Annals of Ecology and Environmental Science
Volume 2, Issue 3, 2018, PP 27-32
ISSN 2637-5338

In-Vitro Induction of Mutation in Groundnut (Arachis hypogea L.) by Using Sodium Azide Mutagens and Its Impact on Selected Agronomic Traits

Gemechis Waktole

Welega University

***Corresponding Author:** Gemechis Waktole, Welega Universit, Email:gamewa2010@gmail.com*

ABSTRACT

Experiment was conducted at Haramaya University experimental field station with the objectives of identifying the quantitative trait variations produced by induced chemical mutation; identify the effect of Sodium Azide on quantitative trait variation (morphological characters) and determine the effectiveness of the mutagenic chemical in groundnut growth. Two varieties of groundnut (Sedi and Werer 961) were used for inducing mutation and planted in complete Randomized Design (CRD) factorial with three replications. The significant reduction (P<0.05) in days to emergence of Sedi and Werer 961 varieties were observed at higher concentration of Sodium azide when compared with control. The plant height, number of leaves per plant, days to 50% flowering, days to 90% maturity, number of pod per plant, number of seed per plant and seed yield was significantly increased in highest concentration (0.05%) of sodium azide treatment. The root nodule number was drastically reduced in 0.01% to 0.03% concentration of sodium azide in Sedi and Werer 961varieties. Moreover, the highest percent oil content was observed in sedi variety (53.43%) treated with 0.05% of sodium azide mutagen as compared with control and rest of the treatments. In this present investigation, 0.01%, 0.02%, 0.03%, 0.04% and 0.05% of sodium azide was played an important role in inducing mutation to improve the quantitative characters of selected groundnut varieties.

Keywords: *Sodium Azide Mutagens, Groundnut, oil content, yield and yield component, root nodule.*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a legume crop belonging to the family Fabaceae. The annual world groundnut production was around 38.2 million tons from 26.4 million ha of production area (FAO, 2012). Groundnut (*Arachis hypogaea* L.) is an oilseed, food and animal feed all over the world (Pande *et al*., 2003; Upadhyaya *et al*., 2006). It is the main source of food in various forms and used as a component of crop rotation in many countries (Gbèhounou and Adengo, 2003). Groundnut is one of the five widely cultivated oilseed crops in Ethiopia (Wijnands *et al*., 2009). However, it is self-pollinating and possesses limited variability. Consequently, the extent to which groundnut cultivars may be improved through conventional breeding methods is limited.

All living organisms have the ability to adapt themselves through natural means in order to overcome the changing environmental conditions. However, it takes hundreds of years before any detectable improvement is obtained.Besides the vital role in plant breeding programs, a new role of induced mutations in releasing of gene silencing in transgenic plants has been reported (Bhatia, 1999). Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield components of various crops. Induced mutations have been used to improve major crops that are mainly propagated by seeds (Mostafa, 2011) and to introduce novel genetic variability in ornamental crops. Sodium azide, a chemical mutagen has become important tool to enhance agronomic traits of crop plants (Adamu and Aliyu, 2007).

Though few researchers have conducted their researches on groundnut, to date the attention of existing scholars seems to be biased to the protection side of groundnut production in Ethiopia. The improvement of groundnut productivity is still stagnant in the country. The total area under groundnut production in Ethiopia was estimated to be 90,155 ha from which about 124,418.7 tons was harvested in 2012/13 (CSA, 2013). Research result showed that groundnut farmers can produce groundnut yields of 2000 kg/ha or more but

the national average yield produced by the farmers in Ethiopia is considerably low, Central Statistics Agency of Ethiopia (2009) survey report revealed that 1123 kg/ha average yield of groundnut per year. FAOSTAT (2010) reveals that, groundnut yield in Africa is lower (980 kg/ha) than the average world groundnut yields. Groundnut production in Ethiopia is found to be constrained by several biotic and a biotic factors i.e. critical moisture stress especially during flowering and then after, lack of improved varieties and appropriate production and post-harvest practices, and diseases affecting both above and underground parts of the plant. It is therefore the aim of the present study to investigate the impact of pretreatment of groundnut seeds with a mutagenic agent, sodium azide, on the yield and yield components.

