Accelerated ageing test reveals quantitative nature of inheritance of seed viability in soybean [Glycine max (L.) Merr]

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Title page

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Abstract

Soybean [*Glycine max* (L.) Merrill] (2n=40), an important source of the premium quality protein (40%) and oil (18-20%), suffers from poor viability of the seeds during ambient storage. The current study aimed to understand the genetic control of seed viability in soybean and its association with other traits through accelerated ageing test. A set of 119 $F_{2:3}$ seeds from a cross between good storing genotype EC1023 (91.87% germination after 1 year of ambient storage) and poor-storing genotype VLS61 (60.87% after 1 year of ambient storage) were tested for viability and vigour through accelerated ageing (AA) test. The parameters of the AA testing, which were initially standardized from six different combinations, were- temperature: 41± 1^0C, duration: 72 hours and relative humidity (RH): ~100%. The tested seeds differed significantly for viability and vigour index I and II, which ranged from 4.16 to 71.42%. The continuous distribution of the viability percent of the $F_{2:3}$ seeds indicated an involvement of more than one gene in controlling the viability of the seeds. The percent seed germination found to be positively and significantly correlated with the average seedling length (r=0.78) and seedling dry weight (r=0.83). Similarly, seedling length was found to be positively and significantly associated with seedling dry weight (r=0.92). The information on genetic control of viability of soybean seeds along with its vigour indices would pave the way for mapping and deploying the genes for improvement of viability in soybean.

Keywords: Accelerated ageing test, Correlation, Germination %, Polygenic Inheritance, Vigour indices.

Declaration

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Introduction

Soybean [*Glycine max* L. Merrill] is the world's *numero uno* oilseed crop accounting for nearly 57% of the global oilseed production. It is also the richest (40-45%) and cheapest source of plant-based protein, which contain nearly all the amino acids required by the human body for its general growth and development. Soybean also contains 18-22% oil rich in poly- and mono-unsaturated fatty acids making it healthier for the consumers. Besides oil and protein, soybean contains carbohydrates, ash, antioxidants, and several other important nutritional elements making it an important food for our health. The de-oiled cake (DOC) of soybean has been the choice of animal growers as nutritious feed for animals, fowl, and fishes. Therefore, soybean is gaining boundless popularity in the food, health, pharmaceutical and cosmetic industries worldwide.

In terms of area and production of soybean, India ranks 5th globally; however, the productivity is very low, which is hovering around 10q/ha as against about 25 q/ha world average. Amongst several other factors, the non-availability of quality seeds during the sowing period is an important factor that affects the production and productivity of soybean in the country. Rapid loss of viability of the seeds during ambient storage decreases the quality of the seeds. Loss of seed viability is often hastened by the genotypic, climatic and storage conditions and it is severe in warm and humid climates (Dargahi et al. 2014). India being a sub-tropical country, the problem of seed viability loss is critical here. From harvest to next planting, seed viability in most of the Indian soybean varieties goes down to around 70% under ambient storage conditions (Singh and Ram 1986). Bhattacharya and Raha (2002) reported a decrease of germination of soybean seeds to zero under 9 months of ambient storage. Poor viability of the seeds demand enhancing the seed rate for maintaining the plant stand in the field, which not only increases the cost of production but also diminishes the farmer's income. It is therefore important to understand the real cause of viability loss in the seeds so that effective measures can be taken up to keep the seeds viable for a longer period.

Seed viability refers to the competence of an embryo to remain alive inside a seed and to show normal germination and growth when sown in the field. The viability of soybean seeds is influenced by lots of factors, viz., physical (seed coat, seed colour, seed size, seed coat permeability, gap between seed coat and cotyledons), biochemical (amount of hydroxylated fatty acids, accumulation of ROS and antioxidants), physiological (electrolyte leaching during imbibition, environmental (temperature, humidity, water stress), and genetics (genes and QTLs) (Sooganna et al. 2016). For enhancing the viability of soybean seeds through the breeding approach, it is essential to understand the genetic control of the trait i.e., whether seed viability is controlled by single or polygenes. However, findings about genetic control of seed viability in soybean are hugely diverse and contradictory. Kueneman (1983) reported the influence of maternal factors while Dao et al. (1999) observed monogenic and di-genic control on seed longevity in ambient storage. Adsul et al. (2018) also observed monogenic control of seed longevity in soybean. Clerkx et al. (2004) considered seed storability as a complex trait controlled by several genes coupled with environmental conditions during seed formation, harvest, and storage. The advent of molecular markers helped in the identification and mapping of several quantitative trait loci (QTL) for seed

