



## CHEMICAL MUTAGENS OF SA, EMS AND PHYSICAL IRRADIATION OF GAMMA RAYS EFFECT ON SEED GERMINABILITY AND LIGNIN CONTENT OF SEED COAT IN SOYBEAN *Glycine Max.* L. Merrill.

<sup>a</sup>Sanjay N. Pathare & <sup>b</sup>Rajendra A. Satpute

<sup>a</sup>Seed Testing Division, Department of Quality Assurance, Ajeet Seeds Pvt Ltd, Chitegaon, Aurangabad.

<sup>b</sup>Department of Biotechnology, Government Institute of Science, Aurangabad.

\*Corresponding author email- sanjay.pathare@ajeetseed.co.in

### ABSTRACT

Lignin content of the soybean seed coat may have an effect on reducing seed deterioration during storage. A study was undertaken to evaluate genotypic differences in storage deterioration of soybean seed and to relate seed coat lignin content with seed quality of soybeans during storage. The Variety of Soybean JS-335 have been employed with different Chemical mutagen and physical Irradiation of gamma rays with different concentrations and these were grown in research plot at Ajeet seeds Pvt. Ltd, Chitegaon, Aurangabad, Maharashtra in June 2013. In M1 Generation we selected 82 Individual plants as a mutant for the multiplication in M2 Generation. Seeds were hand harvested at M2 generation and stored for 9 month at room temperature 29°C and 75 % RH (Ambient condition) for each generation. While in this storage period we tested all 82 Genotypes in three months interval. In this periodically testing we evaluate Germination up to 9 month and lignin analysis after 3 month. Out of 82 genotypes again we have selected 12 genotypes which derived from M2 generation those which retaining the germinability and higher lignin percentage were sown in M3 Generation and these are taking for multiplication and we have been tested all these storage testing of M3 harvested 12 genotypes for further confirmation of deterioration in seed quality. There were substantial differences among the concentrations and derivatives in the rate of seed deterioration during storage. Seeds of GR-100 Kr and EMS-10Mm (M2 & M3 Generation) derived genotypes exhibited the highest storage potential. The seed coat lignin content was significantly and negatively correlated with membrane deterioration associated with decline in soybean seed quality in storage.

**KEYWORDS:** Mutagens, Rising of M1, M2, M3 Generation, and Storage condition, Germination, Lignin.

### INTRODUCTION

Lignin is a complex heteropolymer that primarily consists of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units formed by the oxidative coupling of p-coumaryl, coniferyl, and sinapyl alcohols, respectively, which are products of the phenylpropanoid pathway. After complete deposition in the secondary cell wall, lignin provides a coating for cellulose-hemicellulose microfibrils, thus ensuring stiffness, strength, and impermeability for the lignified tissues. These properties protect the energetic polysaccharides against attack from pathogens and herbivores. Breeding soybean for high seed quality is an important approach for developing cultivars for tropical regions, and the lignin content in the seed coat was found to be one of the screening parameters for this trait. Lignin is a chemical compound found in the seed coat tissue since it is a constituent of cell walls. Its occurrence affects the rate of water absorption throughout the seed coat and the impermeability of soybean seeds of certain genotypes as well. Since lignin determines the rate of water absorption throughout the seed coat, its occurrence may have an effect on soybean seed deterioration during storage. However, few studies have considered the relationship between seed coat lignin content and resistant to soybean seed deterioration during storage. The objective of the study is to relate seed

coat lignin content with seed quality of soybeans during storage. Three commonly employed methods for lignin determination, i.e., the thioglycolic acid (TGA), the acetyl bromide (AB), and the acid detergent fiber (ADF) method, all of these three methods, the ADF method is very easy and suitable for soybean seed lignin determination. Lignin content of the soybean seed coat may have an effect on reducing seed deterioration during storage (Marwanto, Marlin, dan Mulya Marlinda, (2003). The seed coat lignin content differed significantly among the cultivars, and did not significantly change after the 12-month storage period (Francisco Carlos Krzyzanowski, Jose De Barros Franca Neto, Jose Marcos Gontijo Mandarino and Milton Kaster (2007).

