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# Field Performance of 30-year-old Soybean Germplasm Conserved in Indian National Genebank

**P Kiran Babu[1*], J Radhamani[2], J Aravind[2], E Varghese[3] and RK Tyagi[2]**
[1]*Division of Plant Genetic Resources, ICAR-Indian Agricultural Research Institute, New Delhi–110012, India*
[2]*Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi–110012, India*
[3]*Division of Design of Experiments, ICAR-Indian Agricultural Statistical Research Institute, New Delhi–110012, India*

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Viability and vigour are the most important indices for assessing the seed quality. The present study was carried out to evaluate the performance of long-term conserved (30 years) soybean germplasm in the genebank. Some 100 accessions of soybean varying in seed morphological characteristics and origin were evaluated for 25 agro-morphological traits in field using standard descriptors. Field performance of freshly regenerated germplasm of the same accessions was that of originally conserved accessions. Analysis of variance showed significant difference between the regenerated and conserved accessions. Variations in the performance of genotypes with respect to field emergence, plant height, number of branches/plant, number of pods/plant, pod length, pod width, 100-seed weight and yield/plant between conserved and the regenerated accessions were observed. The mantle correlation between the two correlation matrices is very high (r = 0.936) and the mantle correlation between the distance matrices for regenerated and conserved accessions were highly significant (r = 0.957). The cophenetic correlation between the hierarchial clustering for regenerated and conserved accessions in the tanglegram was highly significant (r = 0.88) showing that the genotypic variation, initial viability and storage period has strong influence on the viability of soybean germplasm under the long-term conservation in genebank.

**Key Words: Agro-morphological characterization, Genebank, Field performance, Long-term conservation, Soybean**

## Introduction

Soybean [*Glycine max* (L.) Merrl.] one of the most important legume crops, rich in protein and oil, has assumed an important position in Indian oilseed cultivation scenario. It occupies an area of 12.2 million hectare accounting to a production of 11.9 million tons (FAO, 2014). Soybean seed contributes 25% of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding and is a valuable ingredient in formulated feeds for poultry and fish. Soybean is grown under varied climatic conditions and geographical locations in India.

Maintenance of seed viability plays a critical role to the sustainability of *ex situ* conserved seed collections in long-term storage. For the best seed management in the genebank, the crop curators have to be careful in handling of the seeds from the receipt up to conservation in the genebank. Orthodox seeds are generally conserved in genebanks at 3-7% moisture content with ≥ 85% seed viability and a similar practice is being followed worldwide in about 1750 genebanks (Rao *et al.,* 2006; FAO, 2013). According to the set standards, long-term conserved seeds are monitored regularly every 10 years for quality assessment and regenerated as and when required. The frequency of regeneration can also be altered depending on the storability of crop species. Studies were conducted by several workers on seed monitoring during long-term conservation in several crops *e.g.* cereals (Ruiz *et al.,* 1999), threatened species (Godefroid *et al.,* 2010), other crops (Van Treuren *et al.,* 2013), rice (Hay *et al.,* 2015). A large extent of variations were observed by several workers during seed testing which may be due to the lower initial quality of seeds, differences in physiological maturity of seed at harvest, post-harvest handling procedures, storage temperatures, relative humidity of the storage environment, poor packaging, estimation error, seed proximate composition, dormancy and its genetic constitution (Godefroid *et al.,* 2010; Hay *et al.,* 2013; van Treuren *et al.,* 2013).

Genebanks, the richest reservoirs of valuable plant genetic resources, hold numerous untapped traits which are useful for crop improvement. Indian National

**Author for Correspondence: Email- kiranbabubot@gmail.com*

Genebank (NGB), New Delhi, India, is the second largest genebank with a total holding of 0.44 millions accessions of various agri-horticultural crops including 3,702 accessions of soybean. Field evaluation of naturally aged seeds conserved in the genebank has been undertaken in soybean (Petrova and Geshava, 2017). Most of the studies on long-term conserved germplasm conducted were confined to the seed quality assessment based on seed viability and vigour (Ruiz *et al.,* 1999; Godefroid *et al.,* 2010; Abdellaoui *et al.,* 2013; van Treuren *et al.,* 2013; Hay *et al.,* 2013, 2015; Desheva *et al.,* 2017). However, information on the field performance of long-term conserved germplasm in the field is not available in literature. During monitoring, soybean germplasm conserved under long-term conditions showed lot of variation amongst accessions in terms of seed viability and vigour in laboratory conditions which cannot be precisely compared to the performance of the conserved germplasm in the field (Kulik and Yaklish, 1982; Wang *et al.,* 2004). Therefore, the present investigation is an attempt to compare the field performance of long-term conserved soybean germplasm (30 years in NGB) with the same set of freshly regenerated accessions for 25 qualitative (14) and quantitative (11) traits.

## Materials and Methods

A total of 100 soybean accessions were selected on the basis of variability in seed colour, seed size and geographical origin which were collected/multiplied during 1985. These were processed as per gernebank standards and conserved under long-term conditions at -18±2°C for 30 years, were used for the present study. Out of 100 germplasm accessions, 37 were indigenous collections from different regions of India and remaining were originally sourced from different countries *viz*., 19 from USA, 15 from Australia, 8 from Germany, 4 from Fiji, 3 each from Brazil and Taiwan Province of China, 2 from Bolivia and 1 each from Argentina, Canada, Hungary, Indonesia, Japan, Morocco, Papua New Guinea, South Africa and Russia (Table 1). Some 100-120 seeds of each of the above accessions were drawn from National Genebank (NGB) and equilibrated to room temperature. This was divided into two sets comprising 50-60 seeds each. One original set (conserved for 30 years in NGB; hereafterward referred to as conserved accessions) was retained as such and another set comprising 50-60 seeds was regenerated (hereafterward referred to as regenerated accessions) at ICAR-NBPGR Regional Station, Akola, during 2014-15.

