"EFFECT OF SODIUM AZIDE ON SEED GERMINATION OF MUNGBEAN."

Project Report

Under

DBT Star College Scheme
Department of Biotechnology, New Delhi

By

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Certificate

This is to certify that the work incorporated in the project report on **"Effect of Sodium azide on Seed Germination of Mungbean."** by **Miss. Shelke Pratiksha Laxmun, Miss. Toge Mayuri Ashok, Miss. Pawar Akanksha Ashok, Miss. Pandule Akanksha Dattatray** are students of Arts, Commerce and Science College Sonai, Tal. Newasa, Dist. Ahmednagar. Affiliated to the Savitribai Phule Pune University Pune successfully completed project.

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Declaration

We hereby declare that the work done in this thesis entitled on "**Effect of Sodium azide on Seed Germination of Mungbean.**" is submitted to Department of Botany, Arts, Commerce and Science College Sonai. This project is completed under the DBTStar College Scheme and the supervision of **Dr. V. E. Darandale.** The works is original and not submitted in part or full by me or any other to this or any other University.

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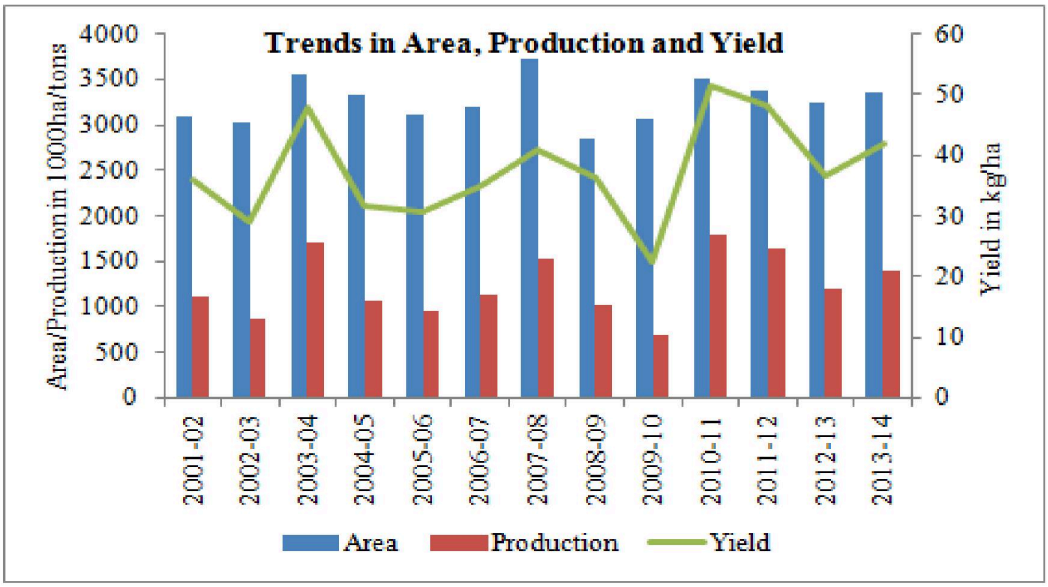
INTRODUCTION:

India grows a variety of pulse crops, also called as grain legumes, under a wide range of agro-climatic conditions and has a pride of being the world's largest producer of pulses. Unique characteristics like high protein content (2 to 3 times more than the cereals), nitrogen fixing ability, soil ameliorative properties and ability to thrive better under harsh conditions make pulses an integral component of sustainable agriculture particularly in dry land areas. Indian population relies on pulses for meeting its protein requirement mainly because of its vegetarian food habit and high cost of animal based protein. A solution to the problem of declining per capita availability has, therefore, to come from a rapid improvement in indigenous production levels. While efforts have been geared up to bring additional area under pulses, more important is to increase the production by exploiting yield potential of the existing varieties through genetic manipulation. India is the largest producer and consumer of pulses in the world accounting for about 29 per cent of the world area and 19 per cent of the world's production (Singh, *et. al,* 2015). The estimates for 2011 indicate that the pulses occupy an area of 23.63 million hectares and produce 14.76 million tonnes with an average yield of 624 kg/ha (ZPKD, 2011). The non-availability of high yielding varieties is a major constraint in achieving higher productivity of pulses. Non synchronous maturity, long duration and flower drop are other problems associated with the varieties of major pulses.