Susceptibility to mechanical damage is related to lignin content of the seed coat, while seed longevity and tolerance to field weathering depends on seed coat integrity. Seed performance in many legumes has been associated with certain seed coat structures, such as the hilum, strophiole and micropyle. In soybean, permeability is also related with porosity, color, and curiosity that affect seed vigor, storage potential, resistance to shrinking and fungi infection, and to susceptibility to imbibitions damage. The seed coat as a modulator of seed-environment relationships in Fabaceae by

Francisco H. Dubbern De Souza and Jtjuo Miarcos-Filho (2001). The lignin contents in sugarcane bagasse, were 266, 242, and 28 mg g<sup>-1</sup> cell wall, In the soybean root, to be 64, 66, and 21 mg g<sup>-1</sup> cell wall, In soybean seed coat 45, 22, and 2.5 mg g<sup>-1</sup> cell wall by the acetyl bromide, Klason, and thioglycolic acid methods, respectively by Flavia Carolina Moreira-Vilar, Rita de Cássia Siqueira-Soares, Aline Finger-Teixeira, Dyonis Matias de Oliveira, Ana Paula Ferro, George Jackson da Rocha, Maria de Lourdes L. Ferrarese, Wanderley Dantas dos Santos, Osvaldo Ferrarese-Filho (2014).

The simultaneous quantification of protocatechuic (PRA), vanillic (VA), ferulic (FA) and p-coumaric (PCA) acids the relationship between these compounds and lignin contents in 13 fibrous feeds. Lignin content was correlated with proportions of FA ( $r = -0.95$ ) and PCA ( $r = 0.90$ ) in the summed phenolic acids and the PCA: FA ratio ( $r = 0.91$ ). By Rui Wang, Hong-Jian Yang, \* Xin Yang and Bing-Hai Cao (2012).

In soybean seeds, from the cultivars Embrapa 48 and FTS Águia with, respectively, 8.08% and 6.06% of lignin on seed coat, which were harvested mechanically and manually mechanical damages in soybean seeds, of immediate effect, can be determined by the use of the 2.0% sodium hypochlorite solution by Tereza Cristina de Carvalho Ana Dionisia da Luz Coelho Novembre (2013). Seed soaking electrical conductivity is influenced by the seed coat lignin content, which is a characteristic that varies among soybean genotypes. There was a significant relationship ( $R^2=0.84^{**}$ ) between electrical conductivity and seed coat lignin content, the latter being a characteristic that varies among soybean genotypes; by M. Panobianco and R.D. Vieira *et al.*, 1999.

## MATERIALS & METHODS

Seed materials were grown in the field of Ajeet seeds Pvt. Ltd. Chitegaon, Aurangabad. The RBD method was used for multiplication of selected lines for their specific characteristics. The hand harvesting of total genotypes individually done and seeds collected in the polythene bags and maintained moisture level 12 % and sealed with their particular tags. Two different parameters were used to test twelve genotypes. 1. Germination Test, 2. Lignin content Test.

### Germination Test

**Germination:** It refers as emergence of plumule and radicle in presence of favourable conditions. After germination period of seed *i.e.* proper days for germination the seedlings are evaluated into Normal seedling, abnormal seedlings, fresh ungerminated seeds, hard seeds, and Dead seeds.

### Equipments

\*Germination walking Chamber with aseptic condition.

\* Evaluation Board/Counting board.

\* Petri plates, Pencil, Eraser, Thread, Scale, Record Book.

### Mounting of seed for Germination

1. Obtain a representative sample of seed.
2. Spread a paper towel on a flat surface and moisten with water until it is thoroughly damp.
3. Place a total of 400 seeds in four Replications *i.e.* 100 seeds in each replication (or other sample size) in rows on the

towel. Make sure you randomly select seeds for your sample; do not cull any damaged, discolored or light seeds, since this will bias your germination test.

4. Moisten a second towel and carefully place onto the first paper towel, leaving the seeds sandwiched between the two towels.
5. Roll up the two towels with the seeds in-between and place in a sealed container that will retain the moisture. Place the container in an area of relatively stable temperature (22-25°C) &RH (65-70%) as per ISTA standards for germination testing; unless otherwise instructed. Avoid areas where direct sunlight with its heating effect strikes the container.
6. Mark the container with the date and variety (Geno) Lots of seed.
7. After the required germination period (fifth days), remove the towels from the container and unwrap the seeds carefully so that the fragile shoots are not destroyed.
8. Count the seedlings that have shoots longer than 1½ inches (and at least one strong root) as viable seeds in the germination rate. Seedlings exhibiting short shoots and/or roots less than 1½ inches would probably not germinate soon enough in our cool soils to contribute significantly to the yield.
9. Determine the actual percent of germination. The germination percentage were calculated with the Formula- Total Number of Germinated Seeds /Total Number of Seeds Tested\*100. The normal seeds exhibiting well developed plumule and radicle.
10. The seedlings categorized into Normal seedling, abnormal seedlings, Fresh ungerminated seeds, hard seeds, Dead seeds.