The experiment to study the field performance of above accessions was conducted during *kharif* (July to October 2015) at Research Farm of ICAR-National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi, India, located at 28°35'N, 77°12'E, altitude 228.6m.The farm is located in semi-arid, sub-tropical climate with average temperature ranging from 19 to 32°C and total rainfall of 706.3 mm recorded during the cropping season (July–October 2015). The soil type was sandy loam to loamy with pH 7.5. Before sowing, FYM (farm yard manure) and recommended dose of fertilizers (20 N, 60 P, 20 K kg/ha) were applied. Four irrigations were given as and when required.

To compare the field performance of seeds of originally conserved accessions and same set of regenerated accessions (control), were grown in the field in two replicates of 30 seeds each. The experimental design was Reinforced Alpha Lattice (Gangopadhyay *et al.,* 2010) with 20 blocks in each replication; 30 seeds were sown in each row of 2 m length (spacing 45 cm × 10 cm). Each block contained 10 accessions and two national checks (SL-688 and PUSA 12). The regenerated and conserved accessions were evaluated based on the 25 standard Distinctness, Uniformity and Stability (DUS) descriptors and minimal descriptors (PPV&FRA, 2009; IBPGR, 1984). Qualitative characters included plant hypocotyl-anthocyanin pigmentation, plant growth type, leaf colour, leaf shape, plant growth habbit, flower colour, presence of pod pubescence, pod pubescence colour, pod colour, pod shattering nature, seed colour, seed lustre, seed shape and seed hilum colour. Observations on quantitative traits were recorded on five randomly selected and tagged plants in the defined block of each genotype. The observations of quantitative characters were recorded for field emergence (%), plant height (cm), days to 50% flowering (No.), days to maturity (No.), number of branches/plant, number of pods/plant, pod length (mm), pod width (mm), number of seeds/pod, 100-seed weight (g) and seed yield/plant (g).

### *Statistical Analyses*

The analysis of variance for Reinforced Alpha Lattice Design was performed using PROC MIXED procedure of SAS® ver. 9.2 (SAS Institute, Cary, NC, USA) and least square means were computed. The rest of the analyses were performed in R package (R Core Team, 2017). Descriptive statistics were computed using 'psych' package (Revelle, 2017) and box plots

**Table 1. List of selected 100 soybean accessions used to study their field performance**

| S. No. | Accession ID | Country of origin | S. No. | Accession ID | Country of origin |
|---|---|---|---|---|---|
| 1 | EC 4486 | Argentina | 52 | IC 24058 | India |
| 2 | EC 14469 | Australia | 53 | IC 24060 | India |
| 3 | EC 14479 | Australia | 54 | IC 501186 | India |
| 4 | EC 36372 | Australia | 55 | IC 24994 | India |
| 5 | EC 76756 | Australia | 56 | IC 25763 | India |
| 6 | EC 109543 | Australia | 57 | IC 501599 | India |
| 7 | EC 109517 | Australia | 58 | IC 501154 | India |
| 8 | EC 109546 | Australia | 59 | IC 501171 | India |
| 9 | EC 109549A | Australia | 60 | IC 501200 | India |
| 10 | EC 109551 | Australia | 61 | IC 501202 | India |
| 11 | EC 109552 | Australia | 62 | IC 501150 | India |
| 12 | EC 109553 | Australia | 63 | IC 501286 | India |
| 13 | EC 109556 | Australia | 64 | IC 501316 | India |
| 14 | EC 109557 | Australia | 65 | IC 501337 | India |
| 15 | EC 109548 | Australia | 66 | IC 501392 | India |
| 16 | EC 95674 | Australia | 67 | IC 501531 | India |
| 17 | EC 93598 | Bolivia | 68 | IC 501468 | India |
| 18 | EC 93602 | Bolivia | 69 | IC 501493 | India |
| 19 | EC 93744 | Brazil | 70 | IC 501220 | India |
| 20 | EC 93745 | Brazil | 71 | IC 501581 | India |
| 21 | EC 93749 | Brazil | 72 | IC 501592 | India |
| 22 | EC 61434 | Canada | 73 | EC 112828 | Indonesia |
| 23 | EC 114522 | Fiji | 74 | EC 62818 | Japan |
| 24 | EC 114523 | Fiji | 75 | EC 38128 | Morocco |
| 25 | EC 114525 | Fiji | 76 | EC 113401 | Papua New Gyinea |
| 26 | EC 114532 | Fiji | 77 | EC 4496 | South Africa |
| 27 | EC 34342 | Germany | 78 | EC 39489 | Taiwan Province of China |
| 28 | EC 34343 | Germany | 79 | EC 113773 | Taiwan Province of China |
| 29 | EC 34374 | Germany | 80 | EC 116055 | Taiwan Province of China |
| 30 | EC 93425 | Germany | 81 | EC 25167 | United States of America |
| 31 | EC 39039 | Germany | 82 | EC 2573 | United States of America |
| 32 | EC 39020 | Germany | 83 | EC 6103 | United States of America |
| 33 | EC 39028 | Germany | 84 | EC 61396 | United States of America |
| 34 | EC 39043 | Germany | 85 | EC 65773 | United States of America |
| 35 | EC 34117 | Hungary | 86 | EC 95280 | United States of America |
| 36 | IC 15539 | India | 87 | EC 95278 | United States of America |
| 37 | IC 501434 | India | 88 | EC 95264 | United States of America |
| 38 | IC 501263 | India | 89 | EC 95281 | United States of America |
| 39 | IC 03 | India | 90 | EC 100783 | United States of America |
| 40 | IC 501267 | India | 91 | EC 100786 | United States of America |
| 41 | IC 501185 | India | 92 | EC 100790 | United States of America |
| 42 | IC 15554 | India | 93 | EC 100794 | United States of America |
| 43 | IC 16561 | India | 94 | EC 100797 | United States of America |
| 44 | IC 16573 | India | 95 | EC 100804 | United States of America |
| 45 | IC 16804 | India | 96 | EC 106992 | United States of America |
| 46 | IC 16816 | India | 97 | EC 107006 | United States of America |
| 47 | IC 16823 | India | 98 | EC 95261 | United States of America |
| 48 | IC 18751 | India | 99 | EC 95269 | United States of America |
| 49 | IC 18756 | India | 100 | EC 37089 | USSR |
| 50 | IC 21755 | India | 101 | Pusa 12 | India (National Check) |
| 51 | IC 21756 | India | 102 | SL 688 | India (National Check) |