Materials And Methods

Study Design

The study was based on laboratory as well as greenhouse experiments. The experimental design was CRD design with factorial arrangements with three replications along with respective control. The selection of the crop plant was based on its economic importance as well as availability. Dried seeds of two varieties of groundnuts Sedi and Werer-961 were obtained from Haramaya University, Babile Research Substation. Accordingly, 108 seeds fromeach variety of groundnuts (Sedi and Werer - 961) were selected for both bioassay and green house study.

Laboratory Examination

The different concentrations of sodium azide (NaN_3) (0.01, 0.02, 0.03, 0.04 and 0.05 %) W/V was prepared in plastic volumetric flask by using distilled water (Dhanavel *et al*., 2008).The seeds of selected varieties (Werer - 961 and Sedi) were surface sterilization with 0.1% mercuric chloride for 1 minute to remove fungal spores on the surface of the seeds. All the seed were soaked in distilled water for about 6 hours. Then the control seeds were removed from distilled water and air dried for about 20 minutes. The control seed for each of the varieties were soaked in distilled water for 6 hrs again. Then the surface sterilized seeds were washed with distilled water several times to remove the mercuric chloride. After this treatment, seeds were pre soaked in plastic petridish which contains distilled water for six hours, and air dried for about 20 minutes and then treated with freshly prepared mutagenic agent of sodium azide solution at different concentrations (0.01%, 0.02%, 0.03%, 0.04% and 0.05%) for 6 hrs. (Dhanavel *et al*, 2008). Then, the seeds treated with sodium azide were thoroughly washed in running tap water for ten minutes to remove excess exudates and chemicals from the seeds. The bioassay study was carried out following the method of Heisey (1990). Three treated seeds were placed on what man filter paper in petriplates (9cm X 2cm). Each petriplate was moistened with 2ml/ plate of distilled water and the control was untreated seeds and incubated at room temperature. Each treatment was replicated three times with respective control. The germination date, plumule and radicle length were measured for each variety. The seeds treated with chemical mutagenic agent in the laboratory were sown in pots (30x 36 cm) in greenhouse followed by CRD design with factorial arrangements with three replications along with respective control. The pots were filled with 12.25 kg sandy loam soil and three plants were sown in the pot. The experimental plants were grown under the greenhouse condition at Haramaya University. The control plants were grown from normal untreated seeds. The Agronomic traits such as Number of leaves per plant, Plant height, Days to 50% flowering, Days to 90% maturity, Number of pods per plant, Number of seeds per plant and Seed yield/plant were measured and determined

Statistical Analysis

Two-way analysis of variance (ANOVA) was carried out using GenStat discovery 15th edition software for the parameters studied (Payne *et al*., 2008), following the standard procedures outlined by Gomez and Gomez (1984). Mean comparisons were conducted using the Fisher's Least Significant Difference Procedure when significant interactions were detected in the ANOVA.

Results And Discussion

Bioassay Studies on Groundnut Varieties

Effects of Sodium Azide on Seed Germination and Seedling Growth of Groundnut varieties

Days to emergence was recorded for the two varieties, Plumule and Radicle length were measured and recorded for each variety. Seed

color and shape were the same before and after the treatments were applied. More than 80% of the seeds were germinated within 4 days in Werer 961 and 5 days in Sedi variety in lower concentrations (0.01% and 0.03%).

Table1. *Effects of Sodium Azide on seed germination and seedling growth of groundnut (Werer 961 variety)*

S.NO	Concentration	Days to Emergence	Radicle Length(cm)	Plumule Length(cm)
1.	0.01%	4.00^{e}	3.47^{bcde}	5.56^{ef}
2.	0.02%	4.00^{e}	3.73^{bc}	5.21^{f}
3.	0.03%	4.00^{e}	3.43^{cde}	6.01^{de}
4.	0.04%	5.00^{d}	3.33^{cde}	6.62^{cd}
5.	0.05%	5.00^{d}	4.36^{a}	7.96^{a}
6.	Control	8.66^{a}	2.69^{f}	4.95^{f}
	CV (%)	5.2	9.9	6.8
	LSD(5% level)	0.5034	0.5782	0.7435