**Normal Seedlings:** The seedlings shows well developed plumule and radicle and further developed into normal plant.

**Abnormal Seedlings:** The discontinued growth of plumule and radicle or the absence of any one of the essential structure.

**Fresh Ungerminated Seeds:** The seed absorbs the moisture after end of test period but not shows any structure and remains as it is.

**Hard Seeds:** At the end of test period seed doesn't absorbs moisture and remains as it is., when pressing it looks hard.

**Dead Seeds:** The decaying matter emerges out from the seed when pressing the seed after test period is dead seeds.

### Lignin content Test

An acidified quaternary detergent solution (CTAB) is used to dissolve cell soluble, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash). ADF is determined gravimetrically as the residue remaining after extraction.

### Safety Precautions

- Always add sulfuric acid to water. Wear face shield and heavy rubber gloves. If acid is splashed on skin, wash immediately with copious amounts of water.
- CTAB powder will irritate mucous membranes, eyes and skin. Wear gloves and dust mask while handling.
- Acetone is highly flammable. Do not let vapors accumulate in work area. Use effective fume removal device. Also

avoid inhaling or contact with skin. Make sure all traces of acetone have evaporated from the crucibles containing fiber residue before placing in the drying oven.

#### Equipments

1. Analytical balance.
2. Erlenmeyer flask.
3. Electric hot plate.
4. Filter paper.
5. Oven.

#### Reagents

1. Cetyltrimethyl Ammonium bromide.
2. H<sub>2</sub>SO<sub>4</sub>.
3. Octan-2-ol (Antifoam agent).
4. Acetone.

For the gravimetric method of lignin determination (After van Soest, 1963; Rowland and Roberts, 1994) 1 gm of milled dry plant material (Seed Coat) which is obtained from the hand harvested seeds of M1 & M2 generation and which is stored for 1 year at room temperature, in which we selected 12 genotypes from screening, and each of this genotypes harvested seed was weighed (W1) into a 250 ml Erlenmeyer flask and boiled for 1 hr in 100 ml Cetyltrimethyl ammonium bromide solution (1gm

Cetyltrimethyl ammonium bromide in 100 ml of 0.5 M H<sub>2</sub>SO<sub>4</sub>) under continuous stirring. A drop of Octan -2-ol was added as an antifoam agent. The solution was filtered over an ignited and preweighed sinter (Robu-glass, No. 2, 16-40 µm, Jena) and washed 3 times with 50 ml of hot distilled water. The filtrate was washed with acetone until further decoloration was not observed.

The filtrate was dried for 2 hr at 105°C. about 10 ml of cool 72 % H<sub>2</sub>SO<sub>4</sub> (15°C) was added to the cooled sinter and mixed with the filtrate. Draining acid was refilled keeping the mixture for 3 hr in 72 % H<sub>2</sub>SO<sub>4</sub>. Thereafter, the acid was filtered off under vacuum, and the residue was washed with hot distilled water until it was acid-free. The sinter was dried at 105°C for 2 hr, cooled, and weighed (W2). The sinter was ignited at 500° C for 2 hr, cooled, and weighed to determine ash content of the residue (W3). Lignin (%) was calculated as-

$$\text{Lignin (\%)} = (W2 - W3) \times 100 / W1$$

Where,

W1- Initial wt (gm) of seed dry coat material

W2- Cooled sinter wt (gm) after drying at 105° C for 2 hr

W3- Residue of ash content wt (gm) after drying at 500° C for 2 hr

#### FLOW CHART OF LIGNIN ANALYSIS

Take 1 gm milled dry seed coat (W1) in 100 ml of CTAB Solution

Boiled these solution for 1 hr

Add a drop of Octan-2-ol in boiling solution as an antifoam agent

Filter the solution, preweighed sinter and wash 3 times with hot water

Filtrate was washed with acetone until decoloration

Filtrate was dried for 2 hr at 105°c

Add 10 ml cooled 72 % H<sub>2</sub>SO<sub>4</sub> in cooled sinter and mixed with the filtrate

Draining acid refilled keeping the mixture in 72 % H<sub>2</sub>SO<sub>4</sub> for 3 hr

Acid was filtered off under vacuum residue was washed with hot water

Sinter was dried for 2 hr at 105°C, Cooled and weighed (W2)