viability in soybean; however, the number of QTLs reported varied considerably, such as two (*Ha1* and *Ha2*) (Kumar et al. 2019; Zhang et al. 2008), three (Dargahi et al. 2014) and five (*VIS 1-5*) (Watanabe et al. 2004). Association of SSR markers *viz*., Satt434, Satt538, Satt281 and Satt598 (Singh et al. 2008), and Satt371, Satt453 and Satt618 (Hosamani et al. 2013) with seed storability have been reported. Sooganna et al. (2016) reported that SSR marker Satt423 could distinctly differentiate good storing soybean genotypes from the poor ones. Permeable seeds are relatively less viable than impermeable ones. Sun et al. (2015) identified a base substitution (T→G) in a gene (*GmHs1-1)* associated with calcium content in the seed coat that transformed the impermeable seed coat to permeable ones. Jang et al. (2015) made a similar observation. Going by these findings, the report of genetic control of seed viability appears to be diverse and inconsistent. Moreover, phenotyping the seeds for seed viability trait is critical for understanding the genetic control of the trait. Usually, viability is expressed in terms of germination after keeping the seeds under ambient storage conditions. However, it is a time taking process and influenced by several factors including storage conditions. Accelerated ageing (AA) has been used as an alternative to the conventional storage method of ageing. Besides AA testing, several other methods *viz.* Electrical conductivity, cold test, sodium hypochlorite etc. are also available for the determination of age of seeds. However, AA test is rapid, precise and the results are comparable to the test under conventional storage (Egli et al. 1978; Hosamani et al. 2013; Sooganna et al. 2016; Tekrony et al. 1980; Usha 2009). Therefore, in this study, an attempt was made to understand the genetic control of seed viability in soybean using an intra-specific segregating population through AA testing.

Materials and Methods

Plant material

The experimental material consisted of 119 $F_{2:3}$ plants developed from an intra-specific cross between soybean genotypes EC1023 (yellow seeded with good seed storability i.e., 91.87% germination after 1 year of ambient storage) and VLS61 (yellow seeded with poor storability i.e., 60.87% germination after 1 year of ambient storage). Parental genotypes were obtained from the Soybean Laboratory, Division of Genetics, ICAR-IARI New Delhi.

Testing seed vigour and viability after accelerated ageing (AA)

Standardization of accelerated ageing (AA) parameters

Accelerated Ageing (AA test) vigour test has been appraised as an index of seed vigour in a wide range of crop species including soybean. However, its parameters need optimization before application to a particular crop. For the standardization of accelerated ageing vigour test in soybean, seeds of DS9712 a popular and commonly grown soybean variety of North India were taken and subjected to two different temperatures viz., 41°C and 43°C for three different time duration i.e., 48hrs, 72hrs and 96hrs, (a total set of six treatments) (Table 1). The artificially aged seeds were subjected to a standard germination test and data on germination percent and other viability related parameters were recorded on the 8th day of germination.

Accelerated ageing vigour test

After the standardization of temperature and time duration for the AA vigour test (Table1 & Fig. 1) for soybean, the viability of the parental genotypes and $F_{2:3}$ populations were examined. Initially, the chamber of the seed

germinator was sterilized with alcohol to avoid fungal contamination of the seeds. The tested seeds were then packed in net cloth bags stapled with a stapler pin and placed in the seed germination chamber (Fig 2). The Age of the seeds (parental and $F_{2:3}$ seeds) was increased artificially by exposing it to high temperature (41°C±1) and relative humidity (~100%) for 72hrs followed by germination test as per ISTA, 2009. Seed germination percent, seedling length, seedling dry weight, vigour index I and II were calculated. As per vigour test protocol, data on germination (%), seedling length, seedling dry weight, vigour index I and II were recorded from the seeds that produced normal seedlings only i.e., seedlings with normal root and shoot growth, having shoot: root ratio nearly unity, healthy and free from seed-borne diseases and pests. Seedlings with abnormal growth (showing high root to shoot ratio, high shoot to root ratio, less root hair development, decayed or deformed radicle of germination seed etc. and infected by pest and diseases) were straightforwardly discarded.

