The Planta RBS: Vol.-2 Issue-1 (April) 2021: 633 - 641
*(Research Book Series – www.pgrindias.in)*

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# EFFECT OF CHEMICAL AND PHYSICAL MUTAGENS ON SEED GERMINATION, SEEDLING HEIGHT AND SEEDLING VIGOUR IN *Glycine max* C.V. Js-9560#

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**Abstract**

Genetically pure and physiologically similar seeds of soybean (*Glycine max* c.v. JS-9560) were treated with different doses of chemical mutagen sodium azide and physical mutagen gamma rays. The doses were determined on the basis of lethal dose 50 ($LD_{50}$). The doses selected for the treatment of seeds with sodium azide were used by two different methods - dry and water pre-soaked. Potency of sodium azide and gamma irradiation was observed on different germination and growth parameters such as percent germination rate, seedling height (shoot length and root length and seedling vigour (vigour index). In sodium azide dry and water pre-soaked treatment, germination percent was minimum at 0.19% i.e. 40.00% and 76.00% respectively; while seedling vigour of 18hrs dry and 12hrs pre-soaked + 6hrs sodium azide treatment was minimum at 0.19% (846.72) and (1678.11) respectively. It was found that germination percentage was minimum at130kr (58.00%) in gamma irradiation treatment. As compare to control and other doses, 130kr shows minimum value (266.00) for seedling vigour. This indicates the effectiveness of sodium azide and gamma rays on the germination rate, seedling height and seedling vigour. The findings reveal that, with the increase in doses of sodium azide and gamma irradiation there was gradual decrease in the seed germination and seedling growth. All treatments found to effective to induce mutation and generate phenotypic as well as genotypic variants growth.

**Keywords:** Seed germination, seedling height, seedling vigour, sodium azide, gamma rays

#Research Article

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## Introduction

Soybean (*Glycine max* (L.) is an annual leguminous species cultivated mainly for its seed as a source of edible oil and protein source. It is used in a variety of industries, providing products for human consumption, livestock feed and industrial purposes. Soybean seed consists of 35% carbohydrate, 5% ash, 40% protein and 20% oil and is a major source of protein and oil for commercial products. It is also used to produce a high protein animal feed. Soybeans were used for tofti, soymilk, soy sprouts, natto, industrial products such as paints, linoleum, printing inks, soaps, plastics, cosmetic, pharmaceuticals, leathers, insecticides and disinfectants. The 'Lecithin' phospholipids obtained as a byproduct of the oil industry, often finds use as wetting and stabilizing agents in the food. The meal and soybean protein are used in manufacture of synthetic fiber (artificial wool), adhesive, textile sizes, waterproofing and fire fighting foam ( Hildebrand *et. al,* 1986).

Research has been concentrated on rich contents soybean oil with high oleic acid, low linolenic acid, high stearic acid and high and low palmitic acid. This can be done by changing the degree of unsaturation and by increasing or decreasing the fatty acid chain length (Topfer *et. al.,* 1995). Stearic and palmitic acids are one of the major saturated fatty acids in soybean oil. A reduction of palmitic acid in soybean oil increases the oil nutritional value, as palmitic acid helps to increase LDL cholesterol in the blood and susceptibility to coronary heart disease. Also, consumption of less palmitic acid shows low occurrences of breast, colon and prostate cancers (Stojsin *et. al.,* 1998).

Soybean builds up the soil fertility by fixing large amounts of atmospheric nitrogen through the root nodules, and also through leaf fall on the ground at maturity. Recently it has been suggested that the consumption of soy foods may contribute to the lowering incidences of breast, colon and prostrate cancers as seen in countries like China and Japan. Soybean contains a number of anti carcinogens. Most recently, interest in soybean has increased due to the presence of isoflavones, compounds that may play an important role in preventing and treating chronic diseases (Munro *et. al,* 2003)

Gamma irradiation and seed treatment with Sodium azide, as potential mutagens can induce physiological and biochemical changes in plants useful as well as harmful mutation in plants (Micke and Domini, 1993; Gupta, 1996). Studies on changes in seed germination pattern in soybean were under the influence of these two mutagens were undertaken during present investigation.