were plotted using 'ggplot2' package (Wickham, 2009). The least square means were used for accession comparison, correlation and cluster analysis. Pearson's linear correlation coefficients among all the traits were computed using the R package 'Hmisc' (Harrel and Dupont, 2017) and plotted using 'corrplot' (Wei and Simko). Principal component analysis was performed in 'stats' package (R Core Team, 2017) and plotted using 'scatterplot3d' package (Ligges, 2003). Gower distance measure implemented in the R package 'cluster' was used to obtain the genetic distance among accessions (Gower, 1971; Maechler, 2017) for hierarchical clustering by UPGMA method. Mantel correlation (Mantel, 1967) between the distance matrices for regenerated and conserved accessions were computed using the 'ade4' package (Dray *et al.,* 2007). Similarly the cophenetic correlation between the hierarchial clustering for regenerated and conserved accessions were computed and a tanglegram was plotted using the 'dendextend' package (Galili, 2015).

## Results

Table 2 resets the data on agro-morphological characters of regenerated ad conserved accessions of soybean. The descriptive statistical analysis of the agro-morphological data of regenerated and conserved accessions (conserved for 30 years in NGB) showed large variation in the mean values of field emergence (%), plant height, number of pods/plant and plant yield (g). Field emergence was observed as highest in seeds of regenerated accession IC 21755 (100%) followed by EC 34342 (98%), whereas highest field emergence was recorded in seeds of conserved accessions in EC 95281 (88%) followed by EC 109556 (86%). These records correlated well with the seed germination, vigour values recorded in laboratory conditions (J Radhamani pers. comm., 2015). The field emergence of the seeds of regenerated accessions varied from 33.3 to 100%, with a mean value of 74.5%, whereas for the seeds of conserved accessions, the range of field emergence was recorded as 16.6 to 88% with a mean value of 48.86%, which is almost 35% lower than that of the seeds of regenerated accessions. The mean values of plant height and yield/plant of regenerated and conserved accessions was 39 cm and 36 cm and 8.08 g and 7.34 g, respectively. Less variation was observed in the days to 50% flowering, days to maturity, pod size, number of seeds/pod and 100-seed weight (Table 2). Number of seeds/ pod ranged between 2-3 and 100-seed weight from 3.45 g to 18.25 g in regenerated and 3.51 to 18.00 g in conserved accessions with a mean value of 10.07 g. Seed yield ranged from 3.21 to 14.02 g in regenerated and 3.03 to 13.87 g in conserved accessions with a mean value of 7.86 g. Irrespective of the regenerated or conserved accessions, the highest number of branches/ plant was recorded in EC 95280 and EC 95674 (8.1); similarly, the highest yield/plant was recorded in IC 501220 and EC 95278 (12.8 g).

The frequency distribution on 14 qualitative traits of regenerated and conserved accessions is presented in Table 3. No differences for qualitative traits between regenerated and conserved accessions were observed.

**Table 2. Mean ± SE and range of agro-morphological data of regenerated accessions and conserved accessions (30 years in NGB) of soybean recorded in field**

| | Field emergence % | | Days to 50% flowering | | Days to maturity | | Plant height (cm) | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SE | Range | Mean ± SE | Range | Mean ± SE | Range | Mean ± SE | Range |
| Check | 93.9 ± 0.5 | 83.3 - 100.0 | 50.3 ± 0.7 | 41.0 - 58.0 | 112.7 ± 0.5 | 105.0 - 119.0 | 67.6 ± 0.5 | 60.5 - 75.4 |
| Regenerated | 74.5 ± 1.2 | 33.3 - 100.0 | 49.0 ± 0.5 | 28.0 - 66.0 | 105.5 ± 0.5 | 83.0 - 121.0 | 39.3 ± 0.7 | 21.3 - 66.5 |
| Conserved | 48.9 ± 1.5 | 16.7 - 93.3 | 49.8 ± 0.5 | 28.0 - 64.0 | 106.0 ± 0.5 | 84.0 - 120.0 | 36.3 ± 0.6 | 20.3 - 61.3 |
| Overall | 61.7 ± 1.2 | 16.7 - 61.7 | 49.4 ± 0.4 | 28.0 - 49.3 | 105.8 ± 0.4 | 83.0 - 105.8 | 37.8 ± 0.4 | 20.3 - 37.8 |
| | No. of branches/plant | | No. of pods/plant | | Pod length (mm) | | Pod width (mm) | |
| Check | 5.0 ± 0.03 | 4.0 - 5.6 | 64.1 ± 0.5 | 56.4 - 72.6 | 41.0 ± 0.2 | 36.5 - 44.9 | 7.231 ± 0.04 | 4.3 - 7.9 |
| Regenerated | 5.4 ± 0.08 | 3.2 - 8.6 | 37.5 ± 0.7 | 15.8 - 69.6 | 35.6 ± 0.3 | 23.4 - 44.4 | 7.277 ± 0.06 | 4.9 - 9.8 |
| Conserved | 4.9 ± 0.07 | 3.0 - 8.4 | 31.3 ± 0.6 | 12.3 - 55.3 | 34.8 ± 0.3 | 20.0 - 45.8 | 7.116 ± 0.06 | 4.6 - 9.7 |
| Overall | 5.2 ± 0.05 | 3.0 - 5.2 | 34.4 ± 0.5 | 12.3 - 34.4 | 35.2 ± 0.2 | 20.0 - 35.2 | 7.197 ± 0.04 | 4.6 - 7.2 |
| | No. of seeds/pod | | 100-seed weight (g) | | Yield/Plant (g) | | | |
| Check | 2.9 ± 0.01 | 2.6 - 3.0 | 9.4 ± 0.1 | 7.9 - 10.6 | 8.6 ± 0.2 | 6.0 - 11.6 | | |
| Regenerated | 2.5 ± 0.02 | 2.0 - 3.0 | 10.3 ± 0.2 | 3.4 - 18.2 | 8.1 ± 0.1 | 3.2 - 14.0 | | |
| Conserved | 2.5 ± 0.02 | 2.0 - 3.0 | 10.1 ± 0.2 | 3.5 - 18.0 | 7.3 ± 0.1 | 3.0 - 13.9 | | |
| Overall | 2.5 ± 0.01 | 2.0 - 2.5 | 10.2 ± 0.1 | 3.4 - 10.2 | 7.7 ± 0.1 | 3.0 - 7.7 | | |

