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The utilization of mutagenesis using sodium azide to improve sesame (*Sesamum indicum* L.)

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Abstract
Sesame (*Sesamum indicum* L.) is considered to be an ancient oil seed crop to generate high quality edible oil having nutritional and health related value but still at an early stage inbreeding. Its chromosome number is 2n=26 and it belongs to the Pedaliaceae family and having significant economic value globally as well as in Ethiopia. In general terms Sesame is unimproved and variety of collections which have been generated of land races, with little or no genetic information that can lead to its application in breeding programs. With the objective of improving the performance of sesame (*Sesamum indicum* L.) by the utilization of mutagenesis using sodium azide, an experiment was conducted. In this experiment, healthy and dry seeds of sesame (*Sesamum indicum* L.), were treated with sodium azide in ascending mutagen concentrations of 0.0165, 0.033, 0.0625, 0.125, and 0.25% targeted at determining the effects of the chemical mutagens to promote genetic variability in terms of the agro morphological parameters of sesame following the appropriate procedures. A gradual decrease was observed in mean root length percentage with increasing concentrations. Reduction in seed germination was observed at 0.0625% concentration in T3 when compared with control and the other treatments but it was increased again in T4 and T5. Root growth was high in the control and in 0.0165% and 0.033% concentrations, when compared to the higher concentration treated plants. This revealed that the reduction in seed germination, root length and shoot length percentage was associated with the increase in the dose/concentration of mutagens even if it shows some increasing in seed germination in T4 and T5. Since the produced mutants from first generation are not adequate for studying the genetic stability these traits should be investigated for the desired traits in subsequent generations and in the field conditions, developing sesame varieties resistant for different biotic and abiotic stresses and assisting the present work with the recent bio-molecular techniques should be future prospects.

Keywords: *Sesamum indicum* L, mutation, quantitative trait, mutagen, sodium azide

Introduction
Sesame (Sesamum *indicum*) is a flowering plant in the genus sesanum, also called *benne*. Sesame (*Sesamum indicum* L., 2n = 26), which belongs to the Pedaliaceae family, is one of the most ancient oilseed crops and is cultivated in tropical and subtropical regions of Asia, Africa and South America (Brar and Ahuja, 1979; Ashri, 1998.) [8, 5]. This family harbors 16 genera and 60 species and is a small family. The most important genus of this family is Sesamum. Sesame (*Sesamum indicum* L.) is considered to be an ancient oil seed crop (Brar and Ahuja, 1979) [8].

Sesame is cultivated in tropical and sub-tropical regions, in plains, up to an elevation of 1200m, and mainly in the dry and hot tropics in the areas with an annual rainfall of 500-1125mm. Sesame draws its importance from the fact that it is a food crop, a raw material for industry, feed for livestock and an export crop (Brar and Ahuja, 1979; Ashri, 1998) [8, 5].

It is an important oil seed crop in the world. Its grain is an excellent source of high quality oil, protein, carbohydrate, calcium and phosphorous, and ranks among the top thirteen oil seed crops, which makes up to 90% of world edible oil production. Sesame oil contains sesamoline and sesame which is used as synergist for insecticides, and its oil which has high contents of oleic (47 %) and linoleic oil (39 %), does not turn rancid unlike other edible oils because of the presence of antioxidant, are used mainly for cooking purposes, salad oils and margarine, and in the manufacture of soaps, paints, perfumes, pharmaceuticals and insecticides.

As reported by Abraham Birara, 2012 it is cultivated for its edible leaves used as vegetables (Mann *et al*., 2003) [17] or oily seeds (Burkill, 1997) [9]. It is an important oilseed crop used to generate high quality edible oil and protein for low income peasants of major sesame growing countries including Sudan, Ethiopia, Uganda, Nigeria, Mexico, Venezuela, India, China, Pakistan, Turkey and Myanmar (Oplinger *et al.,* 1990) [18]. India, with 2.5 million hectares under cultivation of this crop, is a leading sesame producer, accounting for 40% of the world's sesame area and 27% of world production. China is another major sesame growing country with about one million hectares under cultivation and 0.4 million tons of seed production per year. Sesame is typically an annual species (Mahandjiev *et al*., 2001) [12].

