**Character association studies based on morphological, biochemical and seed quality parameters for identification of good storer genotypes in six crosses of soybean [*Glycine max* (L.) Merill.]**

**Abstract:** The present study was taken up to analyze seed longevity as a function of traits such as germination percentage, seed index, wrinkled seed percentage, cracked seed percentage, smooth and shiny seed percentage, catalase and superoxide dismutase content so that an effective selection regime can be formulated for this trait.Low seed longevity is a major concern in most soybean varieties which leads to low production of the crop. Inadequate supply of quality seed is a bottleneck in the expansion of soybean cultivation. Seed viability starts declining right after physiological maturity, forcing farmers to increase the seed rate which further leads to shortage of quality seed and a hike in cultivation cost. This study was conducted to analyze the variability in seed quality traits correlated with seed longevity in six soybean crosses *viz.* DT-21 × PS-24, PS-1347 × PS- 124, DT-21 × BHATT, AGS-25 × JS-335, PS-1556× EC-389148 and PS-1592× PS-1241. Germination percentage in methanol stress test had significant positive correlation with shoot length, root length, catalase activity, superoxide dismutase activity, smooth and shiny seed percentage and seed viability index. It was found negatively correlated with seed index, wrinkled seed percentage and cracked seed percentage. According to these results, methanol stress test, seed index, cracked and wrinkled seed percentage can be evaluated for selecting good storer genotypes. Selection for smaller seed size, less wrinkled and cracked seed percentage can lead to a concomitant selection for good storer genotypes in the segregating generations of crosses. This study thus established how germination tests, biochemical tests and morphological characters can be used to indirectly select for seed longevity. Biochemical analysis can be further applied to confirm the performance of the selected good storers.

**Keywords -** seed longevity, soybean**,** genotypes, seed viability

**Introduction**

Soybean is an important source of food, protein, and oil, and hence more research is essential to increase its yield under different conditions, including stress. Microbial interactions may have important functions in soybean production and health. It is also important to evaluate the abiotic factors which interact with the growth and yield of this crop. This chapter explores the currently available information relevant to the benefits of soybean production worldwide. Among the major factors affecting the production of soybean is the appropriate use of inocula (Pagano & Miransari, 2016; Hamza et al., 2024). Storage of soybean is a challenge for the seed industry in tropical and subtropical countries owing to the loss of seed viability below the minimum seed certification standard (70 *per cent*) before the next growing season. Poor seed quality and loss of viability during storage lead to uneven plant stand, spotty fields and reduced seed production per hectare. This is more pronounced in the case of soybean as compared to other crops. Losses due to seed quality deterioration can stretch up to 25 *per cent* of the harvested crop annually.

Inadequate supply of quality seed is a bottleneck in the expansion of soybean cultivation. Seed viability starts declining right after physiological maturity, forcing farmers to increase the seed rate which further leads to a shortage of quality seed and a hike in cultivation cost. A critical evaluation of the underlying causes for reduced seed longevity needs to be done for cultivated species of soybean in India.

Storability Storage of seed is a genetically regulated character and is influenced by initial quality, pre-storage history, moisture content, relative humidity and temperature of storage environment, duration of storage and biotic agents (**Khatun *et al*., 2009; Biabani*et al.* 2008**). Seed longevity is a result of the interplay of several morphological, genetic, biochemical and environmental factors, because of which direct selection for this trait may not be fruitful. Soybean production cannot increase further without significant improvement in seed longevity. Thus, it is necessary to identify traits that can be indirectly selected so that genotypes with good seed longevity can be easily identified after harvesting the crop.

Correlation gives the direction and nature of character association, thus indicating which traits to select indirectly to effect cumulative improvement in the desired direction. The current study was taken up to analyze seed longevity as a function of traits such as germination percentage, seed index, wrinkled seed percentage, cracked seed percentage, smooth and shiny seed percentage, catalase and superoxide dismutase content so that an effective selection regime can be formulated for this trait.