SE, Standard error, Mean values were rounded off to the nearest integer up to one place of decimal

**Table 3. Frequency distribution of 14 qualitative traits of soybean germplasm**

| Trait | State | No. of accessions |
|---|---|---|
| Hypocotyl anthocyanin colour | Present | 73 |
| | Absent | 27 |
| Plant growth type | Determinate | 100 |
| Leaf colour | Green | 82 |
| | Dark green | 18 |
| Leaf shape | Pointed | 92 |
| | Round | 5 |
| | Lanceolate | 3 |
| Growth habitat | Erect | 32 |
| | Semi erect | 67 |
| | Spreading | 1 |
| Flower colour | Purple | 77 |
| | White | 27 |
| Pod Pubescence | Present | 100 |
| Pod pubescence colour | Brown | 82 |
| | Grey | 18 |
| Pod colour | Brown | 71 |
| | Yellow | 26 |
| | Black | 3 |
| Pod shattering nature | Shattering | 74 |
| | Non-shattering | 26 |
| Seed colour | Yellow | 59 |
| | Yellow green | 24 |
| | Black | 10 |
| | Brown | 7 |
| Seed shape | Elliptic | 57 |
| | Spherical | 43 |
| Seed lustre | Shiny | 62 |
| | Dull | 38 |
| Seed hilum colour | Brown | 65 |
| | Black | 35 |

Hypocotyl anthocyanin pigmentation recorded on the 9- or 10-day-old seedlings, showed that 73% of the accessions were pigmented and 27% non-pigmented. All the 100 accessions were found to be determinate and the most of the accessions (67%) were semi-erect type. Flower colour varied from white (27%) to purple (77%). Pod colour also varied from black (3%), brown (71%) and yellow (26%). Pods of all the accessions showed pubescence, of which 82% showed brown pubescence and 18% grey. Most of the accessions (92%) exhibited pointed tips with green leaves (82%). Pod shattering habit was recorded at the physiological maturity stage and 74% of the accessions were non-shattering and remaining were of shattering type. Distinct seed coat colours were observed among the accessions; 59% accessions were observed as yellow, 26% yellow green and remaining were either black or brown. Some 67% accessions were elliptical and 43% had spherical shape. Seed of 62% of accessions were lustrous and remaing were dull in nature. In 65% of genotypes hilum colour was brown and the rest had black colour.

ANOVA of all the 11 quantitative characters with replication are non-significant between the regenerated and conserved accessions. However, block (replication) is significant for plant height. Accession-wise differences are highly significant for all the 11 quantitative characters. The interaction between regenerated *vs* conserved germplasm is significant for most of the characters but non-significant for days to 50% flowering, days to maturity and number of seeds/pod (Table 4).

### *Correlation Analysis*

Correlations are used to quantify the relationships between different characters, which are important for selection. Comparison of the correlations between regenerated and conserved accessions revealed a similar trend of relationships. For example, significant positive correlation was observed between days to 50% flowering × days to maturity, number of branches/ plant x number of pods/plant, 100-seed weight × yield/ plant, plant yield x number of pods/plant. A highly negative correlation was observed between days to 50% flowering × pod width, plant height × pod width, pod length × pod width, days to 50% flowering × 100-seed

**Table 4. ANOVA of quantitative traits used to evaluate the field performance of soybean germplasm**

| Source | DF | Field emergence (%) | Days to 50% flowering | Days to maturity | Plant height (cm) | No. of branches/ plant | No. of Pods /plant | Pod length (mm) | Pod width (mm) | No. of seed/ pod | 100-seed wt (g) | Yield/ plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | 1 | 0.05$^{NS}$ | 0.74$^{NS}$ | 12.41$^{NS}$ | 1.52$^{NS}$ | 0.06$^{NS}$ | 0.06$^{NS}$ | 2.98$^{NS}$ | 0.32$^{NS}$ | 0.07$^{NS}$ | 0.03$^{NS}$ | 0.21$^{NS}$ |
| Block (Replication) | 38 | 37.04$^{NS}$ | 15.79$^{NS}$ | 17.79$^{NS}$ | 15.08* | 0.11$^{NS}$ | 18.16$^{NS}$ | 7.89$^{NS}$ | 0.41$^{NS}$ | 0.03$^{NS}$ | 0.06$^{NS}$ | 0.21$^{NS}$ |
| Acc. No. | 201 | 1151.91** | 101.29** | 113.82** | 440.94** | 2.36** | 459.4** | 36.84** | 0.81** | 0.19** | 17.88** | 10.45** |
| Regenerated vs Conserved | 1 | 25152.7** | 3.4$^{NS}$ | 0.34$^{NS}$ | 251.71** | 9.55** | 1479.27** | 32.08* | 1.79* | 0.01$^{NS}$ | 1.63** | 19.43** |
| Error | 239 | 53.46 | 13.61 | 15.84 | 9.2 | 0.11 | 14.76 | 6.9 | 0.42 | 0.03 | 0.05 | 0.22 |

**significant at the 0.01 probability level; *significant at the 0.05 probability level; NS, Non-significant weight (Table 5). The mantle correlation between the two correlation matrices was very high (r = 0.936) and the mantle correlation between the distance matrices for regenerated and conserved accessions were highly significant (r = 0.957). The cophenetic correlation between the hierarchial clustering for regenerated and conserved accessions in the tanglegram was highly significant (r = 0.88) (Fig. 1).