The African continent is naturally gifted with suitable weather conditions that can enhance sesame production. The crop requires only 500-650 mm of rainfall per annum. Ethiopia is known to be the origin of diversity for cultivated sesame (Ashri, 1994, 1998; Weiss, 2000; Uzun and Cagirgam, 2006) [5, 6. 23, 22]. Its seed harbors 50-60% oil and 25% protein with *antioxidants* lignans such as sesamolin, sesamin and has been used as active ingredients in antiseptics, bactericides, vermicides, disinfectants, moth repellants, anti-tubercular agents and considerable source of calcium, tryptophan, methionine and many minerals.

There are lots of varieties of *Sesamum indicum* L. according to the size, form and color of flowers, seed size, color and composition. Variation can also be manifested in such a way that some varieties are highly branched whereas others are unbranched (Peter, 2004) [14]. It has been demonstrated by many workers that genetic variability for several desired characters can be enhanced successfully through mutations and its practical value in plant improvement programs has been well established.

Mutations are the tools at hand exploited by the geneticist to study the nature and function of genes which are the basis of plant growth and development, hence producing raw materials for genetic improvement of economic crops (Adamu *et al*., 2007) [3]. Primarily the advantage of mutational breeding is the probability of improving one or two characters without amending the rest of the genotype. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001) [12].

Sodium azide is the inorganic compound with the formula NaN_3. This colorless salt is the gas-forming component in many car airbag systems. It is used for the preparation of other azide compounds. It is an ionic substance, is highly soluble in water, and is very acutely toxic. Sodium azide is an ionic solid. Two crystalline forms are known, rhombohedral and hexagonal. Both adopt layered structures. The azide anion is very similar in each form, being centrosymmetric with N–N distances of 1.18 Å. The Na^+ has octahedral geometry. Each azide is linked to six Na+ centers, with three Na-N bonds to each terminal nitrogen center. It is used in agriculture for pest control of soil-borne pathogens such as *Meloidogyne incognita* or *Helicotylenchus dihystera*.It is also used as a mutagen for crop selection of plants such as rice, barleyor oats. The successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in groundnut (Mensah and Obadoni, 2007) [8], barley (Kleinhofs and Sander, 1975) [10] and other crops (Avila and Murty, 1983).

Unfortunately, average world yield of sesame is still low at 0.46 ton ha^{-1}. Low yield had been attributed to cultivation of low yielding dehiscent varieties with low harvest index values, significant yield loss during threshing and shortage of agricultural inputs such as improved varieties, fertilizers and other agro-chemicals (Ashri, 1994, 1998; Weiss, 2000; Uzun and Cagirgam, 2006) [5, 6, 23, 22]. However, non- dehiscent sesame varieties with yield potential of over 1 ton ha^{-1} and appropriate for mechanical combine harvest have been developed by Sesame Coordinators SESACO in USA. Germination of sesame seed is moderately slow and seedlings grow slowly until they reach a height of 10 cm, the growth habit is generally indeterminate but determinate cultivars have been selected (Adamu *et al*., 2007) [3].

The crop can tolerate periods of drought, but does not tolerate water logging and stem breakage which depend on a number of *factors* including stem strength, head weight, plant height and type of root system could result in poor stands at harvest, and prefers light, well drained soils that will retain adequate moisture and it does reasonably well on poor soils. The Sesame plant is about 60 to 120cm in height and the fruit is a dehiscent capsule held close to stem and when ripe, the capsule shatters to release a number of small seeds which are protected by a fibrous hull or skin, which may be whitish to brown or black depending on the variety. Sesame is deep rooted and well adapted to withstand dry conditions. The crop is grown under a range of environments, which probably affects its performance (Peter, 2004) [14].