Means followed by the same letter(s) within column are not significantly different at P=0.05

Table2. *Effects of Sodium Azide on seed germination and seedling growth of groundnut (Sedi variety)*

S.NO	Concentration	Days to Emergence	Radicle Length(cm)	Plumule Length(cm)
1.	0.01%	6.00^{c}	2.99^{ef}	6.98^{bc}
2.	0.02%	5.00^{d}	3.08^{def}	6.74^{cd}
3.	0.03%	5.00^{d}	3.29^{cde}	5.58^{ef}
4.	0.04%	6.00^{c}	3.66^{bcd}	7.54^{ab}
5.	0.05%	6.33^{c}	4.02^{ab}	8.10^{a}
6.	Control	7.66^{b}	2.69^{f}	6.26^{cde}
	CV (%)	5.2	9.9	6.8
	LSD(5% level)	0.5034	0.5782	0.7435

Means followed by the same letter(s) within column are not significantly different at P=0.05

The number of days for seed germination was increased at higher concentration in both varieties exposed to mutagenic agent. Moreover, sodium azide had stimulatory effect on seed germination especially at lower concentration (0.01 to 0.03%) as compared with control groups. These findings agree with those in black gram (Sharma *et al*., 2005; in cowpea (Dhanavel *et al*., 2008), in little millet; (Thilagavathi & Mullainathan, 2009) and in sesame (Begum and Dasgupta, 2010). However, reductions in seed germination percentages at the highest concentration (0.04 and 0.05%) may be due to the effect of mutagens on various crop plants have earlier been reported by Mensah *et al.* (2005).

Similar results were also cited by (Siddiqui *et al*., 2007). Accordingly, the mutagenic chemical such as sodium azide decreases the seed germination percentage and increases the chromosomal aberrations in root tip mitotic cells of plant which was a dose-dependent manner. In contrast to this report, reduction in seed germination was not observed and rather it increases number of days to emergency of the seed. Reduction in seed germination in mutagenic treatments has been explained due to delay or inhibition of physiological and biological processes necessary for seed germination which include enzyme activity (Kulkarni, 2011). Moreover, similar kinds of results have been recorded in groundnut by Animasaun, *et al.* (2014). The plumule and radicle growth was observed and recorded in table 1 and 2. The maximum plumule and radicle length was recorded at 0.05% SA concentration in both varieties. In addition to this, all the treatments have shown stimulatory effect on Radicle and Plumule as compared to control groups. These findings are in close agreement with the earlier reports of Kumar and Selvaraj (2003). Moreover, Adamu *et al.* (2007) reported that the mutagenic effect on tomato plant treated with sodium azide and it was very effective in inducing mutations with respect to Radicle and Plumule growth.

Pot Experiments

Effects of Sodium Azide on Morphological and Yield Traits of Groundnut

Plant height

Plant height was recorded in both the varieties and presented in table 3 and 4. In groundnut Werer 961 variety showed the ranges for plant height range in between 13.67 to 17.33 cm. More over 0.05% sodium azide treated seeds showed the highest plant height (17.33 cm) as

compared with control plant. The same pattern of the plant height was observed in variety Sedi. The increase in plant height of the two groundnut varieties induced by the chemical mutagen (sodium azide) may be due to the alteration of their genome integrated by environmental signals as reported by Uno *et al*. (2001); probably by increasing the rates of cellular division and expansion at their meristematic regions.

Number of leaves per plant

The effect of sodium azide on number of leaves per plant was recorded in table 3 and 4. It indicated that sodium azide stimulate the leaf number in all the treatment as compared with control group. Meanwhile, highest leaf number (114.3) was observed at 0.05% sodium azide treatment in Sedi variety. Whereas, Werer 961 variety showed the highest number of leaves (109.3) at 0.04% treatment as compared with control.