Seed Germination Test

The germination of the seeds was tested as per ISTA protocol. The test was conducted in two replications of 100 seeds each following the 'between paper method'. The seeds were incubated in the seed germination cabin maintained at a temperature of 25 ± 1 0°C and RH ~95%. Germination data were recorded on the 5^{th} and 8^{th} days after sowing. The number of germinated seeds with normal seedlings was only counted and the percentage of germination was obtained. The viability of the seeds was expressed in terms of seed germination (%) i.e., the higher the seed germination (%), the higher is the seed viability of the plant/genotype.

Vigour indices

Seed vigour is the summation of all properties that determine the overall activity and performance of the seed lots having significant germination in a variable environment (ISTA). Seed lot showing the higher seed vigour indices are regarded to be more vigorous (Abdul-Baki and Anderson 1973). The dry weight of $F_{2:3}$ seedlings was taken after 16 hours of oven drying. The formula for vigour index I and II were as follows:

Vigour Index I = Standard germination (%) × Average seedling length (cm) (Maisuria and Patel 2009); Vigour Index II = Standard germination (%) × Average seedling dry weight (mg or g) (Dezfuli et al. 2008).

Results

Standardization of accelerated ageing parameters

While standardizing the temperature (^{0}C) and duration of treatment (hour) under ~100% relative humidity (RH), it was found that the germination of the seeds treated with 41°C for 48hrs was 22% with very high vigour index I (320.76) and II (3007.40). It indicated failure of the temperature and duration of treatment in making a significant impact on the ageing of the seeds. Similarly, germination of the seeds treated with 41°C for 72hrs was 14% with moderate vigour index I (163.24) and II (1691.48). The seeds subjected to 41°C for 96hrs had 12% germination and low vigour index I (126.54) and II (660.00). With the increase in temperature and duration, the ageing caused to the seeds also increased (Table 1 and Fig 1). Correspondingly, seed viability, vigour, and percentages of normal seedlings were decreased. It was observed that lower temperature and shorter duration was not sufficient to cause ageing effect on the seeds, while higher temperature and longer duration was too much damaging the seed. By evaluating the seed germination (%) and vigour index I and II, temperature 41^0C and duration 72 hrs under ~100%

RH was chosen for accelerated ageing of the seeds. The highly viable and vigorous seeds produced higher percentages of normal seedlings (>germination percentages after AA) even after this stressful treatment.

Inheritance of seed viability in soybean

An F_2 generation plants segregating for seed viability trait was developed by crossing two diverse genotypes of soybean viz., EC 1023 and VLS61, which were highly viable (91.87% germination) and less viable (60.87% germination), respectively. The seeds of the F_2 plants i.e., $F_{2:3}$ seeds were subjected to viability test after accelerated ageing [at 41±1°C and nearly 100% RH for 72 hrs.] as per ISTA (2009) and Daraghi et al. (2014). After accelerated ageing, the viability of the seeds was measured through germination and vigour test.

In this study, the germination of EC1023 (high viable genotype) and VLS61 (poor viable genotype) was 40% and 14%, respectively, which clearly indicated the significant differences in the viability between two parental lines. The germination of the $F_{2:3}$ seeds ranged from 4.16% to 71.42% with a mean of 17.31 (Table 2). Thus, the range of seed germination in the $F_{2:3}$ seeds went beyond the range of germination of the parental genotypes i.e., 14 to 40%. Based on germination percentage, the seedlings were categorized as highly viable (>40% germination), intermediate (30-40% germination) and poor (<30% germination). The chi-square test directed to identify the involvement of one two genes in controlling the trait failed to deliver any significant results. However, while plotted in the bar diagram, the viability data showed continuous distribution keeping the parental types within the range (Fig. 3). The continuous distribution of the data indicated the involvement of more than one gene in controlling the seed viability trait in soybean. The presence of a greater number of phenotypic classes and appearance of the transgressive segregants in the segregating generation also confirms the involvement of a large number of genes and their recombination in the expression of the phenotypes.

Vigour indices and character association

In the AA test, vigour of the seeds was measured in two indexes in which vigour index I is the product of germination percentage and average seedling length (cm) of normal seedlings (Maisuria and Patel 2009). Similarly, the vigour index II is the product of germination percentage and average seedling dry weight (mg) (Dezfuli et al. 2008). In this test, the vigour index I ranged from 6.6 to 1049.66, and the vigour index II ranged from 13.07 to 1694.88 (Table 2), which indicated inherent variation among the seeds in its field performance potentialities. The seedlings having both the indices high would perform better in the field than others. In this test, plant no. C13-P11 was found to have both vigour indexes I (1049.66) and II (1452.49) high (Table 3 and Fig. 4). Contrarily, plant no. C10-P7 was found to have both the indices low i.e., 32.03 and 13.31, respectively.