### *Principal Component Analysis*

Through principal component analysis, 11 principal components were extracted from the 11 quantitative traits. However, among these, the first three components explain 88.7% of the total variation and hence can be used to summarize the original 11 variables. The variability contributed by first component was 62.98% followed by second component (14.45%) and third component (11.28%) (Fig. 2a). Clustering on the basis of these three components revealed overlapping of regenerated and conserved accessions (Fig. 2b). An examination of the loading values in the eigen vectors for these principal components revealed the contribution of individual characters towards clustering. For the first component, field emergence had the highest positive loading, greater in magnitude than that for other traits. Similarly, for the second component plant height and number of pods/plant had high positive loadings. In the third component, days to maturity and days to 50% flowering had high positive loading and number of pods/plant had high negative loading (Fig. 2c). So these traits contribute more to the diversity observed in the studied accessions of soybean. Hence, the marginal grouping observed among the accessions with respect to their storage status may be due to fact that the seed vigour is an influenced trait of field emergence and other related traits such as number of pods/plant and plant height.

## Discussion

Under long-term storage orthodox seeds are generally stored *ex situ* at -18±2°C conditions. *Ex situ* storage influences the seed germination, vigour, rate of seed germination based on the seed quality, proximate composition, seed moisture content and the genetic constitution. The seeds are best stored at an optimum moisture level of 3-7% at sub-zero temperature as most of the metabolic processes of the seeds slow down. Loss in seed vigour precedes loss in seed germination eventually leading to seed deterioration (Brutting *et al.,* 2013).

**Table 5. Correlation coefficient among qualitative and quantitative descriptors between regenerated and conserved accessions of soybean**

| | | FE | DF | DM | PH | NBPP | NPPP | PL | PW | NSPP | HSW | YPP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FE | REG | 1 | | | | | | | | | | |
| | CON | 1 | | | | | | | | | | |
| DF | REG | 0.12 | 1 | | | | | | | | | |
| | CON | 0.26** | 1 | | | | | | | | | |
| DM | REG | 0.11 | 0.97** | 1 | | | | | | | | |
| | CON | 0.23** | 0.98** | 1 | | | | | | | | |
| PH | REG | 0.02 | 0.11 | 0.09 | 1 | | | | | | | |
| | CON | 0.17* | 0.18* | 0.17* | 1 | | | | | | | |
| NBPP | REG | -0.11 | 0.17* | 0.17* | 0.20** | 1 | | | | | | |
| | CON | 0.05 | 0.1 | 0.11 | 0.27** | 1 | | | | | | |
| NPPP | REG | 0.09 | 0.13 | 0.16* | 0.23** | 0.48** | 1 | | | | | |
| | CON | 0.16* | 0.05 | 0.06 | 0.26** | 0.46** | 1 | | | | | |
| PL | REG | -0.19** | -0.20** | -0.18* | -0.07 | -0.18* | -0.01 | 1 | | | | |
| | CON | -0.11 | -0.06 | -0.06 | -0.05 | -0.19** | -0.05 | 1 | | | | |
| PW | REG | -0.08 | -0.33** | -0.31** | -0.32** | -0.17* | -0.14 | 0.40** | 1 | | | |
| | CON | -0.18* | -0.24** | -0.23** | -0.20** | -0.12 | -0.13 | 0.38** | 1 | | | |
| NSPP | REG | -0.19** | -0.21** | -0.18** | 0.05 | 0.06 | 0.08 | 0.24** | 0.1 | 1 | | |
| | CON | -0.1 | -0.15* | -0.11 | -0.01 | 0.07 | 0.21** | 0.05 | 0.07 | 1 | | |
| HSW | REG | -0.04 | -0.31** | -0.27** | 0.01 | 0.09 | 0.04 | 0.16* | 0.15* | 0.03 | 1 | |
| | CON | 0.02 | -0.17* | -0.17* | -0.02 | 0.09 | 0.06 | 0.17* | 0.19** | -0.08 | 1 | |
| YPP | REG | -0.02 | -0.11 | -0.05 | 0 | 0.20** | 0.32** | 0.14* | 0.05 | -0.02 | 0.79** | 1 |
| | CON | 0.11 | -0.07 | -0.04 | -0.06 | 0.20** | 0.34** | 0.19** | 0.08 | -0.02 | 0.76** | 1 |

FE: field emergence, DF: days to 50% flowering, DM: Days to maturity, PH: Plant height, NBPP: No. of branches/plant, NPPP: No. of pods/plant, PL: Pod length, PW: Pod width, NSPP: No. of seeds/pod, HSW: 100-seed weight. YP: Yield/plant