Days to 50% flowering

Days to 50% flowering were observed in werer 961 and Sedi varieties and indicated in table 3 and 4. The mutagenic agent sodium azide showed the significant effect on days to 50% flowering in both the treated varieties. Especially, at the highest concentration (0.05%) showed less number of days (29.33) for werer-961 variety as compared with control group and the rest of the treatments.

Days to 90% maturity

The effect of sodium azide on days to 90% maturity was recorded for Werer-961 and Sedi varieties. The effect was concentration dependent. It showed the ranges 139.33 to 110 for werer-961 and 115.67 to 137.67 for Sedi varieties. Moreover, the highest (0.05%) concentration showed less number of days for both the varieties as compared with control groups. In the highest doses of mutagenic treatments and their combinations, days required for flowering and days required for maturity were found to be significantly decreased, especially at the highest concentration. Similar result was suggested in pre-soaked seeds of green chilli variety treated with sodium azide (Neeraj and Anis 1995).

Number of pods per plant

Number of pods per plant was observed; it showed the effect was concentration dependent. The highest pod numbers were observed in 0.05% treatment with Sodium Azide in Werer 961 varieties. However, the lowest pod number was observed in 0.02% treatment as compared with control groups. In addition to this the same pattern of results were also observed in sedi variety. Among the two varieties, sedi showed the highest pod number as compared with control groups. In line with this, an increase in variability for number of pods per plant following mutagenic treatments of sodium azide in *Vigna mungo* was suggested by Singh *et al*. (2000). Moreover, Animasaun, *et al*. (2014) noticed that the application of sodium azide concentration at higher concentration at 0.05% can increase pod and peg number of groundnuts.

Number of seeds per plant

The effect of sodium azide on number of seed per plants was recorded in table 3 and 4. The mutagenic agent, sodium azide showed the stimulatory effect on number of seed per plant. It showed the ranges 79.3 to 141.0 for werer-961 and 102 to137 for Sedi variety. Among the two varieties, Sedi showed the highest number (178.3) of seed per plant in 0.05% treatment as compared with control group (137).

Seed yield per plant

Seed yield per plant was observed and recorded in table 3 and 4. It indicated that, yield was increased with increasing the concentration of sodium azide treatments. The highest seed yield was observed in 0.05% treatment in both the varieties. Moreover, among the two selected varieties, sedi showed highest seed yield (76.0gram) as compared with control groups.

Table3. *Effects of various treatment of Sodium Azide on morphological and yield traits of Werer 961 variety*

S.NO	Concentration	Plant Height	Number of leaves per plant	Days to 50% flowering	Days to 90% maturity	No of total pod/ plant	Number of seed per plant	Seed Yield(g/plant)
1.	0.01%	13.67^{c}	91.3^{cd}	38.33^{a}	139.33^{a}	34.00^{g}	79.3^{fgh}	36.2^{def}
2.	0.02%	15.33^{ab}	94.3^{bc}	36.67^{a}	122.67^{ab}	33.67^{g}	78.3^{fgh}	36.0^{def}
3.	0.03%	16.67^{a}	104.7^{ab}	34.00^{b}	122.00^{ab}	40.00^{f}	92.0^{def}	41.9^{cde}
4.	0.04%	17.00^{a}	109.3^{a}	31.67^{bcd}	116.00^{b}	51.33^{cd}	136.7^{bc}	62.3^{ab}
5.	0.05%	17.33^{a}	105.3^{ab}	29.33^{d}	110.00^{bc}	53.00^{c}	141.0^{bc}	63.4^{ab}

6.	Control	13.00^{cd}	91.7^{cd}	32.33^{bc}	132.67^{a}	45.00^{ef}	90.0^{dfg}	41^{cde}
	CV (%)	8.2	6.2	4.4	2.2	6.6	17.6	22.3
	LSD(0.05)	2.13	10.42	2.46	4.67	5.88	44.72	20.4

Means followed by the same letter(s) within column are not significantly different at P=0.05

According to Animasaun *et al*. (2014) the lower concentration of mutagen sodium azide (0.01 to 0.03%) were probably too low to induce desirable agronomic characters in the groundnut crop. Similarly, seed yield also significantly influenced by effects of sodium azide concentration and varietal difference. Many researcher have reported the efficiency values to be higher at lower dose of mutagens such as sodium azide (Roychowdhury *et al*., 2012; Khan and Goyal, 2009)