The information related to character association is of profound importance in any crop improvement programme. For the positively correlated traits, improvement in one leads to the corresponding improvement in the other trait and vice-versa. In this experiment, the correlation of seed viability measured through germination percentage was studied with seedling length and seedling dry weight. It was found that germination was positively and significantly associated with average seedling length (r=0.78) and seedling dry weight (r=0.83) (Table 4). Similarly, seedling length was found to be positively and significantly associated with seedling dry weight

(r=0.92). It indicated that seed viability is associated with several other traits and hence selection for this trait would be relatively easy.

Discussion

One of the principal constraints in soybean cultivation is the sustention of seed viability until subsequent planting, as the viability of the seeds begins declining after physiological maturity (Crookston and Hill 1978) followed by fast decline during ambient storage (Surki et al. 2012). Loss of viability is more acute in tropical and sub-tropical regions (Hang et al. 2015) including India. Poor longevity of the soybean seeds affects seedling vigour, crop stands in the field and ultimately the seed yield (Zhang et al. 2019). Therefore, improving seed viability in soybean is important to increase overall crop production (Dargahi et al. 2014). Studies attempting to figure out the component(s) responsible for viability loss hinted that numerous genetic and non-genetic factors such as moisture content, relative humidity, oxygen pressure, temperature of storage etc. influence directly or indirectly inflicting the seeds to lose viability (Groot et al. 2012; Potts et al. 1978). Seed size, seed composition, integrity of the seed coat, mechanical damage, field weathering, etc. deteriorates the seed quality leading to delayed seed germination, abnormal plant growth and poor plant stand in the field thereby reducing crop yield (Ghassemi Golezani et al. 2010). The factors causing loss of viability became more damaging with the increased period of ambient storage; however, response to it varied with genotype, species and other varietal characters (Kurdikeri et al. 2000). The wild species of soybean are the excellent reservoirs of longevity-related genes, maintain viability for a longer period of time (Chandra et al. 2021) and need to be used in the breeding programs to introgress this trait into cultivated soybean (Kumar et al. 2019; Talukdar and Shivakumar, 2016; Zhou et al. 2010). Thus, understanding the genetic basis and its deployment could offer a long-lasting solution to the problem of rapid viability loss in soybean. In the present study, the $F_{2:3}$ seeds of the cross EC1023 x VLS61 were subjected to accelerated ageing followed by germination test. The germination in the seeds ranged from 4.16% to 71.42% indicating variability in the seeds for viability. The range of seed germination in the $F_{2:3}$ seeds (4.16-71.42%) went beyond the range of germination of parental genotypes i.e., 14-40%, which indicated transgressive segregation. The germination data while plotted in a bar diagram showed continuous distribution keeping the parental data within the range. It thus indicated the involvement of polygenes called quantitative trait loci (QTL) in controlling the seed viability trait in soybean. Clerkx et al. (2004) indicated the seed viability to be a complex trait controlled by several genes and also affected by environmental conditions during seed formation, harvest and storage. Hosamani et al. (2013) indicated the genetic makeup of soybean genotypes to determine the viability of the seeds during storage. The numbers of QTL reported for seed viability traits were found to vary considerably from two (*Ha1* and *Ha2*) (Kumar et al. 2019; Zhang et al. 2008), three (Dargahi et al. 2014) to five (*VIS 1-5*) (Watanabe et al. 2004). Verma and Ram (1987) reported the involvement of 2 to 4 genes for seed longevity in soybean. In this study, variation in germination percentage and appearance of the transgressive segregants in the segregating generation confirmed the involvement of a large number of genes in controlling viability in soybean seeds.