## Material and Methods

In the present investigation, different concentrations/doses of physical mutagens i.e. gamma rays were 10Kr, 30Kr, 50Kr, 70Kr, 90Kr, 110Kr and 130Kr. Determination of lethal dose 50 of chemical mutagen were carried out at Cytology and Genetics laboratory, Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati. On the basis of evaluation of lethal dose 50, three doses i.e. 0.15%, 0.17% and 0.19% were determined for the two chemical mutagen treatments i.e. 18hrs for dry sodium azide treatment and 12 hrs for pre-soaked in water ,+ 6hrs sodium azide treatment. After mutagen treatments, seeds were rinsed for 30 min. with running tap water to completely remove mutagens and post soaked for 1Hrs. in Distilled water.

For seed germination 50 seeds of each dose along with control were kept in petri-dishes on blotting paper in triplicates. The emergence of radical was taken as indication for germination of seeds. Germination percentage was calculated by counting the germinated seeds and total number of seeds sown. Further percent over control and reduction over control were also calculated. For seedling growth experiments 50 seeds were placed in three replica for each dose along with control arranged in slots on combs placed in a plastic tray containing water as shown in photographs.

**Seedling Height (cm)**

Seedling height (root and shoot length) was measured on 13th day after sowing in slots. The emergence of radical was taken as indication for germination of seeds, considered germinated when the radicle was at least 4 mm long. Germination percentage was calculated after three days by counting the germinated seeds and total number of seeds sown. Further percent over control and reduction over control was also calculated.

**Seedling Vigour Index**

Seedling vigour index = Seedling injury: Number of seedlings injured were counted for the seedling injury.

**Survivability Percentage**

Number of seeds sown in slots.

## Results and Discussion

**Germination Percentage**

In all the treatments germination percentage was taken on third day after treatment. Germination percentage results showed higher doses. In gamma ray irradiation treatment maximum germination was recorded at control (95.33%) and minimum germination was recorded at 130KR treatment (58.00%) as shown in Table no. 1.

**Seedling Height and Seedling Vigour**

Seedling height and seedling injury were recorded on thirteenth day after sowing in slots. Height was recorded at 130KR as shown in Table no. 1.

In 18hrs dry sodium azide treatments maximum germination percentage was recorded at control. In 18hrs dry sodium azide treatments seedling height and seedling vigour index was increased than control in 0.15% treatment (10.47cm) and gradually reduced to (6.72 cm) in 0.19% treatment (88.60%) and minimum was recorded at 0.19% treatment (60.60%) as shown in Table no. 2. Maximum seedling injury was recorded at 0.17% treatment and minimum at control as shown in Table no. 2.

**Table: 1. Data on effect of Gamma rays seed treatment on *Glycine max* C.V. Js-9560.**

| **Concentrations** | **Germination Parameters** | | | **Seedling Parameters** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **% G** | **% G OC** | **% R OC** | **Shoot Length (cm)** | | **Root Length (cm)** | | **SH (cm)** | **SVI** | **%SS** | **SI** | **CV** |
| | | | | **Range** | **Mean** | **Range** | **Mean** | | | | | |
| Control | 95.33 | 100 | 0.00 | 14.04 - 12.24 | 13.03 ±0.53 | 10.2 - 9.24 | 9.61 ±0.30 | 22.64 | 1630.08 | 97.22 | 0 | 0.53 |
| 10KR | 90.6 | 95.04 | -4.96 | 12.36 - 9.8 | 11.19± 0.75 | 7.04 - 5.6 | 6.37 ±0.42 | 17.56 | 1246.76 | 91.30 | 8 | 0.75 |
| 30KR | 87.30 | 91.58 | -8.42 | 12.92 - 10.28 | 11.35± 0.80 | 5.48 - 4.76 | 5.11 ±0.21 | 16.46 | 1135.74 | 86.76 | 9 | 0.80 |
| 50KR | 78.60 | 82.45 | -17.55 | 7.68 - 6.76 | 7.07±0.31 | 5.08 - 3.08 | 3.83 ±0.63 | 10.90 | 599.5 | 74.55 | 14 | 0.31 |
| 70KR | 79.30 | 83.18 | -16.82 | 9.04 - 7.16 | 8.17±0.55 | 4.4 - 2.56 | 3.69 ±0.57 | 11.86 | 664.16 | 76.67 | 11 | 0.55 |
| 90KR | 77.30 | 81.09 | -18.91 | 9.64 - 8.52 | 8.99±0.34 | 3.64 - 2.6 | 3.27 ±0.33 | 12.26 | 613.00 | 92.00 | 23 | 0.34 |
| 110KR | 58.60 | 61.47 | -38.53 | 7.68 - 6.52 | 7.03±0.34 | 2.36 - 2.16 | 2.28 ±0.06 | 9.31 | 437.57 | 95.74 | 21 | 0.34 |
| 130KR | 58.00 | 60.84 | -39.16 | 4.76 - 4.24 | 4.41±0.17 | 3.08 - 2.28 | 2.59 ±0.25 | 7.00 | 266.00 | 82.93 | 30 | 0.17 |