**Material and Methods**

Plant material and Experimental design

The experimental material in this study comprised of 23 genotypes including ten parents that were used to make six crosses*viz.*DT-21 × PS-24, PS-1347 × PS- 124, DT-21 × BHATT, AGS-25 × JS-335, PS-1556× EC-389148 and PS-1592× PS-1241., sixF1s, six F2s and the standard check PS-1572. These 6 crosses were made in *kharif* 2017-2018 and in the next year i.e. *kharif* 2018-2019, parents along with crossed seeds (F1s) were planted in two replications for evaluation and same crosses were again made for more F1 seeds. In *kharif* 2019-2020, the six crosses including 10 parents, 6 F1s, 6 F2s were sown in a randomized complete block design with three replications in 4m rows with row to row distance of 45cm and plant to plant distance of 10cm. Each replication had 1 row of F1 and 5 rows of the F2 generation along with one row of parents and three rows of standard check PS-1572 at the borders. All recommended cultural practices were followed to raise a healthy plant stand.

Germination test

Towel Paper Germination Test was adopted for evaluating the germination percentage of genotypes. The test was conducted according to the guidelines recommended by ISTA (International Seed Testing Agency). The tests were conducted in three replications. The seeds harvested for the year 2018-19 were subjected to these germination tests. All the genotypes along with the check PS-1572 were subjected to a germination test.

Methanol stress test

Three sets of hundred seeds of each genotype were soaked in twenty per cent methanol for two hours **(Musgrave *et al.* 1980)**. Thereafter, the seeds were washed and placed in ten rows with ten seeds in each row with the radicle end of each seed oriented downwards on moist towel paper. The seeds were again covered with another sheet of moist towel paper, rolled and fastened with a rubber band and kept in an incubator for 5 to 7 days. After 5 to 7 days the seeds were examined and classified into four categories *viz*., normal seedlings (well-developed primary roots and hypocotyls), abnormal seedlings (the seeds having rudimentary radicle and hypocotyls, these seeds were classified as abnormal seeds and such seeds were not included while calculating the germination per cent), hard seed (seeds which do not imbibe water) and dead seed (swollen, discoloured, radicle and hypocotyls not emerged) **(AOSA, 1988)**. These tests were conducted in triplicate and their mean was used for analysis.

**Calculation of correlation**

Mean values of the parents, F1 and F2 individual plants for nine characters were used to compute correlation for germination and seed quality characters. The formula given by **Pearson (1885)** was used to compute correlation**.**

**Results and discussion**

Germination percentage in methanol stress test had a significant positive correlation with shoot length, root length, catalase activity, superoxide dismutase activity, smooth and shiny seed percentage and seed viability index. It was found negatively correlated with seed index, wrinkled seed percentage and cracked seed percentage. This trait also registered positive correlation with germination percentage carried out for freshly harvested seed. Similar observations were also reported by **Pallavi *et al.* (2018)** while evaluating the association between seed longevity and seed yield-related traits in 24 genotypes of soybean. Positive association of seed longevity with germination percentage has also been reported by **Kumar *et al.* (2019).HuaWei*et al.* (2014)** and **Kadurappa (2016)** also confirmed that good seed longevity is reflected in high germination percentage of soybean genotypes after storage. Germination percentage was found to be positively correlated with catalase and SOD activity both before and after storage. These observations were in concordance with the results presented by **Hosamani*et al.*(2013), Naik *et al.*(2019), Adsul*et al.*(2018) and Sooganna et al. (2021)** while they analyzed catalase and SOD activity in relation to seed longevity. Seed viability index in methanol stress test recorded a significant positive correlation with shoot length, root length, catalase activity, superoxide dismutase activity and smooth and shiny seed percentage. This character also showed a significant negative correlation with seed index, wrinkled seed percentage and cracked seed percentage. Similar observation has also been reported by **Chandra *et al.* (2021)** who studied the correlation between germination percentage and seed quality traits in different species of the genus *Glycine*.

Catalase activity recorded after eleven months of storage had a significant positive correlation with shoot length, root length, superoxide dismutase activity and smooth and shiny seed percentage. This trait was negatively correlated with seed index, wrinkled seed percentage and cracked seed percentage.Superoxide dismutase activity recorded after eleven months of storage showed significant positive correlation with shoot length, root length and smooth and shiny seed percentage. This character was negatively correlated with seed index, wrinkled seed percentage and cracked seed percentage.