Testing the viability of seeds through ambient storage is a time taking process. Contrarily, accelerated ageing mimicking the ambient storage is a rapid and effective approach of viability testing in seeds including soybean. Now-a-days, the AA test is one of the most lucrative tests for seed vigour. Artificial exposure of the seeds to higher temperature and humidity for a prescribed time period provides the simulation results with natural ageing (Egli et al. 1978; TeKrony et al. 1980). In this study, the temperature and duration of treatment were optimized

for accelerated ageing, and 41±1°C and nearly 100% RH for 72 hours were used for the treatment. This condition matched with that reported earlier (Daraghi et al. 2014; ISTA, 2009). Highly vigorous seed lots are more tolerant to stressful conditions and produce higher percentages of normal seedlings (Rastegar et al. 2011). The germination characteristics such as germination percentage, germination uniformity and germination indices are remarkably influenced by the ageing treatment (Rastegar et al. 2011; Ruzrokh et al. 2003; Verma et al. 2003) which leads to the reductions in seed quality and performance (Mc Donald 1999). Unlike germination data, the vigour indices reflect the true potential of seed during germination and field emergence (Te Krony et al. 1989); the higher the vigour indices better is the field performance and stand establishment (Finch Savage and Bassel 2016). In this study, we tried to measure the seed viability in term of their seed vigour and the vigour index I and II of the seeds ranged from 6.6 to 1049.66 and 13.07 to 1694.88, respectively. The seedlings having both the indices high would perform better in the field than others. Accordingly, plant no. C13-P11 with both the vigour indices high (1049.66 and 1452.49) is expected to perform better in the field. The effectiveness of accelerated ageing in testing viability was proved in several other crops including mungbean (Bishnoi and Santose, 1996) and chickpea (Dahiya et al. 1997). Accelerated ageing in cowpea was found to affect all physiological parameters such as germination percentage and vigour index (Kapoor et al. 2010). The decrease in germination percent and other indexes can be related to physiological and biochemical changes during seed ageing (Ghassemi-Golezani et al. 2010). Thus, the vigour index offers the possibility of categorizing seed lots into classes of seed quality.

Conclusion

In this study, the condition for accelerated ageing was optimized and $F_{2:3}$ seeds from a cross between good and poor storing genotypes were phenotyped through the accelerated ageing system. Germination of the artificially aged seeds indicated that the viability in the soybean seeds was controlled by more than one gene. Further, the viability trait was found to be associated with the seedling length and seedling dry weight. The findings of this study would pave the way for mapping and genetic improvement of seed viability in soybean.

Author contributions: Conceptualization of research (AT, MS); Designing of the experiments (AT, MS); Contribution of experimental materials (AT, SKL); Execution of lab experiments and data collection (MS, RRY, AK, NKKR, SS, MY, RK, MT); Analysis of data and interpretation (MS, RRY, SC, SB); Preparation of the manuscript (MS, AT).

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Figures

Figure 1

Standardization of AA vigour test in soybean. Treatment of seeds with A: 41oC for 48hrs and G was 22%. B: 41°C for 72hrs and G was 14%, C: 41°C for 96hrs and G was 12%. D: 43°C for 48hrs and G was 10%, E: 43°C for 72hrs and G was 8%, F: 43°C for 96hrs and G was 0%

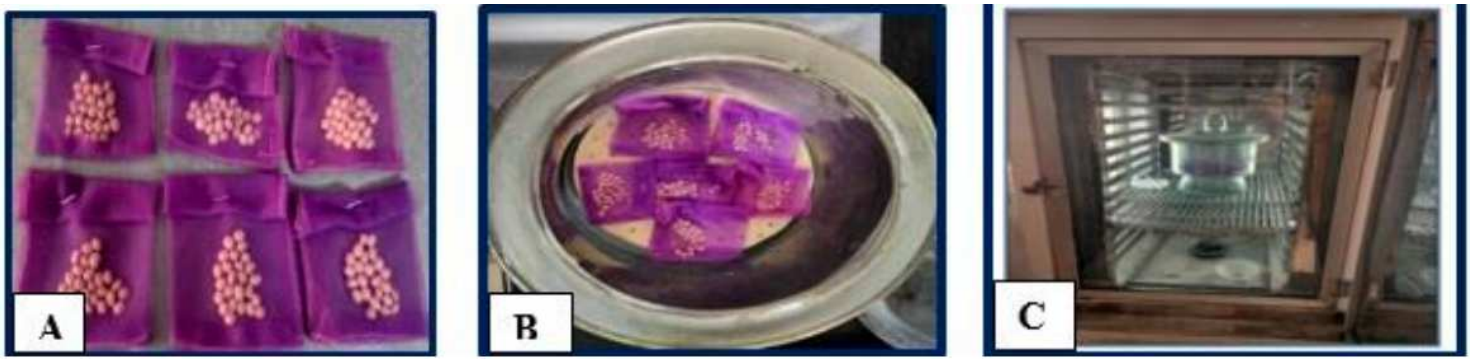

Figure 2

Accelerated ageing vigor test (A) Preparation of seed bags (B) Seed bags kept in desiccator (C) Desiccator containing seed bags along with thermometer kept inside the seed incubator or germinator