Table 4. *Effects of various treatment of Sodium Azide on morphological and yield of traits Sedi variety*

S.NO	Concentration	Plant Height	No.of leaves/ plant	Days to flowering (50%)	Days to maturity (90%)	No. of total pod/ plant	No. of seed/ plant	Seed Yield(g/Plant)
1.	0.01%	12.33^{d}	86.3^{cd}	34.00^{b}	137.67^{a}	34.33^{g}	102.7^{cdf}	46.7^{cd}
2.	0.02%	14.67^{bc}	91.3^{bcd}	31.33^{cd}	127.00^{ab}	40.67^{f}	122.0^{cd}	55.6^{c}
3.	0.03%	15.67^{ab}	100.3^{b}	31.33^{cd}	127.00^{ab}	47.33^{de}	127.0^{bcd}	57.5^{bc}
4.	0.04%	16.67^{a}	110.3^{a}	34.00^{b}	117.00^{bc}	61.00^{b}	163.7^{ab}	71.4^{a}
5.	0.05%	17.00^{a}	114.3^{a}	30.00^{cd}	115.00^{bc}	66.67^{a}	178.3^{a}	76.0^{a}
6.	Control	14.33^{bc}	98.0^{b}	32.00^{bc}	135.67^{a}	52.00^{cd}	137.0^{bc}	61.3^{ab}
	CV (%)	8.2	6.2	4.4	2.2	6.6	17.6	22.3
	LSD(0.05)	2.13	10.42	2.46	4.67	5.88	44.72	20.4

Means followed by the same letter(s) within column are not significantly different at P=0.05

CONCLUSIONS

The current needs of mutation breeding are to strengthen the food security and increase the food production due to increasing world human population. To meet this requirement, several experiments have been done throughout the world. One of them is mutation breeding by chemical mutagens. In view of this, an experiment was conducted in laboratory as well as greenhouse experiment for studying seed germination and identifying the quantitative variations and effectiveness of mutation in ground nut. The experiment was laid out in Complete Randomized Design (CRD) factorial with three replications using two groundnut varieties (Sedi and Werer 961). In bioassay study, the number of days for seed germination was increased at the higher concentration in both varieties exposed to mutagenic agent (sodium azide). Moreover, sodium azide showed the stimulatory effect on seed germination especially at 0.02 and 0.03% concentration as compared with control groups. The maximum plumule and radicle length was recorded at 0.05% SA concentration in both the varieties.

In the pot experiment results were revealed that, high concentration of sodium azide 0.05 % had positive stimulatory effects on selected vegetative traits and yield parameters considered in this study. Lower doses of 0.01-0.03 % were probably too low to induce desirable agronomic characters in these crops. Moreover, the highest quantity of oil content was observed in Sedi variety (53.43%) treated with 0.05% of sodium azide mutagen as compared with control and rest of the treatments. Therefore, it is concluded that the effectiveness of chemical mutagens (sodium azide) in groundnut selected varieties is associated with the concentration of sodium azide, which requires different experiments for more generation in order to improve the crop plants of groundnut. In addition further studies were also recommended for concentrations more than 0.05% of sodium azide arrive at optimum concentration for mutation of both groundnut varieties. However, there is a need to repeat the experiment for more than one generation to arrive at conclusive recommendation.

ACKNOWLEDGEMENT

I am thankful to the Oromia Agricultural Research Institute for funding this research and providing necessary facilities.

BIOGRAPHIE

Mr. Gemechis Wektole received the MSc. degree Biotechnology from Haramaya University, in 2015. Currently, the Author is Lecturer in Wellega University, Ethiopia.

REFERENCE

[1] Adamu AK, Aliyu H. 2007. Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill.). *The Scientific World Journal* 2(4), 9-12.