Mungbean or green gram *(Vigna radiata* (L.) Wilczek) which ranks third to chickpea and pigeon pea is an important pulse crop in Southeast Asia and the Indian sub-continent. Mungbean is grown in almost all the states of India and is cultivated as a kharif (monsoon) and summer season crop in different agro-ecological regions. Loam to sandy loam soils with good internal drainage are considered ideal for mungbean cultivation (Singh, *et. al,* 2015).

Production in India –

India is the world's largest producer as well as consumer of green gram. It produces about 1.5 to 2.0 million tons of Mungbean annually from about 3 to 4 million hectares of area, with an average productivity of 500kg per hectare. Green gram output accounts for about 10-12% of total pulse production in the country.



Source: Ministry of Agriculture, GoI

Mungbean production in the country remained stable more than a decade through the 2000s at around 10 to 15 lakh tons. But a sudden jump in output was noted in 2010-11 to 17.5 lakh tonnes primarily on account of rise in production from Madhya Pradesh, Rajasthan and Tamil Nadu.

Botanical description:-

Mungbean belongs to the genus *Vigna* of the family Fabaceae (Papilionaceae). The plant has tap root, provided with nodules. Stem is erect or suberect, fiirrowed and branched. Leaves are trifoliate, ovate with large petiole. Inflorescence is axillary raceme. Flowers are cleistogamous, bisexual with papilionaceous corolla, 10 stamens [(9) + 1], monocarpellary, unilocular and superior ovary. Pollination takes place before the opening of the flower bud. Mature pods are round and have grey or brownish colour. Seeds are globular, green but sometimes marbled with yellow brown, purple brown or black; hilum is white, round and more or less flat. Germination is epigeal (Wani, 2007).

Economic importance:-

Legumes are next to cereals as a source of food. They constitute an important part of people's diet. The beans, peas, soybeans etc. are the sources of high protein rich seeds. These vegetables proteins are very much essential for balancing the carbohydrates rich cereals.

Seeds of mungbean are highly nutritious containing 24% protein, 1.0 - 1.5% fat, 3.5 - 4.5% fibre, 4.5 - 5.5% ash and 59 - 65% carbohydrate

on dry weight basis and provide 334 - 344 k cal energy (Azad, 1998). The mineral component is high in phosphorous (370 mg/100 g), calcium (118 mg/100 g) and iron (8 mg/100 g). Mungbean protein is considered to be easily digestible. Being rich in quality proteins, minerals and vitamins, it is inseparable ingredient in the diets of a vast majority of Indian population. The dried grains of mungbean can be split or eaten whole after cooking and made into a soup or dhal (porridge). Mungbean is also eaten as sprouts. Green pods and seeds can be cooked as vegetables. It is also recommended as a medicinal diet in case of flatulence and to the sick. It is rich in vitamin B and is regarded as a remedy for beri-beri. Dried and green stalk and leaves of mungbean are used as fodder (Wani, 2007).

Mutagenesis:-

Plant breeding particularly Chemical mutagenesis is a creative work essential to solving the global problem of food provision. The Green revolution emphasized by the fact that permanent increase of yield per unit area is a solution to the problem of providing food to the rapidly expanding population. The variations encountered with many of the agronomic characters such as yield are of continuous type and have resulted from polygenic interaction with the environment. Most of the plant attributes of interest to a plant breeder are quantitative characters which are controlled by polygenic interaction. In such situation the efficiency of selecting the desired mutant is generally lower than for specific characters which are controlled by a single gene. Micro mutations produce genetic variability in quantitative characters of the crop plants. Hence, they deserve full attention of plant-breeders. Such mutations should be useful for improving quantitative inherited traits (e.g. grain yield) without disturbing the major part of the genotypic and phenotypic architecture of crop (Azad, 1998. Shu, 2011).