Sinter was ignited at 500°C for 2 hr, cooled and weighed (W3)

Calculate the Lignin percentage by the formulae

#### RESULT & DISCUSSION

On the basis of seed quality of the 12 genotypes selected from the treatments 100gy, 10Mm, in M2 & M3 generation. The genotypes after 3 month storage period at room temperature were about 29°c and 75% Relative Humidity (Ambient condition). The genotype deteriorates in significant level of germination. Four Genotypes from 100gy and one from 10Mm show stable germination percentage in M2 & M3 generation up to sixth months. Also testing Lignin percentage among twelve genotypes after 3 month, it showed significant correlation in M2 and M3 generation. The four genotypes from 100gy *i.e.* Geno-01, Geno-02, Geno-03 & Geno-04 Showed germination 76,74,66; 79,75,71; 75,71,67; 82,75,63; for 3,6,9 month in M2

generation respectively; one genotype from 10Mm *i.e.* Geno-08 showed 81,78,71 for 3,6,9 month in M2 generation respectively; The tests followed in comparison with control which showed germination 79,69,63 in M2 generation after 3,6,9 month, it is similar to that of genotypes. (Table-01, Fig.-01).The tests are followed in M3 generation and germination percentage were calculated .The germination percentage recorded 79,75,71; 82,78,70; 78,75,69; 77,74,71; For genotypes GR-100gy showed in M2 generation; while 83,77,66; for EMS-10Mm in M3 generation respectively in 3,6,9 month. The M3 control showed 81, 76, 66; germination percentage in 3, 6, 9 month. In M2 and M3 generation showed; the germination percentage is stable at particular level *i.e.* 6month; and slightly declined after the

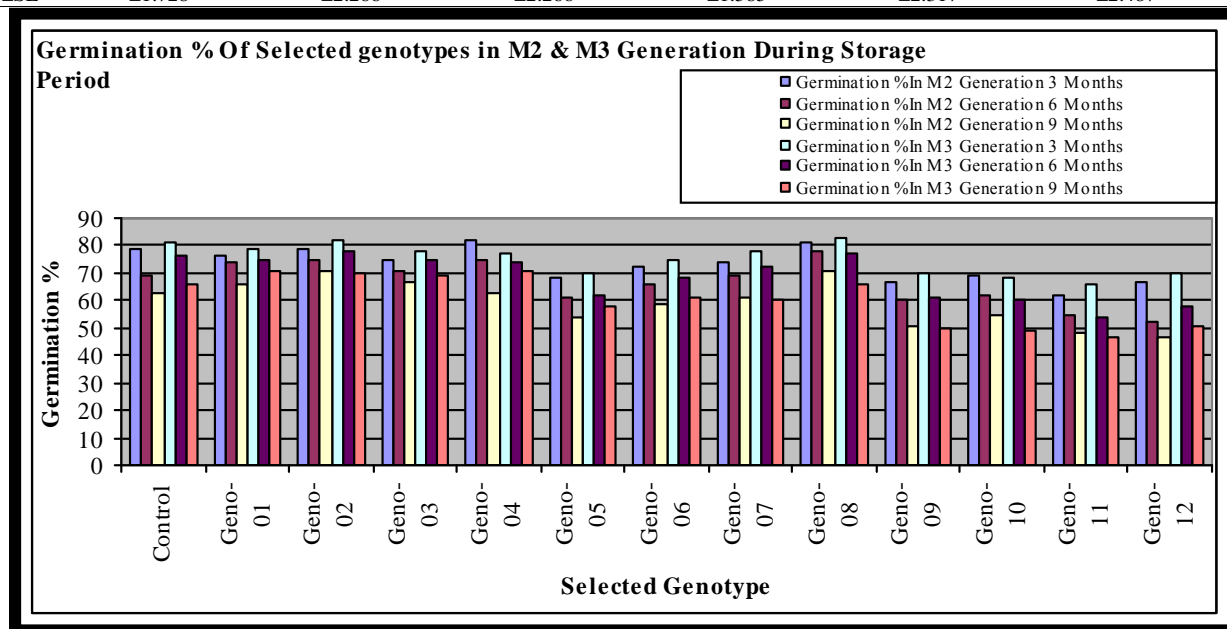
Gamma rays effect on seed germinability and lignin content of seed coat in soybean

9month. (Table-01, Fig.-01) The lignin percentage determination by ADF method showed significant correlation in M2 and M3 generation of selected genotypes. The Lignin determination calculated after 3 month of storage period for each generation. The lignin level stable during this period in M2 and M3 generation; there is no difference in lignin % in among generations.