[2] Animasaun,S.Oyedeji, A. Azeez, and O. Onasanya 2014.Evaluation of the Vegetative and Yield Performances of Groundnut (*Arachis hypogaea*) Varieties Samnut 10 and Samnut 20 Treated with Sodium Azide. *International Journal of Scientific and Research Publications*, 4(3).

[3] Begum T, Dasgupta T(2010). A comparison on the effect of physical and chemical mutagenic treatments in sesame (*Sesamum indicum* L.) *Genetics and Molecular Biology* 33: 761-766

[4] Bhatia, CR. 1999. Release of gene silencing in transgenics a new role for induced mutations. *Mutation Breeding Newsletter*. 44: 3-5.

[5] CSA, 2009. Agricultural sample survey of area and production of crops of 2008/2009 in Ethiopia. Government annual report on area and production of crops, Addis Ababa, Ethiopia, pp. 1-128.

[6] CSA, 2013. The Federal Democratic Republic of Ethiopia Central Statistical Agency. Agricultural Sample Survey 2012 / 2013. Volume I: Report on Area Under production of Major Crops. Pp 14.

[7] Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M and Thilagavathi C, 2008. Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* L.) Walp.) *African Journal of Biotechnology*, 7: 4116-4117.

[8] FAOSTAT. 2010. Groundnut world production. http://www.faostat.fao.org.

[9] Gbèhounou, G. and Adengo, E. (2003). From a crop rotation experiment at Akron. Effect of citric acid on aflatoxin degradation and on functional and textural. Food Research International, Volume 42, Issue 8.

[10] Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research*, 2nd edition, John Wiley and Sons, New York, p.680.

[11] Heisey R.M.1990. "Allelophatic and herbicidal effects of extracts from tree of heaven (Ahanthus attissima)", *American Journal of Botany*, 77, 662-670,

[12] Kulkarni GB, 2011. Effect of mutagen on pollen fertility and other parameters in horse gram (*Macrotyloma uniflorum*(Lam.) Verdc). *Biological Science Discovery*. 2 (1): 146-150.

[13] Mensah JK,Akomeah PA. Ekpekurede EO 2005. Gamma irradiation induced variation of yield parameters in cowpea *Vigna unguiculata (*L) Walp.) *Global journal of pure applied science* 11(3):327-330.

[14] Mostafa, G.G. 2011. "Effect of sodium azide on the growth and variability induction in *Helianthus annus* L.", *International Journal of Plant Breeding and Genetics. 5: 76-85.*

[15] Neeraj and M. Anis. 1995 "Effects of methyl methane sulphonate on seed germination and quantitative characters in chilli", *Advanced Plant Science* 8(1) pp.100-105,

[16] Pande, N., Saxena, J. and Pandey, H. 2003. Natural occurrence of mycotoxins in some cereals. *Mycoses* 33:126-128.

[17] Sharma,S.K.,Ritu Sood and Pandey, D.P. 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper). *Indian Journal of Genetics*, 65: 20-22.

[18] Siddiqui, S., Meghvansi and H. Zia-ul, 2007. Cytogenetic changes induced by Sodium Azide (NaN3) on *Trigonella foenum*- graecum seeds. *South African Journal of Botany*, 73: 632-635.

[19] Thilagavathi, C. and L. Mullainathan, 2009. Isolation of macro mutants and mutagenic effectiveness, efficiency in black gram (*Vigna mungo*(L.)Hepper).*Global Journal of Molecular Science*, 4 (2): 76-79.

[20] Upadhyaya, H.D., Reddy, L.J., Gowda, C.L. and Singh, S. 2006. Identification of diverse groundnut germplasm: Sources of early maturity in a core collection. *Field Crops Research* 97: 261-271.

[21] Wijnands, J.H.M., Biersteker, J. and Van Loo, E.N. 2009.Oil seed business opportunity in Ethiopia. *Oil Seed Research Report*. Addis Ababa, Ethiopia.

Citation: *Gemechis Waktole (2018)." In-Vitro Induction of Mutation in Groundnut (Arachis hypogea L.) by Using Sodium Azide Mutagens and Its Impact on Selected Agronomic Traits". Annals of Ecology and Environmental Science, 2(3), pp.27-32*