Mutation breeding, a much heralded short cut breeding method, brings novel genotypes through heritable changes in genotype and phenotype of a particular trait. Often ever, desired variation is lacking in grain legumes especially in Mungbean. In the present study, the effects of Sodium Azide were studied on percent germination and early seedling growth of Mungbean (Auti and Apparao, 2009, Shu, *et.al,* 2011).

Objective -

- To study the effect of Sodium azide as a mutagenic treatments on seed germination in Mungbean.

MATERIAL AND METHODS:

Experimental Plant Material-

Experimental plant material selected for the present investigation on Effect of chemical mutagen on mungbean (*Vigna radiata* L.). Germplasm of the cultivar was obtained from the pulses improvement division of mahatma Phule Krishi Vidhyapith (M.P.K.V.) Rahuri (District Ahmednagar, Maharashtra, India). The cultivar is desi type, commercially and wide cultivated in the area of Ahmednagar district.

Chemicals –

1) **Sodium Azide AR** – (NaN_3) Mol wt – 65.01
   Loba Chemie Pvt. Ltd. Mumbai

Mode of Treatment with Mutagens-

The pilot experiments were conducted to determine the lethal dose (LD50), suitable concentration of the mutagens and duration of treatment for Mungbean. From the pilot experimental result concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 % Sodium azide for duration of 4 hours were used for mutagenic treatments. For treatment with chemical mutagen, the seeds were first sterilized with 0.01% of $Hgcl_2$ and then were treated with distilled water, and soaked in chemical mutagen like Sodium azide for about Four hours.

Methods of administration of Chemical mutagens-

Healthy and uniform seed of the Mungbean were surface sterilized with 0.1% mercuric chloride solution for about one minute and washed thoroughly with distilled water. They were pre-soaked in Chemical mutagen treated with freshly prepared mutagenic solution for Four hours all chemical mutagenic treatment were carried out at room temperature 30 ± 5^0C with intermediate shaking. The seed soaked in distilled water served as control.

Post treatment Handling –

The seeds treated with various concentrations of chemical mutagens were thoroughly washed under running tap water before sowing about an hour to terminate the reaction of the chemical mutagen and to leach out the residual chemical. The treated and control seeds were blotted. Each treatment and untreated (Control) was carried out 50 seeds were germinated in Between Paper method in germination paper.

Mutagenic studies

Observations-

1. **Percent seed germination –**

50 seeds each treatment along with control was germinated in five replications on moist germinating paper in using distilled water. Seeds were allowed to germinate in at room temperature 30 $\pm$ 5^0C for 8 days. The germination parameter recorded after 4^{th} days. Seeds which produced both plumule and radicals were considered as germinated. The percent seed germination calculated as follows.

Formula –

$$\textbf{\% Seeds germination} = \frac{\textbf{Total No.Of germinated seeds}}{\textbf{Total No.of seeds}} \textbf{ X 100}$$

2. **Seedling injury-**

Seedling height was recorded on the 8^{th} day only in the germinated seeds. Reduction in the means seedling length of the treated seedlings as compared to the control was regarded as seedling injury and expressed as percentage.

