi *et al*. (2017); Wadikar *et al*. (2018); and Shiva *et al*. (2024) have reported low genetic advance as percent of mean as their findings. The characters displaying high heritability with high genetic advance as percent of mean as typically indicates that the traits are under strong genetic control with less influence by environmental factors.

A moderate genetic advance as percent of mean signifies the complexity of the trait which is majorly governed by genotype but environment also plays a crucial role in the expression of the trait.

The characters with low genetic advance as percent of mean can not be suggested for selection because of the proportion of environmental contribution towards the trait.

## Conclusion

The present study was in effort to identify the capacity of a mutagen sodium azide in the creation of effective mutation and to identify potential of sodium azide in variability for the characters under the study.

Among the varieties used under, the most responsive variety was found to be Kadiri-6. The magnitude of varieties induced by sodium azide was observed to be variable with respect to the doses (0.10%, 0.15%, 0.20%, and 0.40%). A general observation was made that with the increase in the dose of sodium azide, the expression of the character ceased relatively. The most effective dose and varietal combination was reported to be 0.10% sodium azide on Kadiri-6. This combination had significantly higher yield (11.32gm) from the control (10.9gm). the contributing characters with magnitudes higher than the control value are seedling height(34.38cm), days to 50% flowering (28.6 days), days to maturity (110.46 days), plant height (34.38cm), number of pods per plant (29.54 pods), pod yield per plant (17.96gm), 100-kernel weight (33.96gm), and number of branches per plant (9.65) enhancing the yield for the combination Kdiri-6 + sodium azide 0.10%.

The high magnitude for heritability and genetic advance as percent of mean was observed for the traits pod yield per plant and kernel yield per plant which signifies the control of the character is genotypic with least environmental influence indicating stability for the character.

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