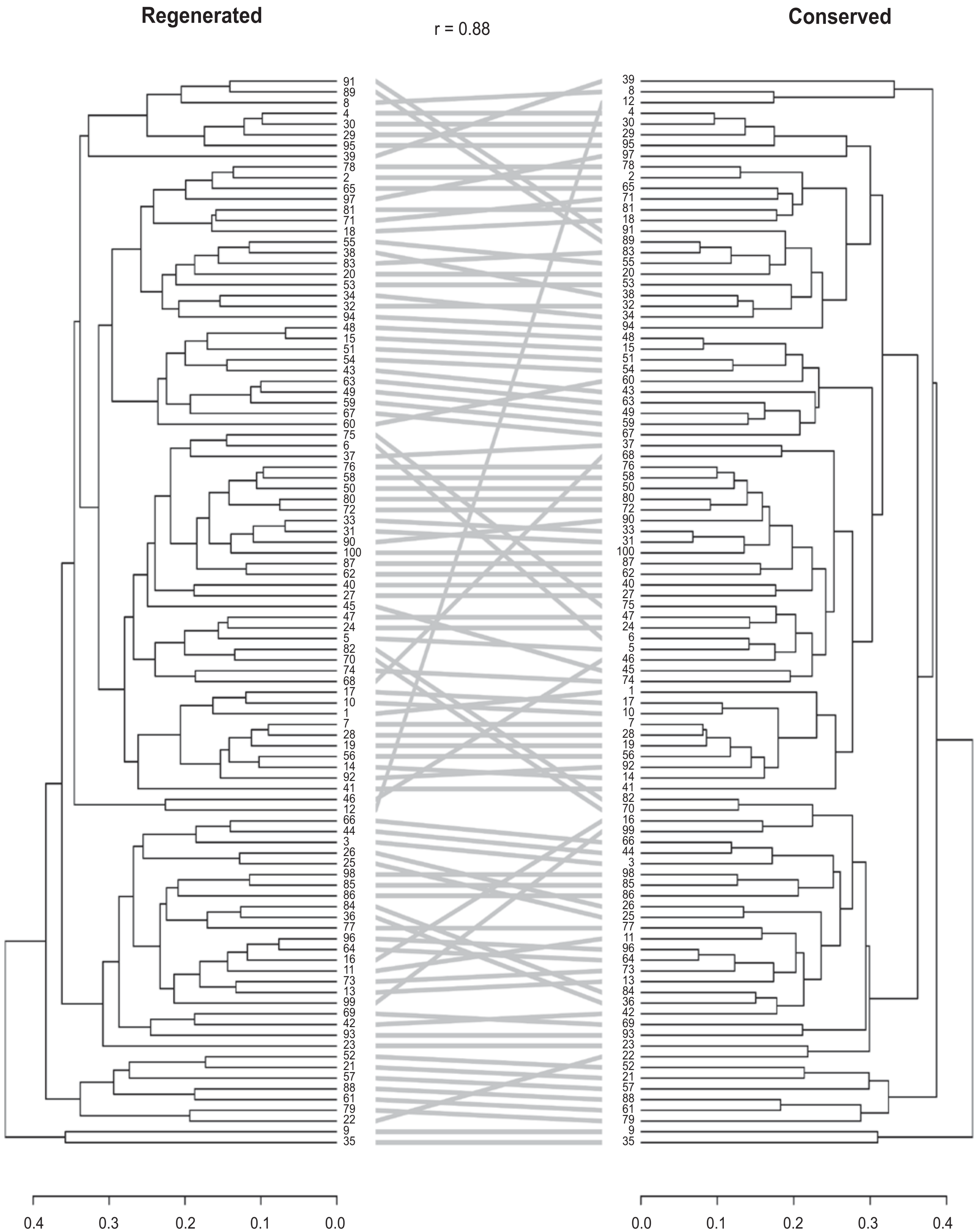


**Fig. 1. Cophenetic correlation between the hierarchial clustering of regenerated and conserved accessions in the tanglegram (Numbers are corresponding to the accessions listed in Table 1)**