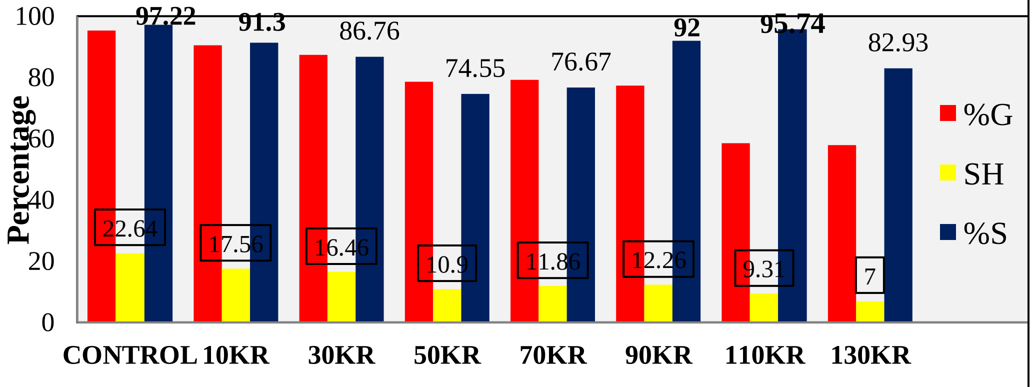
**%G** - Percent Germination, **%GOC** - Percent Germination Over Control, **%ROC** – Percent Reduction Over Control, **SH** - Seedling Height (cm), **%SS** - Percent Seedling Survival, **SVI** -Seedling Vigour Index, **SI** – Seedling Injury**, Cv** – Coefficient Of Variation.

**Table: 2. Data on effect of 18 Hrs. Dry seed treatment on *Glycine Max* C.V. Js-9560.**

| Concentrations | Germination Parameters | | | Seedling Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | %G | %GOC | %ROC | S L (cm) Range | S L (cm) Mean | RL (cm) Range | RL (cm) Mean | SH(cm) | SVI | %S | SI | cv |
| Control | 88.6 | 100 | 0.00 | 6.08 - 4.92 | 5.49±0.34 | 4.4-3.26 | 3.93 ± | 9.42 | 1215.18 | 100.00 | 0 | 10.58 |
| 0.15% | 84 | 86.42 | -13.58 | 6.66 - 5.44 | 6.02±0.35 | 4.78 - 3.18 | 4.05 ± | 10.07 | 1319.17 | 66.41 | 32 | 10.17 |
| 0.17% | 68.6 | 56.79 | -43.21 | 5.28 - 3.48 | 4.59±0.56 | 3.58 - 1.68 | 2.89 ± | 7.48 | 957.44 | 45.31 | 41 | 21.12 |
| 0.19% | 60.6 | 49.38 | -50.62 | 4.24 - 4.04 | 4.12±0.06 | 2.68 - 2.48 | 2.60 ± | 6.72 | 846.72 | 46.03 | 39 | 2.57 |

**%G** - Percent Germination, **%GOC** - Percent Germination Over Control, **%ROC** – Percent Reduction Over Control, **SH** - Seedling Height (cm), **%SS** - Percent Seedling Survival, **SVI** -Seedling Vigour Index, **SI** – Seedling Injury**, Cv** – Coefficient Of Variation.