The lignin percentage was recorded 0.1940, 0.1380, 0.1870, and 0.1670 in M2 genotypes of. GR-100gy *i.e.* Geno-01, Geno-02, Geno-3 & Geno-04 respectively and 0.2120 for EMS-10Mm Geno-08. In M3 generation analysis of lignin content after 3 month storage period were 0.2120, 0.2490, 0.2140, 0.2010 for same genotypes of 100gy and 0.2780 for 10Mm. Lignin content of control is 0.162 and 0.148 in M2 and M3 generation. (Table-02, Fig.-02)

**TABLE 1:** Showing periodical testing of Germination % of selected genotypes in M2 & M3 Generation during Storage Period

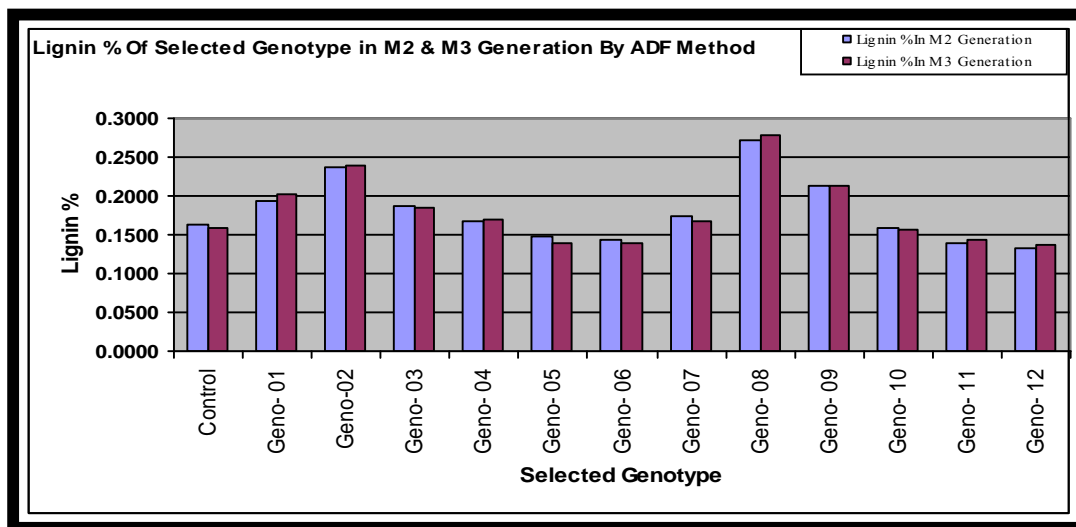
| Sr. No. | Selected Genotype | Germination % In M2 Generation |               |          |               |          |               | Germination % In M3 Generation |               |          |               |          |               |
|---------|-------------------|--------------------------------|---------------|----------|---------------|----------|---------------|--------------------------------|---------------|----------|---------------|----------|---------------|
|         |                   | 3 Months                       | Shift in mean | 6 Months | Shift in mean | 9 Months | Shift in mean | 3 Months                       | Shift in mean | 6 Months | Shift in mean | 9 Months | Shift in mean |
| 1       | Control           | 79.00                          | 0.00          | 69.00    | 0.00          | 63.00    | 0.00          | 81.00                          | 0.00          | 76.00    | 0.00          | 66.00    | 0.00          |
| 2       | Geno- 01          | 76.00                          | -3.00         | 74.00    | 5.00          | 66.00    | 3.00          | 79.00                          | -2.00         | 75.00    | -1.00         | 71.00    | 5.00          |
| 3       | Geno-02           | 79.00                          | 0.00          | 75.00    | 6.00          | 71.00    | 8.00          | 82.00                          | 1.00          | 78.00    | 2.00          | 70.00    | 4.00          |
| 4       | Geno- 03          | 75.00                          | -4.00         | 71.00    | 2.00          | 67.00    | 4.00          | 78.00                          | -3.00         | 75.00    | -1.00         | 69.00    | 3.00          |
| 5       | Geno- 04          | 82.00                          | 3.00          | 75.00    | 6.00          | 63.00    | 0.00          | 77.00                          | -4.00         | 74.00    | -2.00         | 71.00    | 5.00          |
| 6       | Geno- 05          | 68.00                          | -11.00        | 61.00    | -8.00         | 54.00    | -9.00         | 70.00                          | -11.00        | 62.00    | -14.00        | 58.00    | -8.00         |
| 7       | Geno- 06          | 72.00                          | -7.00         | 66.00    | -3.00         | 59.00    | -4.00         | 75.00                          | -6.00         | 68.00    | -8.00         | 61.00    | -5.00         |
| 8       | Geno- 07          | 74.00                          | -5.00         | 69.00    | 0.00          | 61.00    | -2.00         | 78.00                          | -3.00         | 72.00    | -4.00         | 60.00    | -6.00         |
| 9       | Geno- 08          | 81.00                          | 2.00          | 78.00    | 9.00          | 71.00    | 8.00          | 83.00                          | 2.00          | 77.00    | 1.00          | 66.00    | 0.00          |
| 10      | Geno- 09          | 67.00                          | -12.00        | 60.00    | -9.00         | 51.00    | -12.00        | 70.00                          | -11.00        | 61.00    | -15.00        | 50.00    | -16.00        |
| 11      | Geno- 10          | 69.00                          | -10.00        | 62.00    | -7.00         | 55.00    | -8.00         | 68.00                          | -13.00        | 60.00    | -16.00        | 49.00    | -17.00        |
| 12      | Geno- 11          | 62.00                          | -17.00        | 55.00    | -14.00        | 48.00    | -15.00        | 66.00                          | -15.00        | 54.00    | -22.00        | 47.00    | -19.00        |
| 13      | Geno- 12          | 67.00                          | -12.00        | 52.00    | -17.00        | 47.00    | -16.00        | 70.00                          | -11.00        | 58.00    | -18.00        | 51.00    | -15.00        |
|         | ±SD               | ±6.230                         |               | ±8.169   |               | ±8.169   |               | ±5.713                         |               | ±8.353   |               | ±8.966   |               |
|         | ±SE               | ±1.728                         |               | ±2.266   |               | ±2.266   |               | ±1.585                         |               | ±2.317   |               | ±2.487   |               |