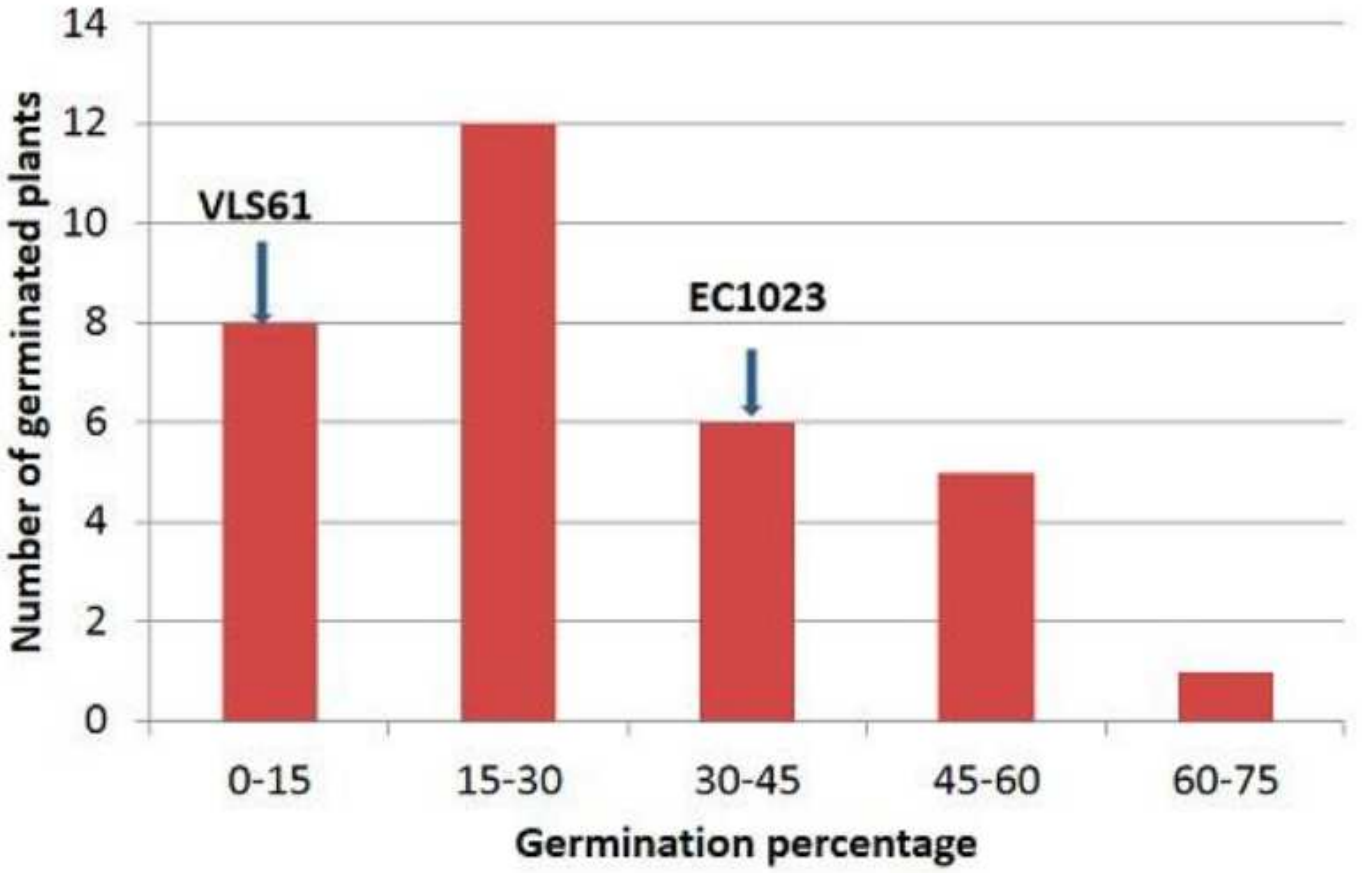

Figure 3

Frequency distribution of germination percentage of the seeds in F2:3 generation

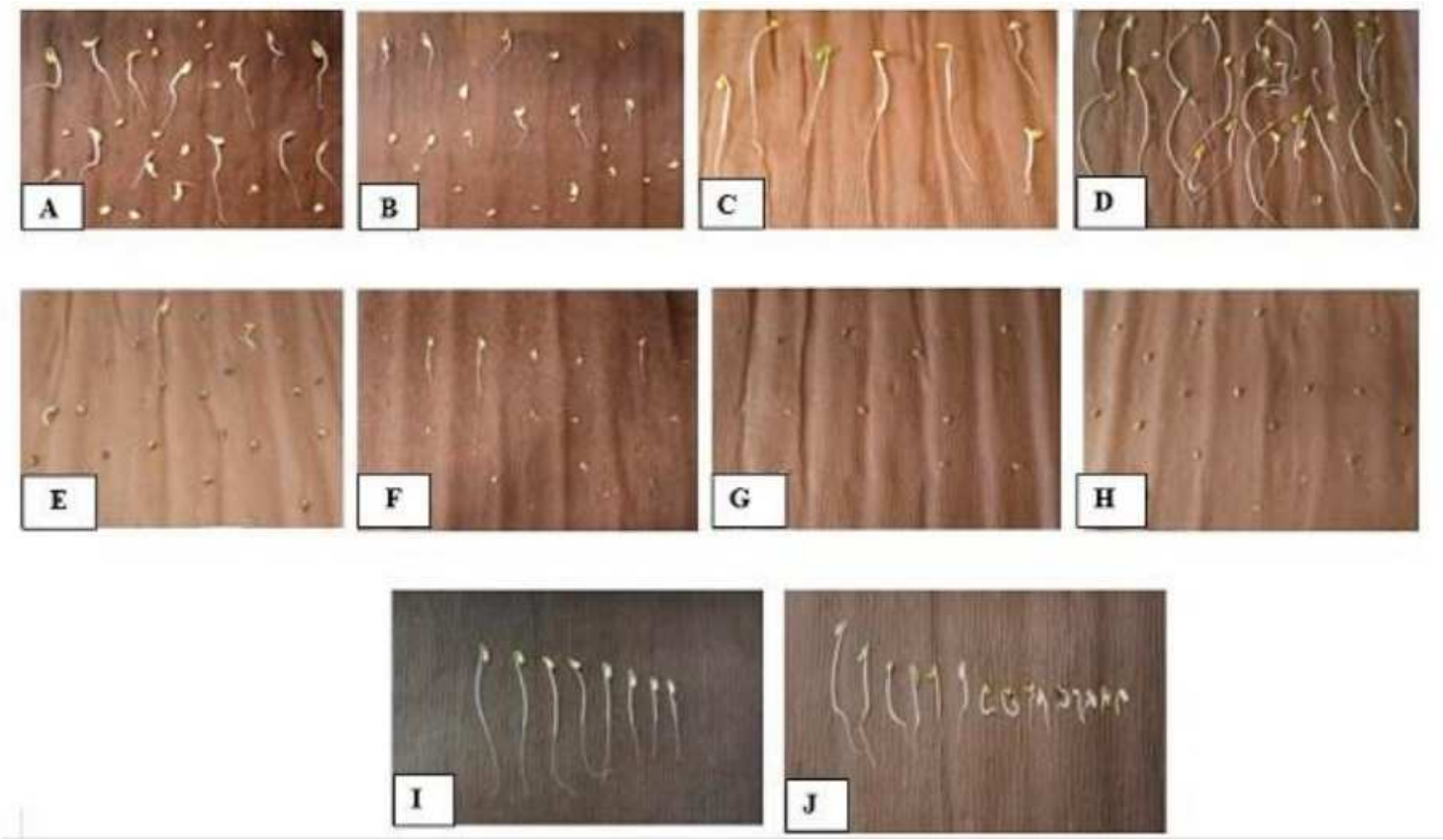

Figure 4

Variation in the germination percentages after AA test in parental genotypes and F2:3 populations. A: High viable parent EC1023. B: Poor viable parent VLS61. C and D: Seedling of highly viable seeds. E and F: Seedlings of poor-viability. G and H: Non-germinated seeds. I: High vigor seedling. J: Seeding with diverse vigor level.

Supplementary Files

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- Tablesinheritanceofseedviabilityinsoybeanfinal.pdf