Generally Sesame is unimproved and variety of collections have been generated of land races, with little or no genetic information that can lead to its application in breeding programs. A number of factors affecting sesame improvement programs have been identified. Firstly, the germ plasm of sesame is not as large as in other crops (1982). Secondly, the genetic architecture of sesame is poorly adapted to mechanized farming system due to its indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Uzun and Cagirgan, 2006) [22].

As reported by Abraham Birara *et al*., 2014 artificial induction of mutation is of scientific and commercial interest as it is one of the methods used in improving the growth and yield of economic plants. It provides raw materials for the genetic improvement of economic crops (Adamu & Aliyu, 2007) [3] and also used to create genetic variability in quantitative traits in various crop plants (Mahla *et al.,* 1990; Shah *et al*., 1990; Mahandjiev *et al*., 2001) [12]. Generally mutation breeding generates a knowledge base that guides future users of mutation technology for crop improvement.

To increase production of the crop there is a need to have a better understanding of its genetic background. However, there is no information on the locally cultivated varieties, which lack variability because of their self-pollination status. The present study was therefore undertaken to fill the gap in knowledge of the genetic background of the crop and assess the effect of the chemical on the plant with the aim of inducing mutation in the seeds of sesame for the improvement of its growth and yield parameters. It is our hope that the mutagen would induce variability and thus provide information on the pattern of variation in mutagenic experiments using these mutagens on sesame plant Mahandjiev *et al*., 2001 [12].

Materials and Methods

Materials used

Distilled water, Bottles with different capacities, Soft, Sodium azide powder, Glove, Alcohol, Labeling paper and marker pen, Sesame seed, Petri plate, Mercuric chloride, sponge, ruler, pipette, Detergent, Measuring cylinder and Beam balance were used to do this research.

Description of the study area

The research was carried out in Ethiopia at Mekelle University Enda Eyesus campus in plant science laboratory.

Study design

The experimental design employed was factorial CRD with three replications for each treatment and control. The design of the study involved laboratory experiments. The laboratory study was based on bioassay aimed to assess the seed germination percentage and length of germinated seeds shoot and root.

Laboratory examination

The induced chemical mutagenesis study utilized one mutagenic chemical, namely sodium azide. The selection of the mutagenic chemicals was based on their effectiveness, availability and inspiration from previous works.

Preparation of mutagenic agents

Ascending concentration gradients of Sodium azide (NaN3) with (0.0163, 0.033, 0.0625, 0.125 and 0.25 %) was prepared in glass beaker using distilled water as a solvent.

Methods of seed Mutagenesis

Seeds of Sesame (*Sesamum indicum*) variety were used for inducing mutation by sodium-azide (NaN3). 180 dry and normal uniform seeds were used; from these 30 was used as a control. The seeds of selected variety were surface sterilized with 0.1% mercuric chloride for 1 minute to remove the fungal spores on the surface of the seeds. Then the seeds were washed with distilled water three times to remove the mercuric chloride. After this treatment seeds were presoaked in glass beaker which contained distilled water for six hours and then 150 seeds were treated with mutagenic agent of sodium aside at different concentrations of NaN3 (0.0163, 0.033, 0.0625, 0.125 and 0.25 %) each with30 seeds for 6 hrs.

Methods of seedling

Five (5) treatments and 1control each with 3 replication was used. Each petri plate was moistened with 2ml/ plate of distilled water. Five treated seeds were placed on soft in a (petri plates) in each replications. Watering 2ml/plate and observation was continued daily until maximum germination would attained on the 10th day after sowing (DAS).

Data analysis

Germination data was recorded randomly on five plants per plot for ten days to determine germination percentage. At the 10thday shoot and root length of each plant was measured and recorded. The collected data was subjected to analysis of standard deviation, standard error and mean.

Results and Discussion

A gradual decrease was observed in mean root length percentage with increasing concentrations. Reduction in seed germination was observed at 0.0625% concentration in T3 when compared with control and the other treatments but it was increased again in T4 and T5. Root growth was high in the control and in 0.0165% and 0.033% concentrations, when compared to the higher concentration treated plants. The result indicates that there is no that much variation of mean shoot length between the treatments and also the control even if the better performed was the control as shown in the figure below.