**FIGURE 1:** Showing Germination deterioration in Selected Genotypes

**TABLE 2:** Showing Lignin % of Selected Genotypes in M2 & M3 Generation by ADF Method

| Sr. No. | Selected Genotypes | Lignin % In M2 Generation | Shift in mean | Lignin % In M3 Generation | Shift in mean |
|---------|--------------------|---------------------------|---------------|---------------------------|---------------|
| 1       | Control            | 0.1620                    | 0.0000        | 0.1580                    | 0.0000        |
| 2       | Geno- 01           | 0.1940                    | 0.0320        | 0.2020                    | 0.0440        |
| 3       | Geno-02            | 0.2380                    | 0.0760        | 0.2390                    | 0.0810        |
| 4       | Geno- 03           | 0.1870                    | 0.0250        | 0.1840                    | 0.0260        |
| 5       | Geno- 04           | 0.1670                    | 0.0050        | 0.1690                    | 0.0110        |
| 6       | Geno- 05           | 0.1470                    | -0.0150       | 0.1400                    | -0.0180       |
| 7       | Geno- 06           | 0.1430                    | -0.0190       | 0.1390                    | -0.0190       |
| 8       | Geno- 07           | 0.1740                    | 0.0120        | 0.1670                    | 0.0090        |
| 9       | Geno- 08           | 0.2720                    | 0.1100        | 0.2780                    | 0.1200        |
| 10      | Geno- 09           | 0.2130                    | 0.0510        | 0.2120                    | 0.0540        |
| 11      | Geno- 10           | 0.1580                    | -0.0040       | 0.1560                    | -0.0020       |
| 12      | Geno- 11           | 0.1400                    | -0.0220       | 0.1430                    | -0.0150       |
| 13      | Geno- 12           | 0.1330                    | -0.0290       | 0.1370                    | -0.0210       |
|         | ±SD                | ±0.0413                   |               | ±0.0432                   |               |
|         | ±SE                | ±0.0114                   |               | ±0.0120                   |               |



**FIGURE 2:** Showing Lignin Stability In Selected Genotype After 3 Month

## CONCLUSION

The given Experimental results indicate that Soybean Genotypes (GR-100Gy & EMS- 10Mm) differed significantly in their ability to maintain seed quality and retaining germinability during prescribed storage period. Seeds of Gr-100Gy & EMS-10Mm Exhibited the highest storage potential. The seed coat lignin content was significantly and negatively related to membrane deterioration associated with decline in seed quality after storage and lignin content remains stable during storage period but not decline in seed viability after specific storage period.

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