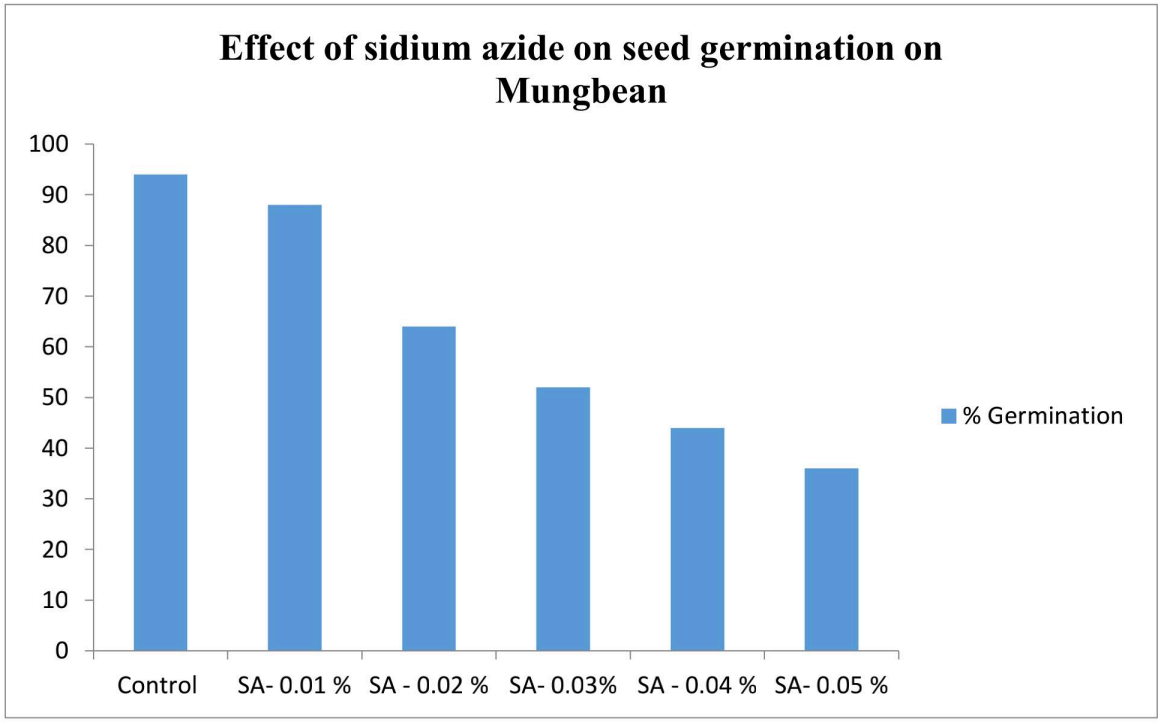
RESULTS AND DISCUSSION:

Experimental results obtained in the present investigation on effect of Sodium azide as a mutagenic treatments on seed germination in Mungbean. *(Vigna radiata* L.).'' Effect of different treatments of Chemical mutagen biological parameters such as percent germination, seedling injury Etc. were studied. An attempt was also made to asses' mutagenic sensitivity of those two cultivars of Mungbean. The results obtained are as follows.

1. **Percent seed germination –**

Data obtained on effect of different concentration / dose of sodium azide, on mean percent seed germination in control and mutagen treated fenugreek cultivar is presented in fig. from the table. It is evident that percent seed germination in the cultivar is subjected to treatment with different concentration of sodium azide, is defiantly less than those of their respective controls. It clearly indicates that both the mutagens have exerted an inhibitory effect on seed germination. Percent seed germination decreased with an increase with a concentration or dose of mutagen. The percent germination was 94% in control. After treatment with sodium azide the percent seed germination has decreased from 94 to 36%. so this concentration of Chemical mutagen was adjusted as LD_{50} for more mutagen for this variety.