Germination percentage (%) of Sesame seeds treated with sodium azide

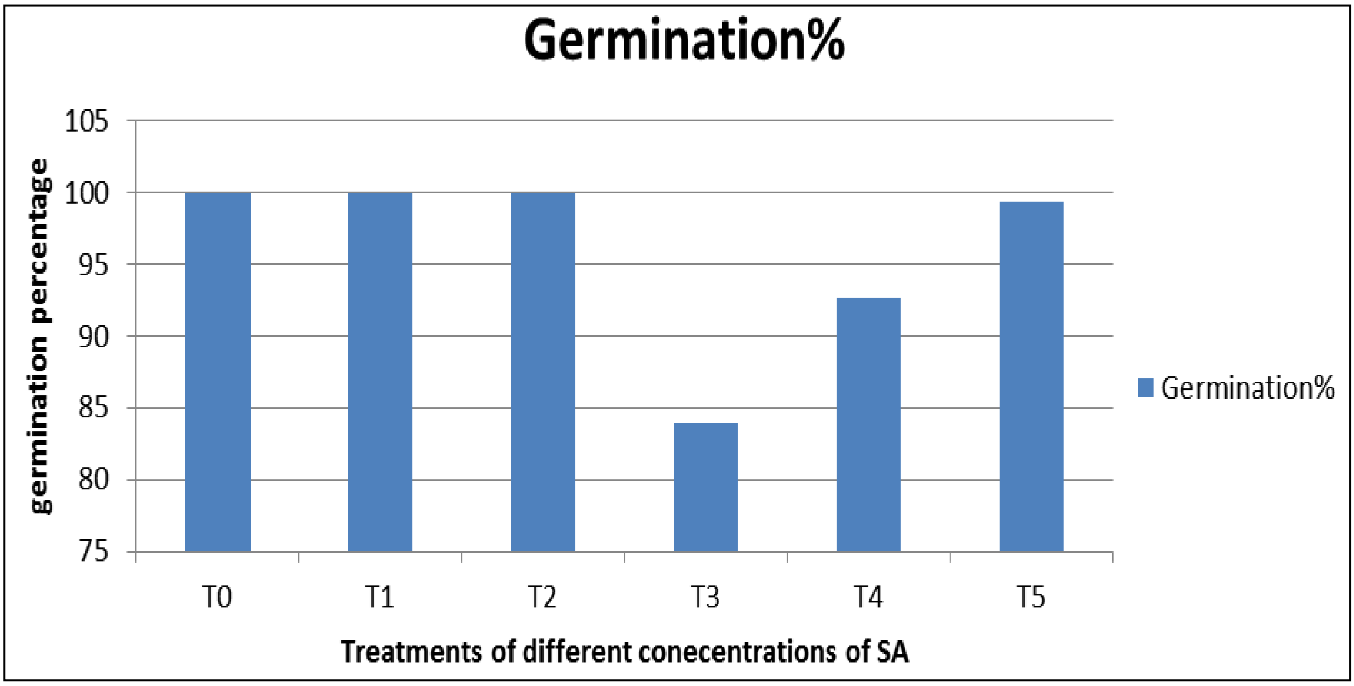

Fig 1: Germination of seed treated with sodium azide A 100% of germination was observed in T1 and T2 which was exactly similar with the control. An 84% of a minimum observation and a successive increase in T4 and T5 with 92.6% and 99.3% were recorded respectively.