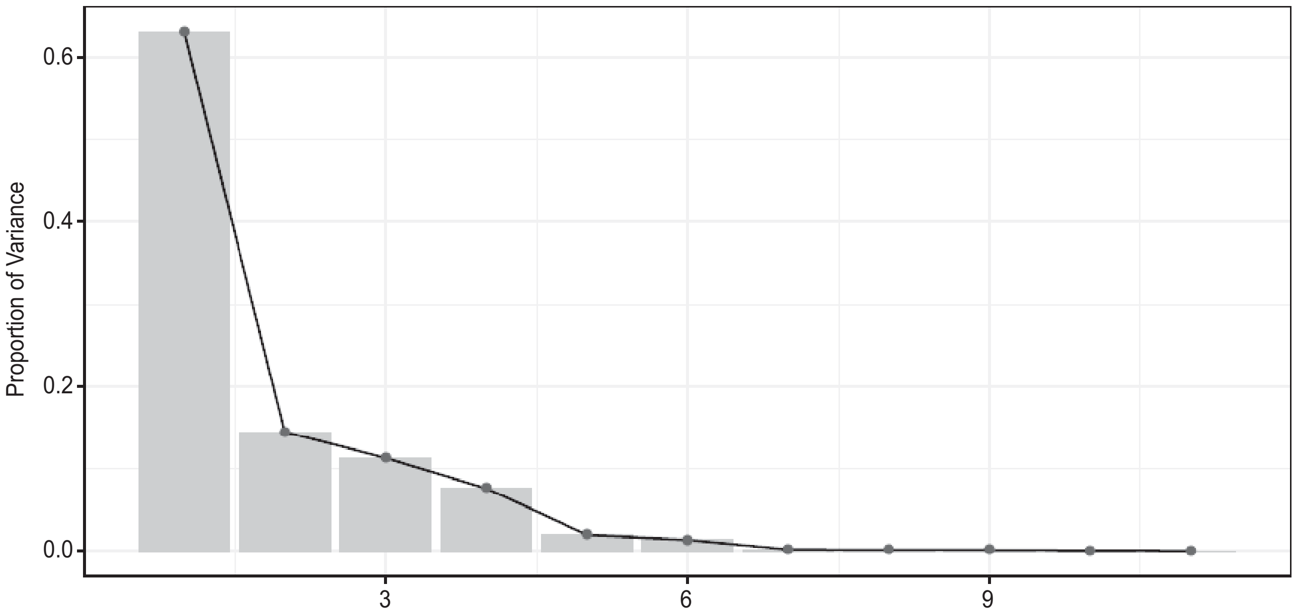


**Fig. 2a. Principal components plot of 100 accessions (regenerated and conserved) of soybean**

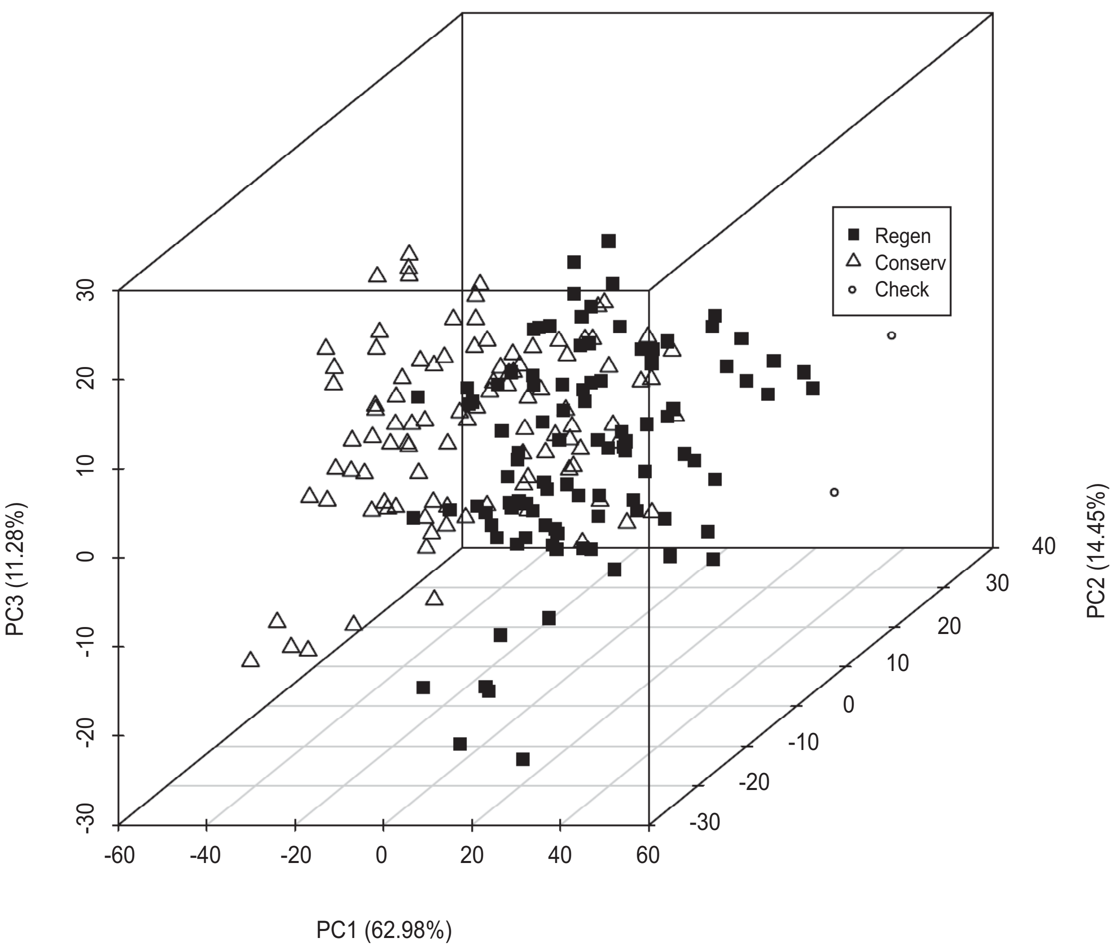


**Fig. 2b. Clustering on the basis of three (PC1, PC2, PC3) components revealed overlapping clusters of regenerated and conserved accessions of soybean (Regn: Regenerated; Conserv: Conserved accessions)**