The seed index recorded a significant negative correlation with smooth and shiny seed percentage, shoot length, root length. It expressed a significant positive correlation between wrinkled seed percentage and cracked seed percentage. Wrinkled seed percentage registered a significant negative correlation with shoot length and root length. This trait also showed significant positive correlation with seed index and cracked seed percentage. Cracked seed percentage showed significant negative correlation with shoot length, root length, and smooth and shiny seed percentage. **Okabe (1996)** reported similar results while studying the inheritance of seed coat cracking in soybean. Smooth and shiny seed percentage registered a significant positive correlation with shoot length and root length. Root length was found positively correlated with shoot length. This observation is supported by the findings of **Zanakis *et al.* 1994, Saha and Sultana (2008)** and **Chourasiya *et al.* (2018).** These results established that early generation selection for traits such as seed index, wrinkled seed percentage, cracked seed percentage and smooth and shiny seed percentage can be practised to indirectly select for genotypes with good seed longevity.These results are indicative of the fact that accelerated ageing tests are reliable for selecting genotypes with good seed longevity. Biochemical tests for catalase and superoxide dismutase can also reveal genotypes with good seed longevity as the amount of these biochemicals was high in good storer genotypes even after the storage period. Selection for smaller seed size, less wrinkled and cracked seed percentage can lead to a concomitant selection for good storer genotypes in the segregating generations of crosses. This study thus established how germination tests, biochemical tests and morphological characters can be used to indirectly select for seed longevity.

**Conclusion**

Improvement in complex traits such as seed longevity requires a detailed study of several traits. Selection can be simplified and speeded up when we target only the traits that show a high correlation with seed longevity. Thus, morphological features of the seed like seed size, percentage of wrinkled and cracked seed can be evaluated for screening putative good storer genotypes. The genotypes can be further evaluated in biochemical analysis for catalase and superoxide dismutase activity. This study also establishes the accelerated aging test as a reliable method to assess genotypes of seed longevity.

**Disclaimer (Artificial intelligence)**

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Details of the AI usage are given below:

1.

2.

3.

**Table 1.Correlation between germination percentage and seed quality characters after eleven months of storage**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No.** | **Seed quality character** | **Germination % in Musgrave test** | **Seed viability index in Musgrave test** | **Wrinkled seed percentage** | **Cracked seed percentage** | **Smooth and shiny seed percentage** | **Seed Index** | **Shoot length in Musgrave test** | **Root length in Musgrave test** | **Catalase activity after storage** | **SOD activity after storage** | **Germination% of freshly harvested seed** |
| **1.** | **Germination % in Musgrave test** | **1** | **0.821\*\*** | **-0.549\*\*** | **-0.423\*\*** | **0.527\*\*** | **-0.352\*\*** | **0.353\*\*** | **0.382\*\*** | **0.478\*\*** | **0.649\*\*** | **0.678\*\*** |
| **2** | **Seed viability index in Musgrave test** |  | **1** | **-0.503\*\*** | **-0.390\*\*** | **-0.506\*\*** | **-0.276\*\*** | **0.704\*\*** | **0.716\*\*** | **0.420\*\*** | **0.525\*\*** | **0.615\*\*** |
| **3** | **Wrinkled seed percentage** |  |  | **1** | **0.731\*\*** | **-0.532\*\*** | **0.226\*\*** | **-0.234\*\*** | **-0.316\*\*** | **-0.298\*\*** | **-0.363\*\*** | **-0.389\*\*** |
| **4** | **Cracked seed percentage** |  |  |  | **1** | **-0.401\*\*** | **0.228\*\*** | **-0.231\*\*** | **-0.269\*\*** | **-0.176\*** | **-0.211\*\*** | **-0.232\*\*** |
| **5** | **Smooth and shiny seed percentage** |  |  |  |  | **1** | **-0.290\*\*** | **0.203\*\*** | **0.204\*\*** | **0.380\*\*** | **0.328\*\*** | **0.412\*\*** |
| **6** | **Seed Index** |  |  |  |  |  | **1** | **-0.148\*** | **-0.163\*** | **-0.178\*** | **-0.328\*\*** | **-0.154\*** |
| **7** | **Shoot length in Musgrave test** |  |  |  |  |  |  | **1** | **0.819\*\*** | **0.179\*** | **0.237\*\*** | **0.276\*\*** |
| **8** | **Root length in Musgrave test** |  |  |  |  |  |  |  | **1** | **0.185\*** | **0.202\*\*** | **0.309\*\*** |
| **9** | **Catalase activity after 11 months of storage** |  |  |  |  |  |  |  |  | **1** | **0.642\*\*** | **0.286\*\*** |
| **10** | **SOD activity after 11 months of storage** |  |  |  |  |  |  |  |  |  | **1** | **0.488\*\*** |
| **11** | **Germination% of freshly harvested seed** |  |  |  |  |  |  |  |  |  |  | **1** |