Effect of Chemical mutagen on seed germination-



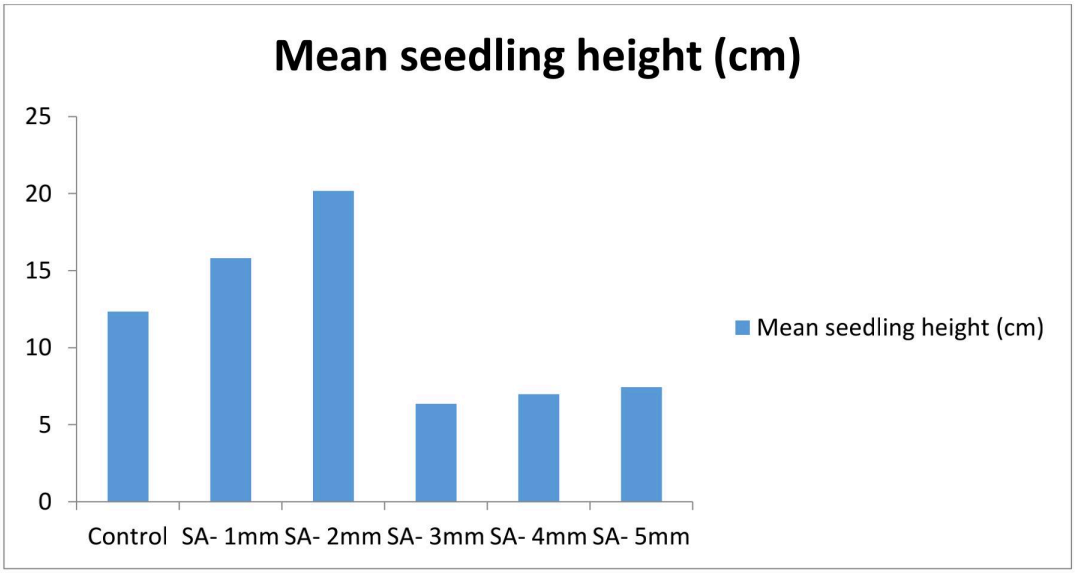
Treatment	Concentration	Seed % Germination
Control	0.0	94
Sodium Azide (SA)	0.01 %	88
	0.02%	64
	0.03%	52
	0.04%	44
	0.05%	36

Seedling injury (Height)

Seedling injury was measured as both increase and reduction in the height of the mutagen treated seedling as compared to the height of the 8 days old control seedlings.

Effect of sodium azide on Mean seedling height

Conc. of Sodium azide	Mean seedling height (cm)
0.0 (Control)	12.35
0.01 %	15.82
0.02%	20.18
0.03%	6.35
0.04%	6.97
0.05%	7.44



CONCLUSION:

In present investigation attempt was made to study effect of Sodium azide as a mutagenic treatments on seed germination in Mungbean. *(Vigna radiata* L.).'' The experimental results obtained were presented in Experimental result. Interpretation of these experimental results in light of the result reported by the other worker on the same or similar mutagens and experimental system on their significance is discussed at length in this chapter. Significance of result obtained on parameter viz., percent seed germination, seedling injury obtained as result of treatment with different concentration of Sodium azide during this result discussed in detail under the following separate heads.

Percent seed germination;-

Results indicated that, percent seed germination in all Chemical mutagen treated plants decrease with increasing concentration of chemical mutagen in Mungbean. This clearly indicates that the mutagens have exerted on inhibitory effect on seed germination. Similar inhibitory effect on seed germination by the mutagens have also been reported earlier by Wani (2007). Mungbean attributed this two physiological damage and chromosomal mutation induce by physical and chemical mutagens. According to (Auti and Apparao, 2009) differential response of cultivars to mutagen could be due to the difference in their eco-morphological, genetic, seed size and physiological organisation. In present investigation in the mutagenic sensitivity of wild verities of Mungbean may be due to difference in size of seed. Pre-soaking of seed increase the sensitivity to chemical mutagen (Shu, et.al,2011). This is subjected to various reasons, such as changing metabolic process.

Seedling injury.

Chemical Mutagen show clearly promontory effect on height of the seedling at lower concentration. The effect is concentration depended. The maximum promoting effect height of seedling was observed 0.03 % concentration of sodium azide show reduction in seedling height (6.35 cm). The reduction in plant height by irradiation and chemical mutagen was described to different factor. According to Wani (2007) reduction in seedling growth is due to slow rate of meristematic action at shoot apex and arresting of mitotic cycle.

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