**Fig.1:Effect of gamma rays on Seed germination, Seedling Height, Seedling Survival in *Glycine max* L.**



| **Table 3: Data on effect of 18 Hrs. Presoaked + 6Hrs. sodium azide seed treatment on *Glycine max* C.V. Js-9560.** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Concn.** | **Germination Parameters** | | | **Seedling Parameters** | | | | | | | | |
| | **%G** | **%GOC** | **%ROC** | **S L (cm)** | | **RL (cm)** | | **SH(cm)** | **SVI** | **%S** | **SI** | **CV** |
| | | | | Range | Mean | Range | Mean | | | | | |
| Ctrl. | 88.00 | 100 | 0.00 | 12.92-11.84 | 12.26±0.33 | 7.7-6.04 | 7.09±0.53 | 19.35 | 2786.4 | 100.00 | 0 | 12.91 |
| 0.15% | 80.66 | 91.66 | -8.34 | 11-8.9 | 9.75±0.64 | 6.02-4.48 | 5.45±0.49 | 15.20 | 2082.4 | 65.69 | 23 | 15.46 |
| 0.17% | 78.00 | 88.64 | -11.36 | 11.1-7.04 | 8.41±1.35 | 6.16-3.26 | 4.40±0.89 | 12.81 | 1678.11 | 54.19 | 33 | 35.14 |
| 0.19% | 76.00 | 86.36 | -13.64 | 12.44-9.36 | 10.39±1.02 | 6.04-4.98 | 5.35±0.34 | 15.74 | 2109.16 | 64.17 | 32 | 11.12 |

**Fig:2 Effect of 18Hrs. Dry Seed Treatment on Seed Germination, Seedling Height, Seedling Survival in *Glycine max* L.**

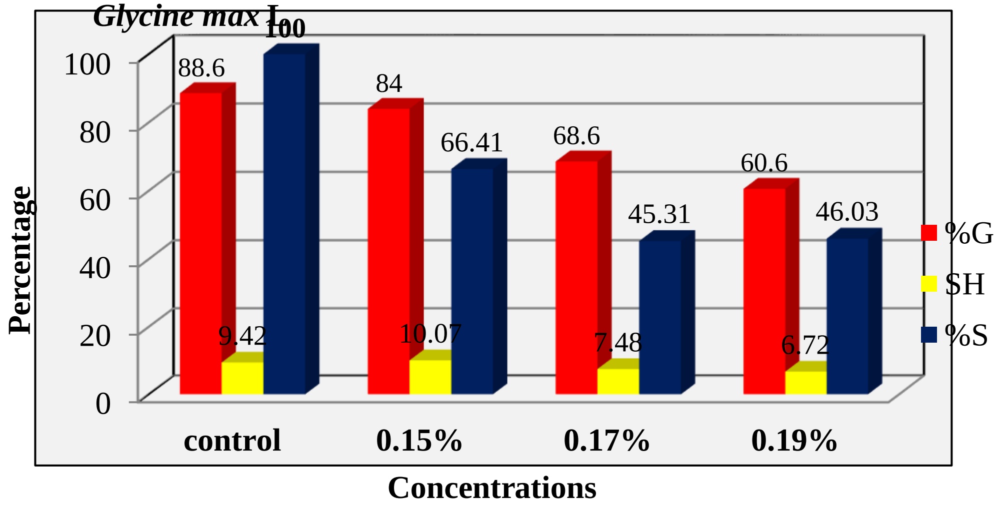
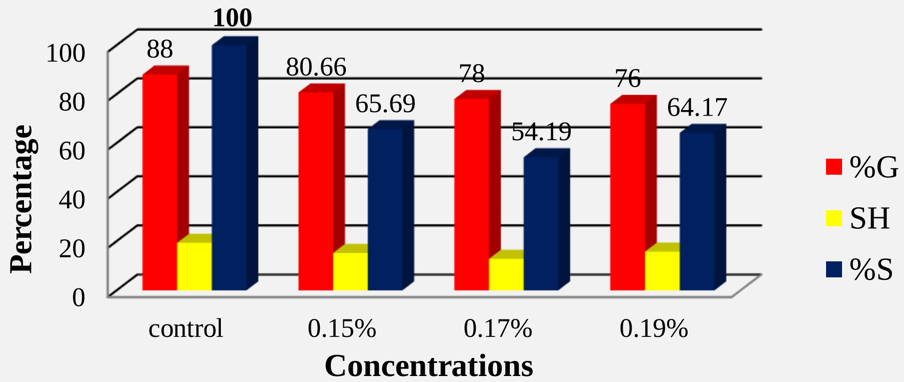


