

## Rescuing and regenerating germplasm in large collections – the case of pigeonpea accession ICP 9820

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### Introduction

The maintenance of seed viability is a basic requirement for successful preservation of germplasm. Seeds intended for storage should have high germinability and viability should be monitored by periodical germination tests during storage. Even for the seeds stored under optimal conditions suitable for long-term storage, viability may decrease as a result of deterioration processes such as: an increase of the free radical content, changes in protein structure, depletion of food reserves, acidification of fat, changes in enzymatic activity, membrane damage, chromosomal changes and an increase in respiration (Justice and Bass 1979). These processes manifest at different levels resulting in decreased germinability, production of abnormal seedlings, and even complete loss of viability.

The Genebank at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Patancheru, India conserves 13,632 accessions of pigeonpea (*Cajanus cajan*) from 74 countries as active collection and 12,138 accessions as base collection for present and future use in crop improvement. An inventory on the active collection representing 12,786 accessions was made during 1999. As part of inventory, seed viability of all the accessions was tested using 50 seeds in each accession. Germination test was performed to determine the proportion of seeds in an accession that will germinate under favorable conditions and produce normal seedlings according to specific criteria for each species (ISTA 2005). The average viability of the collection was 95%. However, about 3% of accessions had <75% viability. The accessions that have <85% viability were considered as critical ones under medium-term storage conditions and were considered for regeneration during the subsequent seasons. Due to their low viability levels such accessions did not meet the requirement for long-term storage and distribution for utilization. The pigeonpea accession ICP 9820 with 78%

viability was one among the several critical accessions that were identified for regeneration. This accession originated from Maharashtra, India. It was initially grown during 1978 rainy season for seed increase and seeds with a viability of 90% were conserved in ICRISAT's medium-term cold room at 4°C and 30% RH, as active collection during 1979.

### Materials and methods

During 2004 rainy season, when 1,497 critical accessions were planted in the regeneration field, there was no germination in ICP 9820 when observations were made 10 days after sowing. In other accessions, germination was normal and plant stand was good. Even after three weeks in the field there was no seedling emergence in ICP 9820. To resurrect this accession, about 200 seeds from the available seed stock of this accession (1978 rainy season harvest) were incubated in the seed laboratory using bacteriological agar. Agar medium is ideal for seed germination and seedling growth compared to paper towels as seedlings from agar medium can be removed without injury for transplantation in the glasshouse or field (Kameswara Rao and Sastry 1996). About 15 seedlings which emerged after >15 days of incubation were initially transferred to paper cups for growing in the glasshouse and later to the field for better establishment. Of these, only four plants survived and special care was taken for securing seeds from them. About 220 g clean seed obtained from these plants was transferred to cold room for conservation.

Germination tests on fresh harvest (2004 rainy season) and the old lot (1978 rainy season) were conducted using between papers method (ISTA 1993). Two replications of 50 seeds each treated with thiram were incubated at 25°C for 20 days. The normal 10-day test was extended to 20 days facilitating the less vigorous seeds from 1978 lot to germinate and grow.

## Results and discussion

Observations on normal and abnormal seedlings, diseased and dead seeds of the two seed lots were recorded (Table 1). After 10 days, the seeds of 2004 lot produced 99% normal seedlings, 1% abnormal seedlings and no diseased/dead seeds. Germination was 0% in the 1978 seed lot after 10 days. However, when the test was extended for 10 more days in both the lots for recording the observations on seedling vigor, few seeds started sprouting in the 1978 lot. Seedling emergence was slow in this seed lot even after 20 days. About 11% of the seeds produced root and shoot growth and were considered as germinated (weak seedlings), 74% were abnormal seedlings and 15% were diseased/dead seeds. A high initial viability is expected when an accession is regenerated under favorable conditions as evident from the fresh harvest. However, the initial viability of harvests from 1978 planting was only 90% indicating that ageing has already occurred in the seed lot and 10% of the seeds are unable to produce normal seedlings in a germination test. There are several factors contributing to seed quality during regeneration (Kameswara Rao and Sastry 1998). The reduction in viability from 90% to 78% after 21 years of storage (1979–99) and further reduction to 0% in 2005 (26 years) followed a normal seed longevity pattern (Harrington 1972). Seed vigor of the two seed lots was compared (Fig. 1). Root and shoot lengths of 10 seedlings were measured randomly after 20 days. The mean root and shoot lengths of 2004 seed lot were 140 and 248 mm respectively and 17 and 32 mm for the 1978 seed lot. A large number of abnormal seedlings lacking in root and shoot growth were observed in the 1978 seed lot indicating its deterioration was for seed germination and vigor attributes.

**Table 1. Germination test of pigeonpea germplasm accession ICP 9820 seed<sup>1</sup>.**

Description	2004 seed lot	1978 seed lot
Seeds tested	100	100
Normal seedlings	99	11 <sup>2</sup>
Abnormal seedlings	1	74
Diseased/dead seeds	0	15
Germination (%)	99	11
Seedling root length (mm)	140	17
Seedling shoot length (mm)	248	32

1. Observations were recorded after 20 days.

2. Weak seedlings.



**Figure 1.** Seed germination test of pigeonpea germplasm accession ICP 9820: 2004 rainy season – normal seedlings (top left); 1978 rainy season – weak seedlings (top right); abnormal seedlings (bottom left); and diseased/dead seeds (bottom right).

A critical review of these observations indicates that seeds lose viability even under favorable storage conditions and the rate of loss is rapid after the seed viability reaches a critical level. Seed viability declined slowly at first and then rapidly as the seeds aged. It is important to know the time of this decline in order to regenerate the accession. Excessive deterioration beyond a level of resurrection will lead to permanent loss of the accession.

## Conclusions

It is quite possible in genebanks that some accessions may show no germination following normal germination tests. However, in such accessions there could be seeds which produce less vigorous seedlings during extended period of germination. Rescuing seedlings of such accessions is important for further growing in ideal conditions of glasshouse/field for obtaining seeds. Seed lots of such accessions when planted directly in the field may not produce seedlings robust enough to penetrate the soil crust and withstand the field conditions, thus, resulting in loss of valuable germplasm.

## References

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