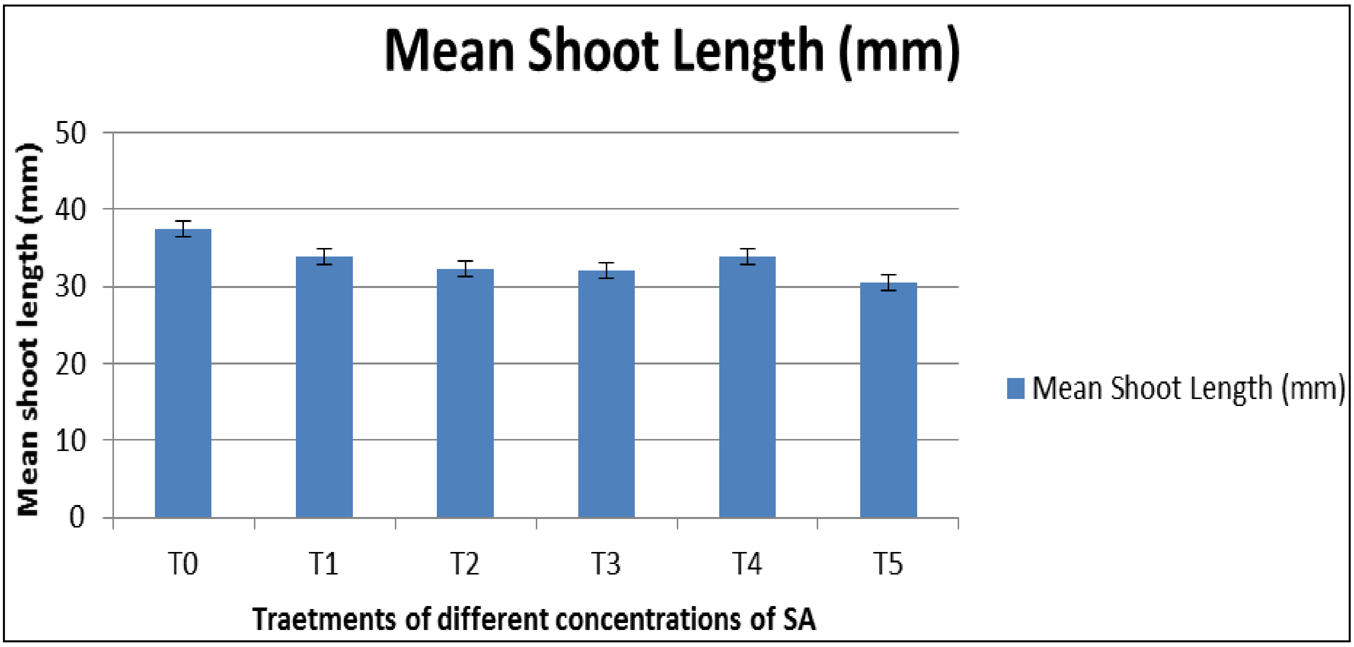

Fig 2: Effect of sodium aside on shoot length of sesame A maximum mean shoot length of 37.6 was observed on the control and a successive decreasing of mean shoot length except T4 with a mean shoot length of 33.85 was resulted. The mean shoot length of T1, T2, T3 and T5 was 33.93, 32.33,32 and 30.46 respectively.