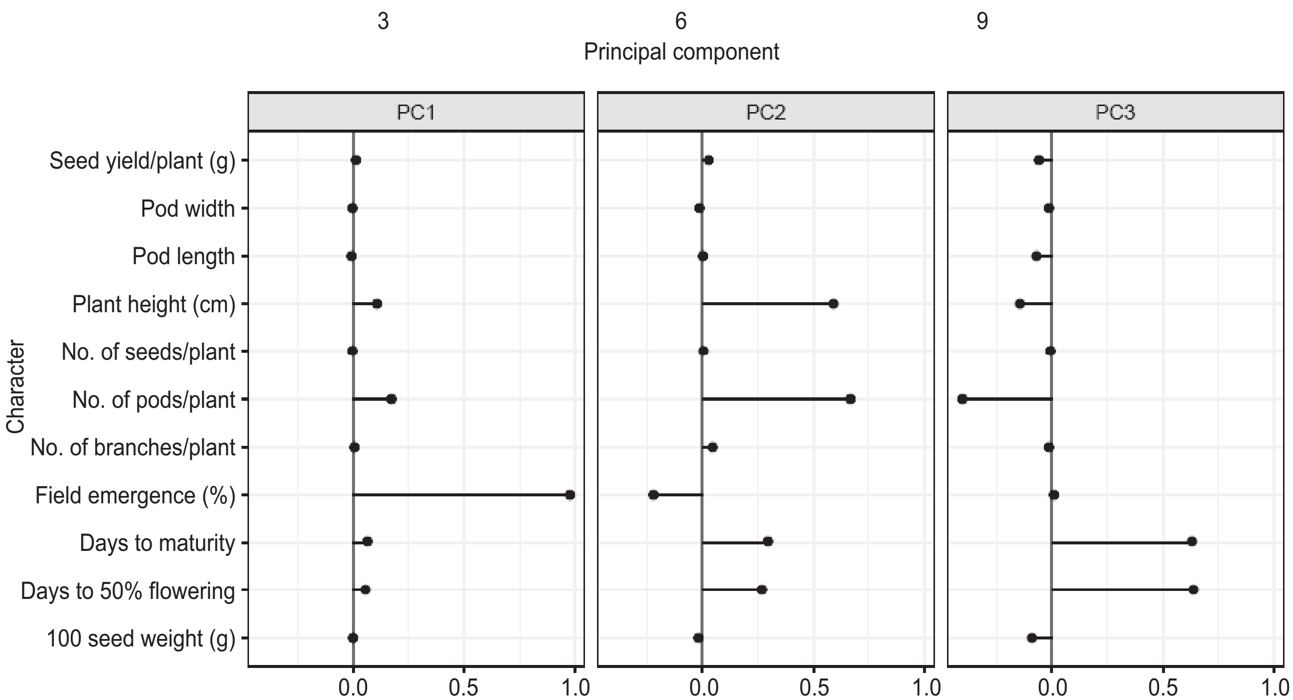


**Fig. 2c. Eigen loading values and eigen vectors for first three principal components**

The vigour is reduced after long-term storage which varies between crops, among plant species, accession to accession complying with the agreement that the phenotypic expressions are influenced by the genetic constitution of the seed. Monitoring the seed viability and vigour is critical for sustainable conservation of germplasm in genebank (Alyaha, 1995). Monitoring of seed viability and vigour in laboratory conditions is common practice to test the seed quality of accessions conserved under long-term storage conditions. However, the performance of conserved germplasm can be better judged based on their field emergence, good vigour, uniform establishment of seedling and ultimately yield by growing in the field and comparing them with freshly regenerated germplasm. The authors report on the field performance of 100 soybean accessions conserved under long-term storage at -18±2$^o$C temperature for 30 years in genebank. Control set comprised of the regenerated set of same original 100 accessions from NGB which comprised the conserved accessions to compare their performance in the field.

Although, under standard genebank storage conditions, high viability is supposed to be maintained even after many decades, it is observed that seeds of several accessions of soybean could not retain viability after storage (30 years) closer to initial viability and suffered natural ageing leading to decrease in seed quality to various extents. Our studies indicate that seeds when stored with moisture (3-7% mc) and germination (75%) at -18°C, the seed metabolic activities do not cease completely but continues to take place at a very slow pace, the rate of which may not be uniform in all the accessions thereby causing difference in viability pattern of these accessions during ageing in genebank. These differences are only expressed during seed germination of different seed lots of stored germplasm. At low moisture contents under long-term storage (3-7% mc) when most of the water content in the seeds is in the bound form, non-enzymatic lipid auto-oxidation might be the primary cause of seed deterioration (McDonald, 1999; Murthy *et al.* 2003; Smith and Berjak, 1995; Walters *et al.,* 2005).

Similarly, in case of wheat, barley, oat germplasm monitored after 10 years in Spanish Genetic Resources Centre (Ruiz *et al.,* 1999), in rice after 30 years in IRRI (Hay *et al.,* 2013, 2015), in several threatened species up to 26 years in National Botanic Garden in Belgium (Godefroid *et al.,* 2010), soybean after 30 years in Bulgarian National Seed Bank (Desheva *et al.,* 2017), several cultivated and wild taxa after 25 years in Centre for Genetic Resources, The Netherlands (van Treuren *et al.,* 2013) it was observed that germination declined to various extents tested in laboratory conditions. Studies conducted by Moyo *et al.,* (2015) showed significant difference in the performance of genotypes with respect to vigour and field emergence in sorghum genotypes conserved for 10 years under long-term storage.

To best of our knowledge, there is no information available on the field performance studies of long-term conserved germplasm in genebank in literature, similar to the results obtained in our studies. However, on the basis of studies on short- or medium-term storage germplasm or artificial ageing conditions, conducted by Potts *et al.* (1983), Alvarez *et al.* (1997) Karmakar *et al.* (1999) it was observed that most of the seed characteristics *viz.* seed size, seed coat colour, thickness, oil content were associated with seed quality traits. Therefore, low seed quality due to deterioration is reported to be the cause of poor stand establishment in the field and consequently yield loss in barley (Abdalla and Roberts, 1969), wheat (Ganguli and Sen-Mandi, 1990), oilseed rape (Ghassemi-Golezani *et al.,* 2010), maize (Ghassemi-Golezani *et al.,* 2011).

For other traits studied, no significant differences were observed in yield and yield contributing components between regenerated and conserved accession indicating that no apparent heritable changes occurred in soybean germplasm during the storage period of 30 years in genebank, as these accessions were multiplied for the first time. There is a great variation in the field emergence of conserved and regenerated accessions. One of the reason for this could be attributed due to the differences in seed size, colour, country of origin and variation of the genotype itself which finally led to the varied range of germination (Dassou and Kueneman, 1984; Mugnisjah *et al.,* 1987; Kuchalan, 2006; Zahid, 2013). The other reason might be due to the poor initial viability of soybean accessions not conforming to genebank standards and also due to the seeds during processing were subjected to lower standards of processing conditions in the genebank. The soybean germplasm were packed in aluminium foil pouches non-hermetically sealed. In non-hermetically seed pouches there might have been presence of more oxygen which could have triggered the process of seed deterioration leading to the observed difference in the pattern of germination of conserved seeds *vis-à-vis* regenerated seeds.

It can be concluded that genotypic variation, pre-harvest conditions, initial viability and storage conditions appear to be most influential factors for maintaining the higher seed viability in genebank also and in turn, the germinability in field conditions which was supported by other studies undertaken by Lu *et al.* (2004) and Benkova and Zakova (2009) in various crops conserved for long-term in genebanks.

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