**Table 2. Mean values for seed quality characters in six soybean crosses**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S No.** | **CROSS** | **Cross family** | **Germination % before storage** | **Germination % in Musgrave test** | **Shoot length in Musgrave test (cm)** | **Root length in Musgrave test (cm)** | **Wrinkled seed %** | **Cracked seed %** | **Smooth and shiny seed %** | **Seed viability index** |
| 1. | DT-21 × PS-24 | C1P1 | 87.44 | 54.66 | 5.22 | 5.79 | 25 | 33 | 67.33 | 665.8 |
| C1P2 | 79.55 | 40.00 | 5.55 | 4.52 | 20.66 | 24.33 | 41.66 | 673.3 |
| C1F1 | 73.30 | 62.44 | 5.20 | 5.80 | 14.33 | 9.66 | 61.33 | 947.4 |
| C1F2 | 87.19 | 60.17 | 4.99 | 5.50 | 22.66 | 20.86 | 44.53 | 825.4 |
| 2. | PS-1347 × PS- 1241 | C2P1 | 87.10 | 57.33 | 4.21 | 5.20 | 25.33 | 24 | 59.33 | 818.7 |
| C2P2 | 88.44 | 74.66 | 5.83 | 5.93 | 23.66 | 14.66 | 61.66 | 1139.33 |
| C2F1 | 88.22 | 58.21 | 5.27 | 6.13 | 25 | 21.33 | 61.66 | 967.69 |
| C2F2 | 82.97 | 51.10 | 6.08 | 5.66 | 28.8 | 23.53 | 55.33 | 898.5 |
| 3. | DT-21 × Bhatt | C3P1 | 97.10 | 57.10 | 6.20 | 5.66 | 19 | 20.66 | 46 | 913.4 |
| C3P2 | 86.21 | 80.88 | 6.50 | 7.75 | 14.33 | 13.33 | 74.66 | 1216.3 |
| C3F1 | 90.44 | 64.44 | 5.24 | 6.07 | 18 | 27 | 39.66 | 895.2 |
| C3F2 | 70.03 | 76.36 | 5.15 | 6.36 | 27 | 20.4 | 45.06 | 948.0 |
| 4. | AGS-25 × JS-335 | C4P1 | 86.22 | 71.88 | 5.72 | 5.27 | 15 | 22.66 | 50.33 | 955.6 |
| C4P2 | 93.77 | 73.33 | 7.13 | 5.82 | 20.66 | 20.66 | 51.66 | 1000.6 |
| C4F1 | 82.66 | 73.77 | 7.34 | 4.78 | 16 | 15 | 73 | 991.0 |
| C4F2 | 76.36 | 51.79 | 4.71 | 5.39 | 28.8 | 21.33 | 53.4 | 775.5 |
| 5. | PS-1556× EC-389148 | C5P1 | 91.44 | 71.66 | 6.20 | 4.57 | 24.66 | 18 | 66.33 | 932.3 |
| C5P2 | 86.66 | 74.00 | 6.14 | 5.14 | 19 | 20.33 | 82 | 982.5 |
| C5F1 | 87.99 | 58.44 | 5.54 | 5.68 | 27.66 | 19.66 | 64 | 842.2 |
| C5F2 | 88.79 | 64.70 | 6.44 | 5.79 | 29.66 | 22.33 | 54.46 | 955.7 |
| 6. | PS-1592× PS-1241 | C6P1 | 88.88 | 49.33 | 4.87 | 6.35 | 25.33 | 19 | 45 | 1039.9 |
| C6P2 | 82.66 | 72.66 | 4.90 | 5.69 | 14 | 15.33 | 49.66 | 855.0 |
| C6F1 | 84.555 | 60.22 | 4.45 | 5.37 | 10 | 9.33 | 62 | 1029.2 |
| C6F2 | 81.28 | 50.57 | 6.54 | 7.13 | 26.3 | 21.26 | 34.8 | 1096.9 |

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