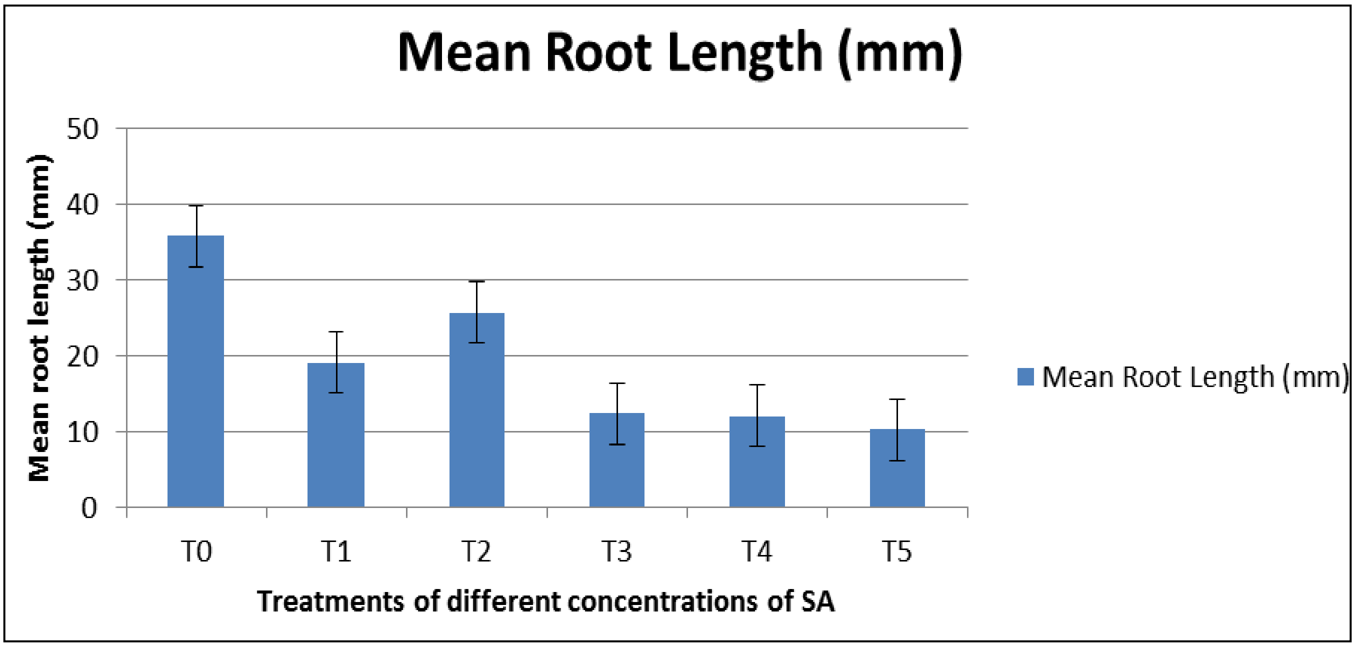

Fig 3: Effect of sodium azide on root length of sesame A gradual decreasing of mean root length was observed from the control to T5 but a little increasing on T2 was recorded. A result of T0 (35.8), T1 (19.13), T2 (25.73), T3 (12.38), T4 (12.07) and T5 (10.2) with a maximum mean root length in the control and a minimum mean root length at T5 was observed.

The present study showed mutagenic effect of sodium azide on Sesame. This revealed that the reduction in seed germination, root length and shoot length percentage was associated with the increase in the dose/concentration of mutagens or may be an error even if it shows some increasing in seed germination in T4 and T5. The stimulatory effect on plant height was in the control than the different concentrations of mutagenic agent, sodium azide.
Reductions in germination and root length percentages due to the effects of mutagens on various crop plants have earlier been documented (Mensah, 1977; Mensah and Akomeah, 1997; Mensah *et al*., 2005) [14]. The control performed better in germination, mean root length and mean shoot length. The observed germination result shows that the control and the treatments with little chemical amount performed the germination better. Almost a similar result was recorded by Mensah *et al*, 2006 [14] that both the germination and root length was gradually decreased as the concentration increases as shown in the figure below.

Conclusion and Recommendation

Generally, a reduction of root length and shoot length were recorded when the treatment concentration increases. The germination at low concentration of T1 and T2 and at the control was exactly similar. But a reduction of germination at T3 was recorded and a gradual increase was observed successively in T4 and T5. The control has performed better in all the three measures; shoot length, root length and germination and reduced when the treatment concentration increases. Therefore, reduction percentage was associated

with the increase in the dose/concentration of mutagens. An error or a mutation occurrence might also be the reason. So we can conclude that the increase in sodium azide concentration affects both the shoot and root length highly and the germination in some extent.
However, there is a need to repeat the experiment for more than one generation to arrive at conclusive recommendation. Since the produced mutants from first generation are not adequate for studying the genetic stability these traits should be investigated for the desired traits in subsequent generations and in the field conditions, developing sesame varieties resistant for different biotic and abiotic stresses and assisting the present work with the recent bio-molecular techniques should be future prospects.

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