**Fig.3:Effect of 12 Hrs. pre-soaked water + 6 Hrs. Seed Treatment on Seed Germination,Seedling Height, Seedling Survival in *Glycine max* L**



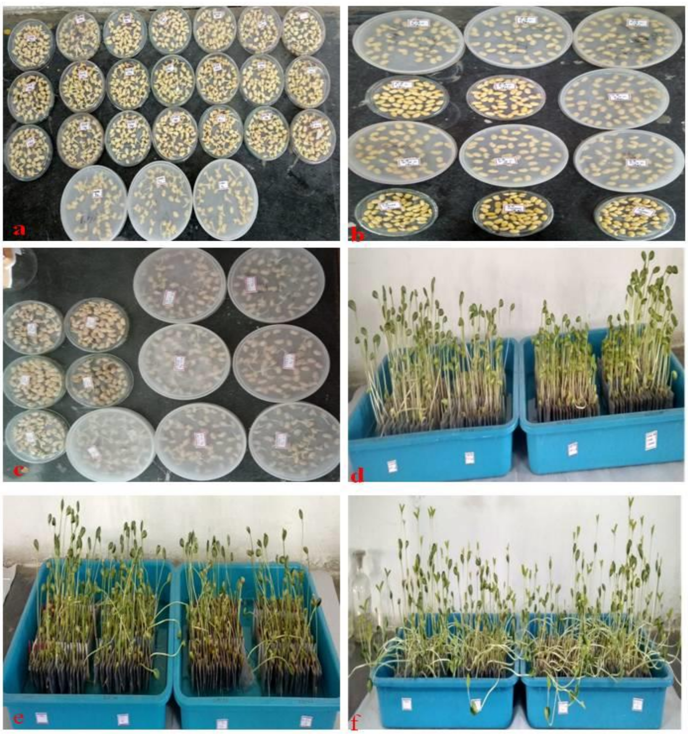



**Plate:1. Micrograph a, b, c showing Physical and Chemical mutagen treatments, seeds arranged in petridishes in count germination percent. Measurement of seedling height d, e, f in different concentrations of Gamma radiations, 18 hrs dry sodium azide, 12 hrs presoaked +6hrs sodium azide treatments.**

**Discussion**

There was gradual reduction in per cent seed germination from 95.3 % in control to 58.0 % due to the treatment with 130Kr gamma rays, while from 88.6 % in control to 60.6 % due to the treatment with 0.19 % sodium azide. Due to the treatment with gamma rays there was gradual reduction in length of the seedling height, as well as seedling vigour index and increase in seedling injury. Control showed Maximum seedling height (22.64 cm) was recorded in control, while and minimum (7.00 cm) due to the treatment with 130Kr gamma rays. Similarly, due to the treatment with sodium azide the seedling length gradually decreased from 9.43 cm in control to 6.72 cm at the concentration of 0.19 %. The decrease in per cent seed germination and seedling length was associated with decreased seedling vigour as well as per cent survivability, while increasing seedling injury. Similar results were observed by Mahla *et.al*. (1999) in Coriander and Sikder *et.al.* (2013) in *Solanum lycopersicum* L.

**Conclusion**

Results obtained showed that with the increase in doses of gamma rays irradiation there was gradual reduction in seed germination percentage, seedling height and seedling vigour index. This indicates that gamma rays (physical mutagen) showed its effectiveness at different doses (10KR, 30KR, 50KR, 70KR, 90KR, 110KR and 130KR) on seed germination percentage, seedling height and seedling vigour index parameters. In case of chemical mutagen sodium azide treatments dry sodium azide and presoaked water sodium azide 18hrs, 12hrs and 6hrs sodium azide showed gradual decrease in germination percentage while seedling height showed fluctuating results with the increase in doses of sodium azide.

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